sip

Society for Invertebrate Pathology

33rd Annual Meeting
Guanajuato, Mexico

University of Guanajuato
13-18 August, 2000

Society for Invertebrate Pathology

LIST OF OFFICERS, 2000

President	Juerg Huber, Germany
Vice President	James Harper, USA
Past President	Brian Federici, USA
Secretary	Ann Hajek, USA
Treasurer	Theodore Andreadis, USA
Trustees	Jennifer Cory, UK
	David Ellar, UK
	Jorge Ibarra, MEXICO
	Leellen Solter, USA
Newsletter Editor	Mark Goettel, CANADA
Assistant Newsletter Editor	Leellen Solter, USA

SIP DIVISIONS

Bacteria

Alejandro Bravo, Chair
Jean-Louis Schwartz,, Vice-Chair
Armelle Delecluse Secretary/Treasurer
Ray Akhurst, Member-at-large
Ruud De Maagd, Member-at-large

Microsporidia

Andreas Linde, Chair
James Becnel, Vice-Chair
Margaret Rotstein, Secretary
Ingemar Fries, Trustee
Louis Malone, Trustee

Microbial Control

Lawrence Lacey, chair
Wendy Gelernter, chair-elect
Jeff Lord, secretary
Michael Brownbridge, Member-at-large (1998-2000)
Nguya Maniania, Member-at-large (1999-2000)
Denis Burges, Member-at-large (1999-2001)

Virus

Peter Krell, Chair
Ian Smith, Vice-Chair
Johannes Jehle, Secretary/Treasurer
Doreen Winstanely, Member-at-large
Nikolai AM Van Beek, Member-at-large

SIP COMMITTEE MEMBERS

MEETINGS BOARD SITE SELECTION COMMITTEE
Just Vlak, Chairman
Mike Adang
Yoshifumi Hashimoto

FOUNDER'S LECTURE COMMITTEE
Dudley Pinnock, Chairman
David Ellar
Harry Kaya
Max Bergoin

MEMBERSHIP COMMITTEE
James J. Becnel, Chairman
Jorge Ibarra
Robert S. Anderson
Lawrence A. Lacey

ENDOWMENT COMMITTEE & STUDENT AWARDS COMMITTEE
Tad Poprawski , Chairman
Nguya K. Maniania
Stephen P. Wraight

NOMINATING COMMITTEE
Wendy Gelernter, Chairman
Bob Granados
Isabelle Thiery
Toshi Iizuka

DATABASE & WEB SITE COMMITTEE
David Onstad, Chairman
Margaret (Peg) Rotstein
John Vandenberg
James Becnel
Mark Goettel
Richard Humber

The Society for Invertebrate Pathology thanks the following sponsors for their generous financial support that helped defray the costs of the 2000 Annual Meeting:

CINVESTAV
Universidad de Guanajuato
Municipal and State Governments ofGuanajuato
Agraquest
Aventis
Dow AgroSciences
Monsanto
Novartis
Pioneer HyBred
Valent BioSciences
Thermo-Trilogy
Becker Microbial Products
Ecogen, Incorporated

Members of the 2000 SIP Organizing Committee:

Jorge E. Ibarra, Chair
Alejandra Bravo, Co-Chair
Ma. Cristina Del Rincón-Castro
Gabriela Olmedo
Eleazar Barboza-Corona
Angélica González
Juan Carlos Torres
Nina M. Bárcenas
Víctor Juárez-Pérez
Trevor Williams
Regina Basurto

Sunday, Aug. 13	Monday, Aug.14	Tuesday, Aug.15
8:30-17:00 Hotel Parador San Javier **SIP Council Meeting**	8:15-17:00 Meeting Headquarters, University of Guanajuato **Registration** 8:30-9:20 Auditorium of the University of Guanajuato **Welcome and Opening Ceremonies** 9:20-10:30 Auditorium of the University of Guanajuato **Founder's Memorial Lecture**	8:15-11:30 Room Pasteur **BACTERIA - SYMPOSIUM II:** **Ecology and Systematics** 8:15-12:20 Room Bassi **NEMATODES - SYMPOSIUM: Nematode/** **bacterium: The Present and Future.** 8:15-10:00 Room Steinhaus **VIRUS II - CONTRIBUTED PAPERS** Room Metchnikoff **FUNGI III - CONTRIBUTED PAPERS**
	Coffee Break 10:30-11:00	Coffee Break 10:00-10:30
	11:00-12:45 Room Pasteur **BACTERIA I - CONTRIBUTED PAPERS** Room Steinhaus **VIRUS I - CONTRIBUTED PAPERS** Room Bassi **FUNGI I -CONTRIBUTED PAPERS**	11:30-12:30 Room Pasteur **BACTERIA III - CONTRIBUTED PAPERS.** 10:30-12:30 Room Steinhaus **VIRUS III - CONTRIBUTED PAPERS.**
	Lunch 12:45-14:00	Lunch 12:30-14:00
14:00-18:00 Hotel Parador San Javier **Registration**	14:00-16:00 Room Pasteur **BACTERIA - SYMPOSIUM I:** **Resistance to Bt Toxins.** Room Steinhaus **VIRUS - SYMPOSIUM I:** **Virus-Insect Host Interactions** Room Bassi **FUNGI II - CONTIBUTED PAPERS**	14:00-16:00 **POSTER SESSION I** University Central Archway Passage
	Coffee Break 16:00-16:30	Coffee Break 16:00-16:30
	16:30-18:30 Room Pasteur **BACTERIA II - CONTRIBUTED PAPERS** Room Steinhaus **VIRUS - SYMPOSIUM II:** **Ecology of Insect Viruses** Room Bassi **MICROBIAL CONTROL WORKSHOP,** **New Products and Technologies**	16:30-18:50 Room Pasteur **BACTERIA - SYMPOSIUM III: Insertion** **of Bt insecticidal toxins into the membrane.** 16:30-17:30 Room Steinhaus **VIRUS - WORKSHOP I: Invertebrate** **Virus Taxonomy and Clasification** Room Metchnikoff **Business Meeting - Fungi** 16:30-17:45 Room Bassi **PROTOZOA I - CONTRIBUTED PAPERS** 17:45-18:45 Room Bassi **Business Meeting - Microsporidia** 17:30-18:30 Room Steinhaus **Business Meeting - Virus** Room Metchnikoff **Business Meeting - Nematodes**
18:00-21:00 Hotel Parador San Javier **Mixer**	Dinner 18:30-20:00 20:00-22:00 Juárez Theatre Cultural Event	Dinner 18:30-20:00 20:00-22:00 Parador San Javier **Business Meeting Microbial Control**

Wednesday, Aug.16	Thursday, Aug.17	Friday,Aug.18
8:00-9:30 Parador San Javier **S.I.P. Business Meeting**	8:15-11:30 Room Pasteur **BACTERIA - WORKSHOP I: Public Concerns about Transgenic Plants**	8:15-13:00 Auditorium **BACTERIA - SYMPOSIUM IV: Bacteria for the Control of Insects of Public Health Importance.**
10:00-11:00 **5 K RACE**	8:30-10:00 Room Steinhaus **MICROSPORIDIA WORKSHOP** 8:30-12:00 Room Bassi **MICROBIAL CONTROL SYMPOSIUM**	8:15-10:00 Room Steinhaus **MICROBIAL CONTROL II - CONTRIBUTED PAPERS** 8:30-9:50 Room Bassi **CROSS - DIVISION SYMPOSIUM: Diseases of non-insecta.**
	Coffee Break 10:00-10:30	Coffee Break 10:00-10:30
10:30-12:30 **EXCURSION** San Miguel de Allende	11:30-12:30 Room Pasteur **BACTERIA IV - CONTRIBUTED PAPERS** 10:30-12:30 Room Steinhaus **VIRUSES IV - CONTRIBUTED PAPERS**	10:30-11:45 Room Steinhaus **MICROSPORIDIA II - CONTRIBUTED PAPERS** 10:30-12:15 Room Bassi **FUNGI - SYMPOSIUM: Entomopathogenic fungi in Mexico and Central America**
	Lunch 12:30-14:00	Lunch 12:30-14:00
	14:00-16:00 **POSTER SESSION II** University Central Archway Passage	BUEN VIAJE !!! SEE YOU IN ISRAEL
	Coffee Break 16:00-16:30	
	16:30-17:30 Room Pasteur **BACTERIA V - CONTRIBUTED PAPERS** 16:30-18:30 Room Steinhaus **SYMPOSIUM: *Hexapoda Aegis*: Current Knowledge of Insect Defense Mechanisms.** 16:30-18:00 Room Bassi **MICROBIAL CONTROL I - CONTRIBUTED PAPERS**	
17:00-20:00 ExHacienda San Gabriel de Barrera **BARBECUE**	17:30-18:30 Room Pasteur **Business Meeting - Bacteria** 19:00-23:00 Real de Minas BANQUET	

PROGRAM

SUNDAY, AUGUST 13
Sunday 8:30-17:00.
Hotel Parador San Javier.
SIP Council Meeting.

Sunday 14:00-18-00.
Hotel Parador San Javier.
Registration.

Sunday 18:00-21:00.
Hotel Parador San Javier.
Mixer.

MONDAY, AUGUST 14

Monday 8:15-17:00
Meeting Headquarters, University of Guanajuato.
Registration.

Monday 8:30-9:20
Auditorium of the University of Guanajuato
Welcome and Opening Ceremonies

Monday 9:20-9:30
Auditorium of the University of Guanajuato
Founder's Memorial Lecture
Introduction: Dudley E. Pinnock

Monday 9:30-10:30
Auditorium of the University of Guanajuato
Founder's Memorial Lecture
Lecturer: Brian A. Federici. Honoree. H. Denis Burges.

10:30. Coffee break

Monday, 11:00-12:45
Room Pasteur
BACTERIA I - CONTRIBUTED PAPERS
Chair: Didier Lereclus

11:00. Evidence for different Cry1Ab resistance genes in a colony of *Plutella xylostella*. Joel González-Cabrera, Salvador Herrero and Juan Ferré. Department of Genetics, University of Valencia, 46100-Burjassot, Valencia, Spain

11:15. *Bt* Resistance and Dominance Levels. Anne Génissel[1,2] Michel Raymond[3] and Denis Bourguet[1]. Unité de Recherches de Lutte Biologique[1], INRA La Minière, 78 285 Guyancourt, France. Station de Zoologie forestière[2], INRA Centre de recherche d'Orléans, 45 160 Ardon, France. Institut des Sciences de l'Evolution[3], CC 065 Université Montpellier II, 34 090 Montpellier, France. **(STUDENT PAPER)**

11:30. Identification of a *Bacillus thuringiensis* ECF (extracytoplasmic function) sigma factor gene involved in ß-exotoxin production and/or secretion. Sylvain Espinasse[1], Michel Gohar[1,2], Didier Lereclus[1,3] and Vincent Sanchis[1,3]. Unité de Lutte Biologique[1], INRA La Minière, 78285 Guyancourt Cedex, France ; Aventis Crop Science[2], Jozef Plateaustraat 22–B 9000 Gent, Belgium & Unité de Biochimie Microbienne[3], Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

11:45. The toxin-coding plasmid of *Bacillus thuringiensis* subsp. *israelensis* is related to the major virulence plasmid of *Bacillus anthracis*. Colin Berry[1], Andrew F. Jones[1], Eitan Ben-Dov[2] and Arieh Zaritsky[2]. [1]Cardiff School of Biosciences, Cardiff University, Wales, UK. [2]Department of Life Sciences, Ben-Gurion University of the Negev, Israel

12:00. A molecular chaperone from *Bacillus thuringiensis* triggering the expression of cryptic ICP-genes. Jianxiu Yu and Yi Pang. State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou, 510275, People's Republic of China.

12:15. Genetic typing of the genus *Bacillus* and presence of two genes coding for the delta-endotoxin of *B. thuringiensis israelensis*. Sophie Chappuis[1,2], Mauro Tonolla[2], Raffaele Peduzzi[2], Nicola Patocchi[1], and Peter Lüthy[3]. Fondazione Bolle di Magadino, Casa comunale, CH-6573 Magadino, Switzerland[1]. Istituto Cantonale Batteriologico, via Ospedale 6, CH- 6904 Lugano, Switzerland[2]. Institute for Microbiology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland[3]. **(STUDENT PAPER)**

12:30 Hybrid *Bacillus thuringiensis* δ-endotoxins provide enhanced spectrum of activity on Lepidopteran pests. Sakuntala Sivasupramaniam[1], Victor T. Kabuye[1], Thomas Malvar[1], James A. Baum[1], Amy Jelen Gilmer[2], Angela Peters[2], Dolores Coyle[2], K. S. Mohan[3], Farah Deeba[3] and K. C. Ravi[3]. [1]Monsanto Co. 700 Chesterfield Parkway North, St. Louis, MO 63198. [2]Ecogen Inc. 2005 Cabot Boulevard West, Langhorne, PA 19047. [3]Monsanto Co. Bangalore, India

12:45. Lunch

Monday, 14:00-16:00
Room Pasteur
BACTERIA - SYMPOSIUM I: Resistance to Bt Toxins.
Chair: David Ellar

14:00. Role of Cyt1A in Managing Resistance to Mosquitocidal Proteins of *Bacillus thuringiensis* and *Bacillus sphaericus*. B. A. Federici[1,2,3], M. C. Wirth[1], D. K. Bideshi[1,2], H. W. Park[1], and W. E. Walton[1]. [1]Department of Entomology and Interdepartmental Graduate Programs in [2]Genetics & [3]Microbiology, University of California. Riverside, California 92521

14:20. *Bacillus thuringiensis* Cry1 toxin interactions with susceptible and resistant *Heliothis virescens*. M.J. Adang[1,2], J.L. Jurat-Fuentes[1], D. Banks[2] and F. Gould[3]. [1]Departments of Entomology, and [2]Biochemistry and Molecular Biology, University of Georgia, Athens, GA, 30602. [3]Department of Entomology, North Carolina State University. Raleigh, NC, 27695.

14:40. Insect proteinases and adaptation to *Bacillus thuringiensis*. Brenda Oppert[1], Juan Ferre[2], Salvador Herrero[2], Mike Grove[3], and Fred Gould[4]. [1] USDA Agricultural Research Service, Grain Marketing and Production Research Center, 1515 College Ave., Manhattan, KS 66502, [2]Department of Genetics, University of Valencia, 46100 Burjassot, Valencia, Spain, [3]Department of Entomology, 501 A.S.I. Building, The Pennsylvania State University, University Park, PA, 16802, [4]Department of Entomology, 2301_A Gardner Hall, North Carolina State University, Raleigh, NC 27695

15:00. Do *Heliothis virescens* and *Plutella xylostella* have the same mechanism of Cry1Ac resistance? Evidence from comparative mapping studies. David G. Heckel[1], Linda J. Gahan[2], Bruce E. Tabashnik[3] and Fred Gould[4]. [1]Dept. Genetics, University of Melbourne, Parkville, Victoria 3052, Australia; [2]Dept. Biological Sciences, Clemson University, Clemson, South Carolina 29634, USA; [3]Dept. Entomology, University of Arizona, Tucson, Arizona 85721, USA; [4]Dept. Entomology, North Carolina State University, Raleigh, North Carolina 27695, USA.

15:20. *Bacillus toxin* (*Bt*) susceptibility and resistance in *C. elegans*. Lisa Marroquin[1], Dino Elyassnia[1], Joel Griffitts[1], Jerald Feitelson[2], and Raffi V. Aroian[1] Department of Biology [1], University of California, San Diego, La Jolla, CA 92093-0349; Akkadix Corportation[2], La Jolla, CA 92037

15:40. Development and management of resistance to Bt toxins in the diamondback moth. M. Shelton. Department of Entomology. Cornell University/NYSAES. Geneva, New York 14456

16:00. Coffee break

Monday, 16:30-18:30
Room Pasteur
BACTERIA II - CONTRIBUTED PAPERS
Chair: Jean-Louis Schwartz

16:30. Ionic selectivity of the pores formed by the *Bacillus thuringiensis* insecticidal toxins Cry1Aa and Cry1Ac in midgut brush border membrane vesicles. Martin Kirouac[1], Vincent Vachon[1], Jean-François Noël[1], Frédéric Girard[1], Jean-Louis Schwartz[1,2] and Raynald Laprade[1]. Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1] and Biotechnology Research Institute, National Research Council, Montreal, Quebec[2], Canada. **(STUDENT PAPER)**

16:45. Analysis of mutants in some conserved residues of helix α-5 from *Bacillus thuringiensis* Cry1Ab δ-endotoxin. M. Eugenia Nuñez-Valdez, Jorge Sanchez, Laura Lina, Leopoldo Güereca and Alejandra Bravo. Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ap. Postal 510-3, Cuernavaca 62250, Mor., México

17:00. Expression of Cry1Aa receptor variants from silkworm in cultured mammalian cells. Satoshi. Ikawa, Yoko Tsuda, Takashi Fukada, Kenji Sugimoto and Michio Himeno. Department of Applied Biochemistry, College of Agriculture, Osaka Prefecture University, 1-1 Gakuen-cyo, Sakai , Osaka 599-8531, Japan

17:15. Differential effects of pH on the pore-forming properties of *Bacillus thuringiensis* insecticidal crystal toxins. Raynald Laprade[1], Le Binh Tran[1], Vincent Vachon[1] and Jean-Louis Schwartz[1,2]. Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1], and Biotechnology Research Institute, National Research Council, Montreal, Quebec[2], Canada

17:30. Estimation of the radius of the pores formed by the *Bacillus thuringiensis* Cry1C δ-endotoxin in planar lipid bilayers. Olivier Peyronnet[1], Brian Nieman[1,2], Francis Généreux[1,3], Vincent Vachon[1], Jean-Louis Schwartz[1,4] and Raynald Laprade[1.] Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1], University of Waterloo, Waterloo, Ontario[2], Université de Sherbrooke, Sherbrooke, Quebec[3] and Biotechnology Research Institute, National Research Council, Montreal, Quebec[4], Canada

17:45. Analysis of the pores formed by α-helix 4 mutants of the *Bacillus thuringiensis* insecticidal toxins Cry1Aa. Vincent Vachon[1], Gabrielle Préfontaine[2], Cécile Rang[3], Florence Coux[1,3], Marc Juteau[1], Jean-Louis Schwartz[1,2], Roland Brousseau[2], Roger Frutos[3], Raynald Laprade[1]and Luke Masson[2]. Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec, Canada[1], Biotechnology Research Institute, National Research Council, Montreal, Quebec, Canada[2] and CIRAD, Montpellier, France[3]

18:00. Role of α-helix 3 charged residues in pore formation by the *Bacillus thuringiensis* insecticidal toxin Cry1Aa. Vincent Vachon[1], Florence Coux[1,2], Gabrielle Préfontaine[3], Cécile Rang[2], Lucie Marceau[1], Luke Masson[3], Roger Frutos[2], Jean-Louis Schwartz[1,3], Roland Brousseau[3] and Raynald Laprade[1]. Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec, Canada[1], CIRAD, Montpellier, France[2], and Biotechnology Research Institute, National Research Council, Montreal, Quebec, Canada[3]

18:15. Expression of *Bacillus thuringiensis* Cry1Ac toxin domain III. Hyun-Ku Lee[1*], Daniela I. Oltean[2], Fred L. Gould[3] and Sarjeet S. Gill[2]. [1*]Department of Entomology & [2]Environmental Toxicology Graduate Program, University of California Riverside, Riverside CA, [3]Department of Entomology, North Carolina State University, Raleigh NC, [*]Department of Immunology, The Scripps Research Institute, La Jolla CA, USA. **(STUDENT PAPER)**

18:30. Dinner

Monday, 11:00-12:45
Room Steinhaus
VIRUS I - CONTRIBUTED PAPERS.
Chair: Just M. Vlak

11:00. The *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus genome sequence. Xinwen Chen[1,2], Sander Peters[3], Wilfred F. J. IJkel[2], Hans Sandbrink[3], Hualin Wang[1], Xiulian Sun[1], Renato Tochini[3], René Klein Lankhorst[3], Douwe Zuidema[2], Zhihong Hu[1] and Just M. Vlak[2].

11:15. Genomic and transcriptional organization of *Cp*DNV, a mosquito densovirus with an ambisense genome. E. Baquerizo, F.X. Jousset, A. M. Abd-Alla, F. Cousserans, and M. Bergoin. Unité de Virologie Moléculaire, Laboratoire de Pathologie Comparée, Université Montpellier II, 34095 Montpellier, France

11:30. Organization of the *Mamestra configurata* nucleopolyhedrovirus genome. Qianjun Li[1], Cam Donly[2], Lulin Li[3], Leslie G. WIllis[3], David A. Theilmann[3] and Martin Erlandson[1]. [1] Saskatoon Research Centre, AAFC-Saskatoon, SK. [2]Southern Crop Protection and Food Research Centre, AAFC, London, Ont. [3]Pacific Agri-Food Research Centre, AAFC, Summerland, B. C.

11:45. Phenotypic variation between genotypic variants of a nucleopolyhedrosis virus attacking pine beauty moth, *Panolis flammea.* D.J. Hodgson[1], R.S. Hails[2] & J.S. Cory[3]. [1,2,3]CEH Oxford, Mansfield Rd., Oxford, OX1 3SR, United Kingdom.

12:00. Genomic heterogeneity of *Cryptophlebia leucotreta* granulovirus is caused by intragenomic recombination of short sequence repeats (SSR). Johannes A. Jehle. State Education and Research Centre Neustadt/Wstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany

12:15. Expression of phenoloxidase genes from a parasitoid wasp in recombinant baculoviruses. Masamitsu Shikata, Ian Smith and Neil Parkinson. Central Science Laboratory, Sand Hutton, York, United Kingdom

12:30. Expression analysis and promoter characterization of a cluster of genes from CfMNPV transcribed late in viral infection. Clifford N. Dominy [1], Basif M. Arif [2] and Peter J. Krell [1]. [1]Department of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada. [2]Great Lakes Forestry Research Centre, Sault Ste Marie, Ontario, P6A 5M7Canada.

12:45. Lunch

Monday, 14:00-16:00
Room Steinhaus
VIRUS - SYMPOSIUM I: VIRUS-INSECT HOST INTERACTIONS.
Chair: Loy Volkman

14:00. Establishment of systemic infection in *Heliothis virescens* by HzSNPV and AcMNPV: A comparison of 'S' versus 'M' strategies. Jan O. Washburn1, James E. Wong2 and Loy E. Volkman1. 1 Department of Plant and Microbial Biology, University of California. Berkeley, CA 94720-3102. 2 DuPont Agricultural Products, Stine-Haskell Research Center, P.O. Box 30. Newark, DE 19714.

14:30. The role of hemocytes in AcMNPV pathogenesis in *Helicoverpa zea* and *Heliothis virescens.* Dominique Trudeau, Jan O. Washburn and Loy E. Volkman. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA.

15:00. Actin rearrangement during baculovirus infection: The role of Arif-1. Dagmar Knebel-Mörsdorf. Max-Planck-Institute for Neurological Research and Department of Neurology, University of Cologne, Cologne, Germany.

15:30. Polydnavirus-mediated alteration of insect physiology. Bruce A. Webb. Department of Entomology. University of Kentucky, Lexington KY 40546-0091

16:00. Coffee break

Monday, 16:30-18:30
Room Steinhaus
VIRUS - SYMPOSIUM II: Ecology of Insect Viruses.
Chair: Jenny Cory and Judy Myers

16:30. Where and when do nucleopolyhedroviruses matter to insect populations? Judith H. Myers. Department of Zoology and Faculty of Agricultural Sciences,University of British Columbia, 6270 University Blvd. Vancouver, B.C., Canada V6T 1Z4

16:50. Using Mathematical Models To Understand Disease Epizootics in Insects. Greg Dwyer. Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago IL 60637.

17:10. The spatial and temporal dynamics of the prevalence of nuclear polyhedrosis in a field population of the mulberry tiger moth, *Spilosoma imparilis* (Lepidoptera: Arctiidae). Yasuhisa Kunimi. Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan

17:30. Inhibition of baculoviral disease mediated by phytochemicals. Kelli Hoover and Heidi Appel. Department of Entomology, The Pennsylvania State University, 501 ASI, University Park, PA 16802 USA

17:50. Sublethal infections – are they important in host-virus dynamics? Jenny S. Cory. Ecology and Biocontrol Section, NERC Centrefor Ecology and Hydrology – Oxford, Mansfield Road, Oxford, OX1 3SR,UK

18:10. The secret life of invertebrate iridescent viruses. Trevor Williams. ECOSUR, A.P. 36, 30700 Tapachula, Chiapas, Mexico

18:30. Dinner

Monday, 11:00-12:45
Room Bassi
FUNGI I - CONTRIBUTED PAPERS.
Chair: Mark S. Goettel

11:00. A *Paecilomyces fumosoroseus* mutant strain with enhanced virulence against *Bemisia tabaci* synthetizes additional chitinase enzyme. Claudia Hernández, Magdalena Iracheta, Reyes Tamez, Carlos Hernández-Luna, Luis Galán-Wong and Benito Pereyra-Alférez. Departamento de Microbiología e Inmunología. Fac. Ciencias Biológicas/UANL. San Nicolás de los Garza, N. L. México. **(STUDENT PAPER)**

11:15. Storage compatibility of *Metarhizium anisopliae* var. *acridum* with other pesticides used for locust and grasshopper control. B. Luke and R. Bateman. CABI Bioscience, Silwood Park, Ascot, SL5 7TA UK. **(STUDENT PAPER)**

11:30. Abiotic factors influencing resting spores of the forest tent caterpillar pathogen *Furia crustosa* (Zygomycetes: Entomophthorales). Melanie J. Filotas and Ann E. Hajek. Department of Entomology Cornell University, Ithaca, NY 14853. **(STUDENT PAPER)**

11:45. Effect of *in vitro* -passage of *Beauveria bassiana* on virulence to silverleaf whitefly. Michael Brownbridge[1], Scott Costa[1] and Stefan Jaronski[2]. [1]Entomology Research Laboratory, University of Vermont, Burlington, Vermont 05405 and [2]Mycotech Corp., Butte, Montana 59702

12:00. Lack of effects of sublethal doses of the chemical pesticides imidacloprid, diflurobenzuron and buprofezin on the susceptibility of thermoregulating grasshoppers to the fungus *Beauveria bassiana*. Mark S. Goettel[1], Grant M. Duke[1] and G. Douglas Inglis[2]. [1]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1, [2] Department of Entomology, Mississippi State University, Mississippi State, MS 39762

12:15. Toxicological assessment of *Beauveria bassiana* against Mexican bean beetle. García-Gutiérrez[1], H. Medrano-Roldán [2] and V. Hernández-Velázquez[3]. [1]CIIDIR-IPN Unidad Durango, Sigma s/n Fracc. 20 de Nov. II 34220 Durango, Mex. [2] Instituto Tecnológico de Durango, Felipe Pescador 1830 Ote. [3]Centro Nacional de Referencia de Control Biológico. Tecomán Colima, 28120. **(STUDENT PAPER)**

12:30. Horizontal Transmission of *Beauveria bassiana* in the Colorado potato beetle. Francis A. Drummond[1], Eleanor Groden[1], and David Long[2]. Department of Biological Sciences[1], University of Maine, Orono, Maine, USA 04469 & 231 Salem Street [2], Lynnfield, MA 01940-2327

12:45. Lunch

Monday, 14:00-16:00
Room Bassi
FUNGI II - CONTRIBUTED PAPERS.
Chair: Judith K. Pell

14:00. Developing *Metarhizium anisopliae* for termite control in Africa: Strain selection and first field trial results. J. Langewald [1], A. Bokonon-Ganta [2], W. Gitonga [3], C. Kooyman [4], J. Maniania [5]. IITA (PHMD) Benin, B.P. 08-0932, Cotonou, Benin[1]; 2 Service de Protection des Végéteaux, Porto Novo, Benin[2]; Kenya Agricultural Research Institute, Nairobi, Kenya[3]; CABI Bioscience, Africa Regional Centre Nairobi, Kenya[4]; International Centre of Insect Physiology and Ecology, Nairobi, Kenya [5]

14:15. Variability and adaptability in mitosporic fungi selected for biocontrol of insect pests. E Watson, N E Jenkins & M B Thomas. Leverhulme Unit for Population Biology and Biological Control, NERC Centre for Population Biology and CABI Bioscience, Imperial College at Silwood Park, Ascot, Berks, SL5 7PY. **(STUDENT PAPER)**

14:30. First experiences with the development of a myco-insecticide based on *Beauveria brongniartii* against the field and forest cockchafer and related species. Kerstin Jung and Gisbert Zimmermann. Federal Biological Research Centre, Institut for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany

14:45. Efficacy of *Beauveria bassiana* for control of *Lygus lineolaris* and the interaction with a *Peristenus* parasitoid. A. Bruce Broadbent. Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford St., London, Ontario, Canada, N5V 4T3

15:00. Steam-exploded agricultural wastes as a novel source of nutrients for production of *Metarhizium anisopliae*. Larry Vaughan[1] and Herman Warren[2]. Office of International Research and Development[1] and Department of Plant Pathology, Physiology, and Weed Science[2]. Virginia Tech, Blacksburg, VA 24061 USA

15:15. Determination of the rate of production and size of conidia of the aphid-pathogenic fungus *Erynia neoaphidis* using image analysis. Simon Gray[1], Tony Bonner[1] and Judith Pell[2]. [1]Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., LU1 3JU, U.K. [2]Entomology and Nematology Dept., IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, U.K.

15:30. Testing a liquid medium for industrial production of submerged spores of *Metarhizium anisopliae var. acridum.* Dietrich Stephan[1], Dirk Mager[1], Helmut Junge[2], Hermann Strasser[3] and Gisbert Zimmermann[1]. Federal Biological Research Centre for Agriculture and Forestry[1], Institute for Biological Control , Heinrichstrasse 243, D-64285 Darmstadt, Germany, FZB Biotechnik GmbH[2], Glienicker Weg 185, D-12489 Berlin, Gemany, Leopold-Franzens-University[3], Institute for Microbiology, Technikerstrasse 25, A-6020 Innsbruck, Austria

15:45. Effect of exogenous nutrients on conidial germination of two hyphomycetes and infectivity of germinated conidia. R. R. James, Kika de la Garza, USDA-ARS Subtropical Agricul. Research Center Weslaco, TX

16:00. Coffee break

Monday, 16:30-18:30
Room Bassi
MICROBIAL CONTROL WORKSHOP. "New Products and Technologies in Microbial Control"
Chair: Lawrence Lacey and Wendy Gelernter

18:30. Dinner

TUESDAY AUGUST 15

Tuesday, 8:15-11:30
Room Pasteur
BACTERIA - SYMPOSIUM II: Ecology and Systematics of Entomopathogenic Bacteria.
Chair: Alejandra Bravo and Víctor Juárez

8:15. *Bacillus sphaericus* in the environment. Allan A. Yousten, Microbiology Section, Biology Department, Virginia Tech, Blacksburg, VA 24061.

8:35. Ecotoxicology of *Bacillus thuringiensis* and *Bacillus sphaericus*, with an emphasis on mammalian safety. Joel P. Siegel. United States Department of Agriculture, Agricultural Research Service, 2021 South Peach Avenue, Fresno, California, 93727

8:55. *Bacillus thuringiensis* conjugation under environmental conditions. Olivia Marcia Nagy Arantes. Bio/CCB, Universidade Estadual de Londrina, 86051-970, Londrina, Brazil.

9:15. Ecology of *Paenibacillus popilliae* and *Serratia* spp., pathogens of soil dwelling scarabaeid larvae. Trevor A. Jackson and Maureen O'Callaghan. AgResearch, PO Box 60, Lincoln, New Zealand

9:35. Control of virulence gene expression in *Bacillus thuringiensis* and *Bacillus cereus*. Didier Lereclus. Unité de Biochimie Microbienne, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, Station de Lutte Biologique, INRA, La Minière, 78285 Guyancourt cedex, France

10:00. Coffee break

10:30. Biodiversity of entomopathogenic sporeforming bacteria. Allan A. Yousten. Microbiology Section, Biology Department, Virginia Tech, Blacksburg, VA 24061

10:50. *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus anthracis*: one species based on genetic evidence. Anne-Brit Kolsto, Erlendur Helgason, Ole Andreas ¯kstad, Dominique Caugant and Ida Hegna. The Biotechnology Centre of Oslo and Institute of Pharmacy, University of Oslo, and National Institute of Public Health, Oslo, Norway

11:10. Molecular characterization of *Bacillus thuringiensis* type strains. Jorge E. Ibarra[1], Víctor Juárez-Pérez[2], Octavio Martínez[1], and Armelle Delecluse[2]. [1]CINVESTAV-IPN, 36500 Irapuato, Gto. México; [2]Bactéries et Champignons Entomopathogènes, Institut Pasteur, Paris, France.

Tuesday, 11:30-12:30
Room Pasteur
BACTERIA III - CONTRIBUTED PAPERS.
Chair: Brian A. Federici

11:30. Structural role for Domain I helix α-7 in Cry3A crystallization *in vivo* in *Bacillus thuringiensis*. Hyun-Woo Park [1] and Brian A. Federici [1,2]. Department of Entomology [1] & Graduate Programs in Genetics and Microbiology [2]. University of California – Riverside, Riverside, California 92521, USA

11:45. Properties of a recombinant *Bacillus thuringiensis* subsp. *israelensis* IPS-82 that produces Cry11B. Hyun-Woo Park [1], Armelle Delécluse [2] and Brian A. Federici [1]. Department of Entomology, University of California, Riverside, California 92521, USA [1]. Unité des Bactéries Entomopathogènes, Institut Pasteur, Paris, France [2]

12:00. Binding of ICP to the BBMVs and midgut epithelia of *Culex pipiens* and *Bombyx mori* larvae. Hiroshi Sakai[1], Kanao Otake[2], Motoyuki Esaki[2], Tohru Komano[3], and Masashi Yamagiwa[1]. [1]Department of Bioscience and Biotechnology, Okayama University, Okayama 700-8530, Japan, [2]Division of Applied Life Sciences, Kyoto University, Kyoto 606-8502, Japan, [3]Department of Genetic Engineering, Kinki University, Wakayama 649-6493, Japan

12:15. Isolation of *Bacillus thuringiensis* from Faeces of Insectivorous Bats from Upper Rhine Valley, Germany. Thania V. Guaycurus[1], Andreas Arnold[2] and Norbert Becker[2]. 1.Department of Genetic, Institut Oswaldo Cruz, FIOCRUZ, Brasil1. 2.German Mosquito Control Association, KABS, Germany

12:30. Lunch

Tuesday, 14:00-16:00
University Central Archway Passage
POSTER SESSION I

BACTERIA

BP1. Binding sites for the Cry1Ac Insecticidal Crystal Protein of *Bacillus thuringiensis* in *Helicoverpa armigera* (Lepidoptera: Noctuidae). C. Angelucci[1, 2], R.A. Akhurst[1] [1]CSIRO Division of Entomology; [2]Faculty of Science, Australian National University, Canberra, Australia. **(STUDENT POSTER)**

BP2. Regulation of the accumulation of the Cry1D toxin in *Bacillus thuringiensis* subsp. *aizawai*. Lily Chang[1], Yu Zinui[1] and Arthur Aronson[2]. [1]Laboratory of Bacillus Molecular Biology, Huazhong Agricultural University, Wuhan 430070, PRC; [2]Department of Biological Sciences, Purdue University, W. Lafayette, IN, 47907

BP3. A new procedure and method of analysis to evaluate the performance of liquid formulations of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) in streams or rivers. Mario Boisvert[1] and Jacques Boisvert[1]. Département de Chimie-Biologie[1], Université du Québec à Trois-Rivières, Trois-Rivières (Québec, Canada), G9A 5H7. **(STUDENT POSTER)**

BP4. Ecological risk of transgenic insect resistance under Canadian field conditions. Lorraine Braun[1], Anne Légère[2], Peter Mason[3], Neal Stewart Jr.[4], and Suzanne Warwick[3]. [1]AAFC, 107 Science Place, Saskatoon SK Canada S7N 0X2; [2]AAFC, 2560 boulevard Hochelaga, Sainte-Foy PQ Canada G1V 2J3; [3]AAFC-ECORC, Ottawa ON Canada K1A OC6; [4]Dept. Biol. U.N.C. Greensboro NC 27402-6174, USA

BP5. Characterisation of the Cry1Ac-binding carbohydrate epitopes on *Manduca sexta* 120 KDa Aminopeptidase N. Catherine E. CHAMBERS, Joseph Carroll and David J. Ellar. Department of Biochemistry, University of Cambridge 80 Tennis Court Road, Cambridge, U.K., CB2 1GA

BP6. The α-glucosidase in *Culex pipiens* midgut which serves as receptor to *Bacillus sphaericus* binary toxin : cloning and expression. Isabelle Darboux[1], Jean-François Charles[2] and David Pauron[1] [1]Institut National de la Recherche Agronomique, Unité Santé Végétale et Environnement, 123 Boulevard Francis Meilland, 06606 Antibes Cedex, France. Institut Pasteur, Laboratoire des Bactéries et Champignons Entomopathogènes, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France

BP7. Response of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to the selection with *Bacillus thuringiensis*. Ovidio Diaz-Gomez,[1] J.

C. Rodriguez-M.,[2] A. M. Shelton,[3] A. Lagunes-T.,[2] N. M. Barcenas-O.,[2] and R. Alatorre R.[2]. Facultad de Agronomia, U.A.S.L.P. Alvaro Obregon 64, San Luis Potosi, S.L.P. 78000, MEXICO[1]. Inst. de Fitosanidad, C.P. Km 35.5 Carr. Mexico-Texcoco. Montecillo 56230, Edo. de Mexico[2]. Entomology, Cornell Univ. NYSAES, Geneva, NY 14456 U.S.A.[3]

BP8. Gene deletion indicates that Vip3A is required for the *Bacillus thuringiensis* spore effect against *Spodoptera exigua*. William P. Donovan, Judith C. Donovan and James T. Engleman Ecogen Inc. Langhorne, PA 19047

BP9. Effect of *Bacillus thuringiensis* toxins on the midgut of the nun moth, *Lymantria monacha*. C. Rausell, [1], N. De Decker[2], I. García-Robles[1], B. Escriche[2], E. Van Kerkhove[2], M.D. Real[1] and A.C. Martínez-Ramírez[1]. [1] Dep. Genética, Fac. CC. Biológicas, Universitat de Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia) SPAIN. [2] Department Medische Basis Wetenschappen, Limburgs Universitair Centrum, B-3590 Diepenbeek, Belgium

BP10. Reliability evaluation of $K_L$ap as a parameter for *Bacillus thuringiensis* scale-up fermentations. Andrés González, Alexandre Restrepo, and Sergio Orduz. Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas, Apartado Aéreo 7378, Medellín, Colombia

BP11. Specificity of *Bacillus thuringiensis* Cry1 proteins Against Colombian *Spodoptera frugiperda* population. GROSSO V, Martinez W, Uribe D and J Cerón. Instituto de Biotecnología, Universidad Nacional de Colombia, A.A. 14-490, Santafe de Bogotá, Colombia. (STUDENT POSTER)

BP12. Effects of *Bacillus thuringiensis* Insecticidal Crystal Proteins on Adult *Heliothis virescens* (F.) and *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Mike Grove [1], Wendy Little [1,2], and William J. McCarthy[1]. Pesticide Research Laboratory and Graduate Study Center, Department of Entomology, The Pennsylvania State University, University Park, PA, 16802. Present address: American Type Culture Collection 10801 University Blvd. Manassas, VA, 20110

BP13. Role of the tryptophans residues in toxicity of Cry1Ab toxin. Georgina Hernández , Alejandra Bravo and Ma. Eugenia Núñez. Instituto de Biotecnología Universidad Nacional Autonoma de México Ap. postal 510-3 Cuernavaca Morelos. **(STUDENT POSTER)**

BP14. Effect of *Bacillus thuringiensis* β-exotoxin on three species of *Anastrepha* (Diptera: Tephritidae). Jorge Toledo[1], Pablo Liedo[1], Trevor Williams[1] and Jorge E. Ibarra[2]. [1]ECOSUR, Apdo .Postal 36, 30700Tapachula, Chis., Mexico; [2]CINVESTAV-IPN, Apdo. Postal 629, 36500 Irapuato, Gto., Mexico

BP15. Analysis of the properties of *Bacillus thuringiensis* insecticidal crystal toxins using a potential-sensitive fluorescent probe. Martin Kirouac[1], Vincent Vachon[1], Sébastien Rivest[1], Jean-Louis Schwartz[1,2] and Raynald Laprade[1]. Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1], and Biotechnology Research Institute, National Research Council, Montreal, Quebec[2], Canada

BP16. Oligopeptide permease is required for expression of the *Bacillus thuringiensis plcR* regulon and for virulence. Myriam Gominet[1], Nathalie Gilois[2] and Didier Lereclus[1, 2]. Unité de Biochimie Microbienne, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris[1], Station de Lutte Biologique, INRA, La Minière, 78285 Guyancourt cedex, France[2]

BP17. Novel low molecular toxin of *Bacillus thuringiensis* (H14) strain. Vladimir E. Likhovidov, Faskhetdin Sh. Isangalin State Research Center for Applied Microbiology, Obolenks, Serpukhov region, Moscow area, 142279, Russia

BP18. New *Bacillus thuringiensis* bacteriophage with wide spectrum of action. Vladimir E. Likhovidov , Faskhetdin Sh. Isangalin State Research Center for Applied Microbiology, Obolensk,region, Moscow area, 142279, Russia

BP19. Screening Of Tropical Strains Of *Paenibacillus popilliae* Against Two Mexican White Grub Species. Najera-Rincon, Miguel R[1], E. Hidalgo[2], P.J. Shanon[3], and L. A. Rodriguez del Bosque[4]. [1]National Research Center for Sustainable Production, CENAPROS-INIFAP. PO BOX: 7-116. Morelia, Michoacan. 58260. Mexico. [2]Tropical Agricultural Research and Higher Education Center (CATIE), Costa Rica. [3]Natural Resources Institute, United Kingdom, [4]North East Regional Research Center, Mexico

BP20. Various levels of Cross-resistanceto *Bacillus sphaericus* strains in four*B.sphaericus* resistant *Culex pipiens* (Diptera : Culicidae) mosquito populations. Christina Nielsen-LeRoux[1]*, D. Raghunatha Rao[2], T.R. Mani[2], SylvianneHamon[1], Jittawadee Rodcharoen[3] & Mir.S.Mulla[3]. [1] BactériesEntomopathogènes, Institut Pasteur, 28, Rue du Dr. Roux, 75724 ParisCedex 15, France, [2] Centre for Research in Medical Entomology I.CM.R.) Post Box 11, 4 SarojiniStreet, Chinna Chokkikulam Madurai- 625 002, India. [3]Department of Entomology, University of California,Riverside,CA 92521-0314, U.S A.

BP21. Isolation and identification of a putative *Bacillus sphaericus* strain producing Cry-like proteins. M. Eugenia Nuñez-Valdez, F. Javier Villalobos, Marco A. Barreda, Angel Romero, Luciano Hernandez[‡] and Alejandra Bravo. Instituto de Biotecnología, Cuernavaca, Mor. and [‡]Facultad de Química, D.F. Universidad Nacional Autónoma de México

BP22. Binding analysis of *Bacillus thuringiensis* Cry11Bb toxin to *Aedes aegypti* brush border membrane vesicles. Cesar Segura[1], Sergio Orduz[1] and Mike Adang[2]. Biotechnology and Biological Control Unit. Corporación para Investigaciones Biológicas. Apartado Aéreo 7378 Medellin, Colombia[1]. Entomology Department, University of Georgia Athens, GA 30602-2605., USA[2].

BP23. A study of the factors determining susceptibility to Cry δ-endotoxins. Johanna S. REES[1], Daniel J. Lightwood[1,2], Catherine E. Chambers[1], Eileen J. Bone[1] and David J. Ellar[1]. Deapartment of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, Cambridgeshire, CB2 1GA, UK[1], Celltech Chiroscience plc, 216 Bath Road, Slough, Berkshire, SL1 4EN, UK[2]. **(STUDENT POSTER)**

BP24. Gene organization of large plasmids of *Bacillus thuringiensis* subsp. *israelensis* and its related strains. Kikuo Sen[1], Hideyuki Katagiri[1], Shin-ichi Kurosawa[1, 2], Hiroshiro Shibai[1], So Takebe[3] and Tohru Komano[3]. [1]Faculty of Agriculture, Shinshu University, Nagano 399-4598, JAPAN; [2]Akita Prefectural University, Akita 010-0195, JAPAN; [3]Department of Genetic Engineering, Kinki University, Wakayama 649-6493, JAPAN

BP25. Development of a bioassay methodology for the evaluation of the biopesticide activity of *Bacillus thuringiensis* native strains against first instar larvae of *Tecia solanivora*. Uribe D, Castelblanco A, Grosso V, Martinez W and J Cerón. Instituto de Biotecnologia Universidad Nacional de Colombia, A.A. 14-490, Santafe de Bogotá, Colombia. **(STUDENT POSTER)**

BP26. Cloning and characterization of four distinct gypsy moth midgut aminopeptidase-N enzymes related to the *Bacillus thuringiensis* Cry1Ac receptor. Karen J. Garner and Algimantas P. Valaitis. USDA Forest Service, Northeastern Research Station. Delaware, OH 43015

BP27. Investigations of Cry4A toxin structure and function. Masashi Yamagiwa[1], Tohru Komano[2], and Hiroshi Sakai[1]. [1]Department of Bioscience and Biotechnology, Okayama University, Okayama 700-8530, Japan, [2]Department of Genetic Engineering, Faculty of Biology-oriented Science and Technology, Kinki University, Wakayama 649-64, Japan

BP28. *Bacillus thuringiensis* Cry1Ac and Cry1Fa δ-endotoxins share aminopeptidase binding proteins in *Heliothis virescens.* David J. Banks[1], Juan L. Jurat-Fuentes[2] and Michael J. Adang[1,2]. [1] Department of Biochemistry and Molecular Biology, University of Georgia, Athens GA, 30602, US, [2] Department of Entomology, University of Georgia, Athens GA, 30602. **(STUDENT POSTER)**

16:00. Coffee break

Tuesday, 16:30-18:50
Room Pasteur
BACTERIA - SYMPOSIUM III: Insertion of Bt insecticidal toxins into the membrane.
Chair: Alejandra Bravo

16:30. Studies on the status of the *Bacillus thuringiensis* Cry1A toxins in *Manduca sexta* larval membranes. Arthur Aronson, Lan Wu and Manoj Kumar. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

16:50. Site-directed Spin Labeling of Cry1Aa and Cry1Ab for Analysis of Membrane Insertion into *Manduca sexta* BBMV. Oscar Alzate1,2 and Donald H. Dean2. Pontificia Bolivariana University, Medellin, Colombia1 and Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210-12922

17:10. Membrane pore architecture of a cytolytic toxin from *Bacillus thuringiensis.* David J. Ellar, and Boonhiang Promdonkoy. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, CB2 1GA, UK

17:30. Visualization of the pore formed by the insecticidal *Bacillus thuringiensis* Cry1Aa toxin in lipid membranes. Jean-Louis Schwartz[1,2] ,Nicole Van Mau[3], Véronique Vié[4], Christian Le Grimellec[4], Frédéric Heitz[3], Raynald Laprade[2], Cécile Rang[5], Roger Frutos[5] and Luke Masson[1]. [1]BRI, National Research Council of Canada, Montreal (Qc), Canada, H4P 2R2, [2]GRTM, Université de Montréal, Montreal, H3C 2J7, [3]CRBM, CNRS UPR 1086, 34293 Montpellier Cedex 5, France, [4]CBS, 34090 Montpellier Cedex, France, [5]CIRAD, 34032 Montpellier Cedex 1, France

17:50. Evidences for inter-molecular interaction as a necessary step for pore-formation activity and toxicity of *Bacillus thuringiensis* Cry1Ab toxin. Mario Soberón Rigoberto V. Pérez, María E. Nuñez-Valdéz, Isabel Gómez, Jorge Sánchez, and Alejandra Bravo. Instituto de Biotecnolog'a, U.N.A.M., Apdo Postal 510-3 , Cuernavaca, Morelos 62271. México.

18:10. Studies on the mode of action of *B. thuringiensis* δ-endotoxin suggest an "umbrella-like" model for its folding and insertion into membranes. Yechiel Shai. The Department of Biological Chemistry. The Weizmann Institute of Science, Rehovot, 76100 Israel.

18:30. Interaction of Cry3A δ-endotoxin with model liposomes. O. I. Loseva[1], E. I. Tiktopulo [2], V. D.Vasiliev[2], A. P. Dobritsa[1] and S. A. Potekhin[2]. State Research Center for Applied Microbiology, Obolensk, Moscow Region, 142279[1]. Institute of Protein Research, Russian Academy of Sciences, Pushchino, Moscow Region, 142292[2], Russia

18:50. Dinner

Tuesday, 8:15-10:00
Room Steinhaus
VIRUS II - CONTRIBUTED PAPERS.
Chair: Robert R. Granados

8:15. The p34.8 (gp37, spindlin) gene is not essential for baculovirus replication. XiaoWen Cheng[1], Peter Krell[2], Qili Feng[1], Arthur Retnakaran[1] and Basil Arif[1]. Great Lakes Forestry Centre[1], Sault Ste. Marie, Ontario and Department of Microbiology [2], University of Guelph, Ontario, Canada.

8:30. The AcMNPV *pe38* gene is not essential but affects DNA synthesis and budded virus production. Maynard L. Milks, Leslie G. Willis, and David A. Theilmann. Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B. C., Canada V0H 1Z0.

8:45. Impact of transposon TCp3.2 and TCl4.7 integration on *Cydia pomonella* granulovirus. Hugo M. Arends and Johannes A. Jehle. State Education and Research Centre Neustadt/Weinstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany. **(STUDENT PAPER)**

9:00. A model of Nucleopolyhedrovirus population genetics applied to co-occlusion. Jim Bull, Charles Godfray and David O'Reilly. Department of Biology, Sir Alexander Fleming Building, Imperial College, South Kensington, London, SW7 2AZ, UK. **(STUDENT PAPER)**

9:15. Functional analysis of the LdMNPV *hrf*-1 gene in recombinant AcMNPV-infected Ld652Y cells. Motoko Ikeda[1], Elizabeth A. Reimbold[2], and Suzanne M. Thiem[2,3]. Graduate School of Bioagricultural Sciences[1], Nagoya University, Chikusa, Nagoya 464-8601, JAPAN; Depts of Microbiology[2] and Entomology[3], Michigan State University, East Lansing, MI 48824

9:30. Three major structural proteins of White Spot Syndrome Virus have evolved by gene duplication. Marielle C.W. van Hulten, Fokko Zandbergen, Marcel Westenberg, Stephen D. Goodall and Just M. Vlak. Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, the Netherlands. **(STUDENT PAPER)**

9:45. *De novo* generation of defective interfering baculoviruses in insect cells. Gorben P. Pijlman[1], Erwin B. van den Born[1], Dirk E. Martens[2], Cornelis D. de Gooijer[2] and Just M. Vlak[1]. Laboratory of Virology[1] and Food and Bioprocess Engineering Group [2], Binnenhaven 11 6709 PD, Wageningen University, The Netherlands. **(STUDENT PAPER)**

10:00. Coffee break

Tuesday, 10:30-12:30
Room Steinhaus
VIRUS III - CONTRIBUTED PAPERS.
Chair: Basil Arif

10:30. Characterization of the 122b isolate of LdMNPV. Holly J.R. Popham[1], David S.Bischoff [2], Melissa J. Mercer[1], and James M. Slavicek[1]. USDA Forest Service, Northeastern Research Station, Delaware, OH, 43015, and AgriVax Inc., USC School of Medicine, Los Angeles, Ca 90033

10:45. EGT activity in granulovirus-infected insect larvae. Ian Smith[1], Robert Weaver[1] and Frances Hunter[2]. [1]Central Science Laboratory, Sand Hutton, York, United Kingdom. [2]School of Animal and Microbial Sciences, University of Reading, United Kingdom

11:00. Replication of Hz-2V in the reproductive tissues of H*elicoverpa zea*. Christopher P. Rallis[1] and John P. Burand[1,2]. Departments of Entomology[1] & Microbiology [2]. University of Massachusetts-Amherst, Amherst, Massachusetts 01003

11:15. Evidence for budded virions in a new baculovirus from the mosquito *Culex nigripalpus.* James J. Becnel, Susan White, Bettina Moser, Tokuo Fukuda and Margaret J. Rotstein. Center for Medical, Agricultural and Veterinary Entomology. U. S. Department of Agriculture, Agricultural Research Service, Gainesville, Florida 32604

11:30. The effect of entomopoxvirus infection on the development and endocrinology of *Mythimna separata* larvae. Madoka Nakai, Akane Ohba and Yasuhisa Kunimi. Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology

11:45. Developmental resistance of *Lymantria dispar* to *Lymantria dispar* Nucleopolyhedrovirus. Mike Grove, Kit O'Connor, Andrea Dreger, Maria Geleskie, Bailey Klinestiver, Heather Emminger, Kelli Hoover. Department of Entomology, The Pennsylvania State University, University Park, PA 16802

12:00. Sublethal baculovirus infections in the Indian meal moth, *Plodia interpunctella*: From Individuals to Populations. Steven M. Sait. Population and Evolutionary Biology Research Group, School of Biological Sciences, University of Liverpool, Nicholson Building, Brownlow Street, Liverpool, L69 3GS, UK

12:15. Baculoviruses and tritrophic interactions: The effects of insect host-plant on a baculovirus from the winter moth, *Operophtera brumata*. Ben Raymond & Rosie Hails. CEH-Oxford, Institute of Virology and Environmental Microbiology, Oxford, OX1 3SR, UK.

12:30. Lunch

Tuesday, 14:00-16:00
University Central Archway Passage
POSTER SESSION I

VIRUS

VP1. Molecular cloning and sequence analysis of the *immediate early 1* gene of *Anticarsia gemmatalis* MNPV. M.F. Bilen[1]; M.G. Pilloff[1]; B. Morais Ribeiro[2]; V. Romanowski[1, 3]; M.E. Lozano[1] and P.D. Ghiringhelli[1]. [1]Departamento de Ciencia y Tecnología - CEI, Universidad Nacional de Quilmes, Saenz Peña 180, B1876BXD-Bernal; [2]Dto. de Biología Celular, Universidade de Brasilia, [3]IBBM, Facultad de Ciencias Exactas, UNLP, Argentina. **(STUDENT POSTER)**

VP2. Stable cell lines expressing baculovirus P35: Resistance to nutrient stress and enhanced production of a secreted reporter protein. G. Lin, G. Li, R.R. Granados, and G.W. Blissard. Boyce Thompson Institute, Cornell Univ., Ithaca, NY 14853-1801.

VP3. Midgut cell cultures from *Pseudaletia unipuncta* and *Trichoplusia ni* larvae for baculovirus studies. J. Garcia, J. Zhong, G. Li, P. Wang and R.R. Granados. Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853

VP4. Searching for nuclear polyhedrosis viruses in the natural populations of *Malacosoma neustria* L. (Lasiocampidae). Jankevica L.[1], Kropa M.[1], Jankevics E.[2]. [1]Department of Experimental Entomology, Institute of Biology, University of Latvia, Miera street 3, Salaspils, LV 2169, Latvia, [2]LU Biomedical Research and Study Centre, Ratsupites street 1, Riga, LV 1067, Latvia

VP5. Development of *Hyphantria cunea* nucleopolyhedrovirus for microbial control: cloning and biological characterization. K. Kamiya[1], N. Okimoto[2], N. Shirata[2], S. Kawamura[1], M. Ikeda[2], & M. Kobayashi[2]. [1]Gifu Prefectural Institute for Bioindustrial Technology, Minokamo, Gifu 505-0004, Japan; [2]Laboratory of Sericulture and Entomoresources, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-0814, Japan.

VP6. Establishment of a new cell line having high phagocytic ability from hemocytes of the beet armyworm, *Spodoptera exigua*. Chisa Yasunaga-Aoki[1], Takeshi Kawarabata[1], Kazuhiro Iiyama[1], and Shigeo Imanishi[2]. [1] Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan, [2] National Institute of Sericultural and Entomological Science, Tsukuba 305-8634, Japan

VP7. *Hyphantria cunea* nucleopolyhedrovirus (NPV) interferes with *Bombyx mori* NPV replication in a cell line from *B. mori* Noriko Shirata[1], Motoko Ikeda[1], Katsumi Kamiya[2], Satoshi Kawamura[2] and Michihiro Kobayashi[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan; [2]Gifu Prefectural Institute of Bio-industrial Technology, Minokamo, Gifu, 505-0004, Japan

VP8. Sequence and organization of the *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (CfMNPV) genome. Jondavid de Jong[1], Clifford N. Dominy[1], Basil M. Arif[2], Hilary Lauzon[2] and Peter J. Krell[1]. [1]Department of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada, [2]Canadian Forest Service, Sault. Ste. Marie, Ontario, P6A 5M7, Canada

VP9. Gene Organization and Sequencing of the CfDEFNPV genome, a defective Nucleopolyhedrovirus of *Choristoneura fumiferana.* Hilary A.M. Lauzon[1], Peter B. Jamieson[1], Peter J. Krell[2] and Basil M. Arif[1]. [1]Canadian Forest Service, Great Lakes Forestry Centre,1219 Queen St. E., Sault Ste. Marie, Ontario, Canada, P6A 5M7; [2] Department of Microbiology, University of Guelph, Guelph, Canada, N1G 2W1

VP11. Biodiversity of *Spodoptera litura* NPVs from apparently healthy *Spodoptera litura* larvae collected in Indonesia from 1995 to 1997. Era Wahyuni[1, 3, 4], Liliane Croizier[3], André Pollet[1, 2], Soeprapto Mangoendihardjo[1], Miguel López-Ferber[3] and Guy Croizier[3]. 1- Research Center for Biological Control. University of Gadjah Mada (UGM). Yogyakarta. Indonesia. 2- Institut de Recherche pour le Développement (IRD). Paris. France. 3- UMR5087 INRA-CNRS-Université MontpellierII. Saint Christol-lès-Alès. France. 4- RILET Malang. Indonesia

VP12. Sequence analysis of the *Cydia pomonella* granulovirus genome. Teresa Luque[1], Ruth Finch[2], Doreen Winstanley[2] and David O'Reilly[1]. [1]Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK; [2]Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

VP13. Toxic effects of *Invertebrate iridescent virus 6* in mosquitoes. C. F. Marina,[1,2] J. I. Arredondo-Jiménez,[2] I. Fernández-Salas,[3] J. Ibarra[4] & T. Williams[1]. [1]ECOSUR, AP 36, Tapachula 30700, Chiapas, México, [2]Centro de Investigación de Paludismo, AP 537, Tapachula 30700, Chiapas, México, [3]Fac. Ciencias Biológicas, Univ. Auton. Nuevo León, AP 109-F, Monterrey, Mexico [4]CINVESTAV-IPN, AP 629, Irapuato 36500, Guanajuato, Mexico.

VP14. Advances in the use of *Spodoptera exempta* nuclear polyhedrosis virus (SeNPV) to control the larvae of *Spodoptera exempta* (East African Armyworm) in Tanzania. Mark Parnell[1], David Grzywacz[1], Charles Dewhurst[1] and Wilfred Mushobozi[2]. 1: Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK. 2: Pest Control Services, PO Box 7473, Arusha, Tanzania.

VP15. Replication of nuclear polyhedrosis viruses under high temperature *in vitro*. Takeru Sato. National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305-8605, Japan

VP16. Deletion of the KDEL ER-retention motif promotes secretion of chitinase from AcMNPV infected insect cells. G. Saville[1], C.J.Thomas[1], S.Mann[1], R.D.Possee[2] and L.A.King[1]. [1]School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, OX3. [2]NERC-CEH, Oxford OX1 3SR. **(STUDENT POSTER)**

VP17. *Cydia pomonella* granulovirus: Horizontal transmission of the virus is closely related to its distribution. Susanne B. Steineke and Johannes A. Jehle. State Education and Research Centre Neustadt/Weinstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany. **(STUDENT POSTER)**

VP18. Effect of sublethal dosages of *Granulovirus* (Baculoviridae) on *Spodoptera littoralis* (Lepidoptera, Noctuidae). E. Vargas-Osuna and A. Fernández-Vilchez. Entomología Agrícola y Forestal. Departamento de Ciencias y Recursos Agrícolas y Forestales. E.T.S.I.A.M. Universidad de Córdoba. Apartado 3048. 14080 Córdoba. Spain

16:00. Coffee break

Tuesday, 16:30-17:30
Room Steinhaus

VIRUS - WORKSHOP I: Invertebrate Virus Taxonomy and Classification
Chair: Peter J. Krell and Johannes Jehle

16:30. An introduction to the ICTV and invertebrate RNA viruses. Anette Schneemann and John E. Johnson. Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

16:50. The Baculoviridae, current taxonomic issues. David A. Theilmann. Pacific Agri-Food Research Centre. Agriculture and Agri-Food Canada. Summerland, B. C. Canada

17:10. On the taxonomy of white spot syndrome virus (whispovirus): a case study. Just M. Vlak and Marielle C.W. van Hulten. Laboratory of Virology, Binnenhaven 11 6709 PD, Wageningen University, The Netherlands.

Tuesday, 17:30-18:30
Room Steinhaus
Business meeting Virus

18:30. Dinner

Tuesday, 8:15-12:20
Room Bassi
NEMATODES - SYMPOSIUM: Nematode/bacterium: the Present and Future.
Chair: Harry K. Kaya and Itamar Glazer

8:15. Introduction (H. Kaya)

8:20. Bacterial symbionts and correlation with entomopathogenic nematode taxonomy. Noël Boemare. Laboratoire de Pathologie comparée, UMR CNRS-INRA n° 5087, CP 101, University of Montpellier II F-34095 Montpellier CEDEX 5, France.

8:45. From Steiner to present: Recapitulations and considerations of entomopathogenic nematode systematics in the 21st century. S. Patricia Stock. Department of Nematology. University of California Davis. One Shields Ave. Davis, CA 95616-8668

9:10. Molecular genetics of EPN: current status and future developments. Ann M. Burnell. Institute of Bioengineering and Agroecology, Department of Biology, National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland.

9:35. Nematode biodiversity: parasitism and speciation. Byron J. Adams, Khuong B. Nguyen and Heather L. Smith. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

10:00. Coffee break

10:30. *Photorahbdus* and *Xenorhabdus* Genes for Transgenic Plants. Thomas Meade, Scott Bintrim, Donald J. Merlo, Jon Mitchell and Jean L. Roberts. Dow AgroSciences LLC. 9330 Zionsville Road, Indianapolis, IN 46268

10:55. Nematode environmental tolerance. Itamar Glazer. Department of Nematology, Volcani Center, Bet Dagan 50250. Israel

11:20. Interactions between entomopathogenic nematode species and other agents. Albrecht M. Koppenhöfer. Department of Entomology, Rutgers University, New Brunswick, NJ 08901

11:45. Molluscicidal nematodes and their potential for slug control in North America. Parwinder Grewal. Department of Entomology, Ohio State University, Wooster, OH 44691

12:10. Conclusion (I. Glazer)

12:20. Lunch

Tuesday, 14:00-16:00
University Central Archway Passage
POSTER SESSION I

FUNGI

FP1. A laboratory trial demonstrating that *Erynia neoaphidis* is able to overwinter in aphid cadavers at low relative humidity. Tony Bonner[1], Simon Gray[1] and Judith Pell[2]. [1]Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., LU1 3JU, U.K., [2]Entomology and Nematology Dept., IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, U.K. **(STUDENT POSTER)**

FP2. Variability of *Beauveria bassiana* isolates from *Hypothenemus hampei*: Germination, sporulation and effect of water activity. Francisco Hernandez-Rosas[1], Raquel Alatorre-Rosas[1] and Gerardo Saucedo Castañeda[2]. [1]Instituto de Fitosanidad, Colegio de Postgraduados, Km 36.5 Carr. México-Texcoco. C.P. 56230. Texcoco, Edo. de México. México. [2]Departamento de Biotecnología. Universidad Autónoma Metropolitana. Unidad Iztapalapa. Apdo. Postal 55-535, México. **(STUDENT POSTER)**

FP3. Liquid culture production of blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus* using portable fermentation equipment. Mark A. Jackson[1] and David A. Odelson[2]. [1]USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Fermentation Biochemistry Research Unit, Peoria, IL, USA; [2]EcoSoil Systems, Inc., San Diego, CA, USA

FP4. Direct Spore Counts vs. Colony Forming Unit Counts As Methods for Quantifying Viable *Beauveria bassiana* Conidia. Stefan T. Jaronski and Judi Liebman. Mycotech Corporation, Butte MT 59701

FP5. Effect of salts, vitamins, sugars and sources of nitrogen on the growing of three Entomophthorales species: *Batkoa* sp., *Furia* sp. and *Neozygites floridana*. Luis G. Leite[1,2,4], Sérgio B. Alves[3], Antonio Batista Filho[1] and Donald W. Roberts[2]. **(STUDENT POSTER)**

FP6. Coating Spores of *Metarhizium flavoviride* for UVB-Protection and Effects on Virulence to African Desert Locust (*Schistocerca gregaria*) Forskal. Jarrod Leland[1], Donald Mullins[1], Herman Warren[2], Larry Vaughan[3], Jacques Fargues[4], Nathalie Smits[4], [1]Department of Entomology, [2]Department of Plant Pathology and Weed Science, and [3]Office of International Research and Development Virginia Tech, Blacksburg, VA, 24061 [4] Institut National de la Recherche Agronomique, 34982 Montferrier-sur-Lez, France. **(STUDENT POSTER)**

FP7. Effect of host plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). T. J. Poprawski, S. M. Greenberg, and M. A. Ciomperlik [1]. Beneficial Insects Research Unit, USDA-ARS Subtropical Agricultural Research Center, and Texas Agricultural Experiment Station, 2413 East Highway 83, Weslaco, Texas 78596;[1] Mission Plant Protection Center, USDA-APHIS Plant Protection and Quarantine, Moore Air Base, P.O. Box 2140, Mission, Texas 78572

FP8. Compatibility of Selected Fungicides with Fungal Pathogens of *Bemisia* Whiteflies. N. A. Silva Uribe, T. J. Poprawski[1], K. Arévalo-Niño and L. J. Galán-Wong. Universidad Autonoma de Nuevo Léon, Facultad de Ciencias Biológicas, Monterrey, Mexico; [1]USDA-ARS, Beneficial Insects Research Unit, Weslaco, TX 78596

FP9. Effects of constant and fluctuating temperatures on sporulation and infection by *Erynia neoaphidis*. Paresh A. Shah*, Markus Aebi and Urs Tuor. Mikrobiologisches Institut, ETH-Zentrum, CH-8092, Zürich, Switzerland *IACR-Rothamsted, Harpenden, AL5 2JQ, England

FP10. Effect of the growth media composition in the biopesticide activity of *Beauveria bassiana* strains against second-third instar larvae of *Spodoptera frugiperda*. Uribe D, Aponte L and J Cerón. Instituto de Biotecnología, Universidad Nacional de Colombia, A.A. 14-490, Santafe de Bogotá, Colombia

FP11. Blastospore growth and infectivity by injection into fall armyworm: a comparison of two strains of the entomopathogenic fungus *Paecilomyces fumosoroseus.* Jennifer A. Altre[1] and John D. Vandenberg[2]. [1]Department of Entomology, Cornell University, and [2]USDA Agricultural Research Service, US Plant Soil & Nutrition Lab, Tower Road, Ithaca, NY 14853.

FP12. Evaluation of isolates of the fungus *Paecilomyces fumosoroseus* with relation to their physiology and effectiveness in the control of *Frankliniella occidentalis*. Figueroa[1], L.M., A. Varela[1] & D. Corredor[2]. [1]Depto. de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana. [2]Facultad de Agronomía, Universidad Nacional de Colombia. Santafé de Bogotá, D.C., Colombia. **(STUDENT POSTER)**

FP13. Study of the alcohol dehydrogenase activity in *Metarhizium anisopliae*. Olga Alicia Callejas-Negrete, Angélica González and Juan Carlos Torres Guzmán. Instituto de investigación en Biología Experimental. Facultad de Química. Universidad de Guanajuato. Guanajuato, Gto. México. **(STUDENT POSTER)**

NEMATODES

NP1. Entomopathogenic nematodes for the control of turfgrass insect pests in Quebec. Louis Simard[1], Guy Bélair[2] & Jacques Brodeur[1]. [1]Département de Phytologie, Université Laval, Ste-Foy (Quebec) Canada, [2]Agriculture & Agri-Food Canada, St-Jean-sur-Richelieu (Quebec) Canada.

NP2. Gene regulation and expression of haemolytic/cytolytic activity from the insect pathogenic bacterium *Xenorhabdus nematophilus.* Brillard J., Boemare N., Bréhelin M. and Givaudan A. Laboratoire de Pathologie comparée, Université Montpellier II - INRA - CNRS UMR5087, 34095 Montpellier Cedex 5, France. **(STUDENT POSTER)**

NP3. Mortality of larvae and pupae of *Galleria mellonella* treated with bacterial symbiont from entomopathogenic nematodes. Ali N. Mahar, Sami A. Elawad, Simon, R. Gowen and Nigel M.G. Hague. Department of Agriculture, The University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT, U.K.

NP4. Influence of abiotic factors on the parasitism of *Steinernema feltiae* (Rhabditida: Steinernematidae) on larvae of *Anastrepha obliqua* (Diptera: Tephritidae). Jorge Toledo [1], Concepción Pérez[1], Pablo Liedo[1], Trevor Williams[1] and Jorge E. Ibarra[2]. [1]ECOSUR, A.P. 36, Tapachula 30700 , Chiapas, Mexico; [2]CINVESTAV-IPN, A.P. 629, Irapuato 36500, Guanajato, Mexico.

NP5. Development of entomopathogenic nematodes for control of codling moth in orchards and fruit bins. Lawrence A. Lacey, Thomas R. Unruh, and Richard Chauvin. Yakima Agricultural Research Lab, USDA-ARS, Wapato, WA 98951 USA

NP6. Controlling white grubs (*Phyllophaga* spp.) with entomopathogenic nematodes and fungi in Oaxaca, Mexico. Ruiz V., J[1]., Aquino B., T.[1] and H. K. Kaya[2]. CIIDIR OAXACA, IPN[1] and University of California, Davis[2]

PROTOZOA

PP1. A multiple infection of entomopathogenic microsporidians in *Bombyx mori.* Mihoko Hashiguchi[1], Rie Teramoto[1], Chisa Yasunaga-Aoki[1], Takeshi Kawarabata[1] and Hidehiro Tomimaru[2]. [1] Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan, [2] Fiacao De Seda Bratac S/A, Caixa Postal 39-Cep, 17690-000 Bastos-Est, Sao Paulo, Brazil. **(STUDENT POSTER)**

PP2. Molecular and morphological investigations on a microsporidium infecting the grape berry moth *Lobesia botrana.* Johannes A. Jehle[1], Charles R. Vossbrinck[2], Regina G. Kleespies[3] [1]State Education and Research Center Neustadt/Wstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr, Germany; [2] Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06504, USA; [3] Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany

PP3. Free radicals generation in the hemolymph of *Anopheles albimanus* and their effect against *Plasmodium berghei* ookinetes. Humberto Lanz–Mendoza, Salvador Hernández, Magdalena Ku, Avigail Gil, Uzziel Roman, María del Carmen Rodríguez and Mario H. Rodríguez. Centro de Investigaciones sobre Enfermedades Infecciosas. Instituto Nacional de Salud Pública. Cuernavaca, Morelos. México. CP 62508.

PP4. Biological and Pathological Studies on the *Helicosporidium* spp. D. Boucias, J. Becnel[*], G. White[*], C. Stokes, A. Tartar, and B. Adams. Department of Entomology and NematologyUniversity of Florida, Gainesville, Florida, 32611, USA. [*]USDA/ARS Center for Medical, Agricultural and Veterinary Entomology P.O. Box 14565,Gainesville, Fl 32604

PP5. Exposure to trypanosomatids slows development in *Drosophila* hosts. Mercedes A. Ebbert, Jennifer J. Burkholder and Jennifer L. Marlowe. Department of Zoology, Miami University, Oxford, OH, 45056, USA.

16:00 Coffee break

Tuesday, 16:30-17:30
Room Bassi
PROTOZOA I - CONTRIBUTED PAPERS.
Chair: Ted Andreadis

16:30. Development of *Vairimorpha* sp. in the fat body tissues of *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae and effects on host food utilizaton values. Leellen F. Solter[1] and Michael W. Henn[2]. [1]Illinois Natural History Survey, 607 East Peabody, Champaign, IL 61820, USA [2]Institute of Applied Zoology, Technical University of Munich, Am Hochanger 13, 85354 Freising, Germany

16:45. Influences of a new microsporidian isolate on development of gypsy moth (*Lymantria dispar* L.) - a comparison with *Nosema "Germany"*. Dörte Goertz, Andreas Linde, Judith Golldack. Fachhochschule Eberswalde, Dept. of Forestry, Applied Ecology, Alfred-Möller-Str.1, 16225 Eberswalde

17:00. The use of heat and drug therapy for the management of *Nosema* disease in *Muscidifurax raptor* (Hymenoptera: Pteromalidae). Carl K. Boohene[1], Christopher J. Geden[2] and James J. Becnel[2]. Department of Entomology & Nematology, University of Florida, Gainesville, Florida USA[1]; and USDA – ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA[2]. **(STUDENT PAPER)**

17:15. Phylogenetic analysis of the Protist *Helicosporidium sp.* A. Tartar, D. Boucias, J. Becnel[*], and B. Adams. Department of Entomology and Nematology. University of Florida, Gainesville, Florida, 32611 USA. [*]Center for Medical, Agricultural and Veterinary Entomology. USDA, ARS, Gainesville, Florida 32604, USA. **(STUDENT PAPER)**

Tuesday, 17:30-18:30
Room Bassi
Business meeting Microsporidia

18:30. Dinner

Tuesday 8:15-9:45
Room Metchnikoff
FUNGI III - CONTRIBUTED PAPERS.
Chair: Tad Poprawski

8:15. Screening Deuteromycete Fungi for the Control of Larval Fleas (Siphonaptera). Stefan T. Jaronski[1], Rex Thomas[2], and Rebecca Lutz[1]. [1] Mycotech Corporation, Butte MT 59701, [2] Heska Corporation, Ft. Collins CO 80521

8:30. Differential susceptibility of *Bt*-resistant and *Bt*-susceptible Colorado potato beetle, *Leptinotarsa decemlineata*, to *Beauveria bassiana*. John D. Vandenberg,[1] Michael H. Griggs,[1] Stephen P. Wraight[1] and Leah S. Bauer[2]. [1] USDA Agricultural Research Service, U.S. Plant Soil & Nutrition Lab, Tower Road, Ithaca, NY 14853 and [2] USDA Forest Service, Center for Integrated Plant Systems, East Lansing, MI 48824

8:45 Persist ence of fungal conidia on different varieties of poinsettia and tomato. Michael Brownbridge, William Reid and Alek Adamowicz. Entomology Research Laboratory, University of Vermont, Burlington, Vermont 05405, USA

9:00 Persistence of *Beauveria bassiana* conidia applied to dorsal versus ventral surfaces of potato foliage. Stephen P. Wraight and Mark E. Ramos. USDA, ARS Plant Protection Research Unit; US Plant, Soil and Nutrition Laboratory, Ithaca, NY 14853

9:15 Multiple exposure and the importance of foliar persistence of *B.bassiana* for control of *Leptinotarsa decemlineata*. E. Groden, F.A. Drummond, H.B.H. Jorgensen, and S. Fernandez. Department of Biological Sciences. University of Maine, Orono, Maine 04469

9:30 On the beginnings of entomophthorous epizootics in insect populations. Svetlana Gouli and Vladimir Gouli. Entomology Research Laboratory, University of Vermont, P.O. Box 53400, Burlington, VT 05405-3400

10:00. Coffee break

Tuesday, 16:30-17:30
Room Metchnikoff
16:30. Business Meeting Fungi

Tuesday, 17:30-18:30
Room Metchnikoff
17:30. Business Meeting Nematodes

18:30. Dinner

Tuesday, 20:00-22:00
Parador San Javier
Business Meeting Microbial Control

WEDNESDAY

Wednesday, 8:00-9:30
Parador San Javier
S.I.P. Business Meeting

THURSDAY

Thursday, 8:15-11:30
Room Pasteur
BACTERIA - WORKSHOP I: Addressing Public Concerns about *Bacillus thuringiensis* Genes in Transgenic Plants.
Chair: Susan MacIntoch

8:15. Environmental, physical and biological factors affecting gene flow from hybrid corn to teosinte. Baltazar M. Baltazar1, Salvador V. Luna1 , Jesus M. Figueroa1, Rodolfo M.Gomez1, Rod Townsend2, and John. B. Schoper2. Hibridos Pioneer de Mexico S.A. de C.V.1, Carr. Guadalajara - Morelia Km. 21 #8601, Nicolas R. Casillas - Mpio. Tlajomulco Jalisco, CP 45635 Pioneer Hi-Bred International, Inc.2, P.O. Box 1000, Johnston, IA, USA 50131-1000.

8:35. (To be announced) Susan MacIntosh. Aventis CropScience.

8:55. Opportunities and challenges of developing and testing Bt maize in developing countries. David Bergvinson[1] and David Hoisington[2]. [1]Maize Program and [2]Applied Biotechnology Center, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 México, D.F., México

9:15. The Mexican experience with insect resistant (Bt) transgenic crops. Ariel Alvarez-Morales. Cinvestav, IPN. Irapuato Unit. Department of Plant Genetic Engineering, Apdo. Post. 629, Irapuato, Gto. Mexico 36500

9:35. Status of food safety evaluation of Cry protein-containing crops. Bruce M. Chassy. Department of Food Science and Human Nutrition, University of Illinois-Urbana Champaign, Urbana, Illinois 61801

10:00. Coffee break

10:30. The role of gene flow in biosafety research on transgenic plants. Detlef Bartsch and Thomas Mücher. Chair of Biology V, Aachen University of Technology – RWTH, 52056 Aachen, Germany.

10:50. Potential effects of Bt expressing crops on beneficial non-target organisms: From laboratory to field scale experimentation and the development of temporal / spatial modelling for optimised use of pest-resistant GM crops in IPM. Dr A.N.E. Birch, Scottish Crop Research Institute, Invergowrie, Dundee DD, 5DA, Scotland, U.K.

11:10. Consequences for Non-Target Insects: Monarch Butterfly Update. Richard L. Hellmich. USDA-ARS, Corn Insects and Crop Genetics Research Unit, and Department of Entomology, Iowa State University, Ames, Iowa.

Thursday, 11:30-12:30
Room Pasteur
BACTERIA IV - CONTRIBUTED PAPERS.
Chair: Jim Baum

11:30. Gene flow in the European Corn Borer: implications for the sustainability of transgenic insecticidal maize. Denis Bourguet[1], Marie Thérèse Bethenod[1] and Frédérique Viard[2]. Unité de Recherches de Lutte Biologique, INRA La Minière, 78285 Guyancourt, France[1], Laboratoire de Génétique et d'Evolution des populations végétales, Université Lille I, 59 655 Villeneuve d'Ascq, France[2].

11:45. Response of bertha armyworm to transgenic canola expressing Bt toxins. Lorraine Braun[1], Elke Diederichsen[2], and Martin Erlandson[1]. [1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan Canada S7N 0X2; [2]Plant Genetic Systems N.V., Josef Plateaustraat 22, 9000 Gent, Belgium

12:00. Insecticidal activity of walnut plants transformed to contain Insecticidal Crystal Protein Fragments of *Bacillus thuringiensis.* P. V. Vail[1], A. Dandekar[2], J. S. Tebbets[1], S. L. Uratsu[2], G. H. McGranahan[2] and C. A. Leslie[2]. [1]USDA-ARS, Horticultural Crops Research Laboratory, 2021 S. Peach Ave., Fresno, CA 93727. [2]University of California, Department of Pomology, 1045 Wickson Hall, Davis, CA 95616

12:15. Cry proteins from *Bacillus thuringiensis* toxic to the cotton boll weevil. Jim Baum[1], Greg Brown[1], Bill Donovan[2], Elysia Joyce[1], Victor Kabuye[1], Anne-Marie Mettus[2], Fred Moshiri[1], and Saku Sivasupramaniam[1]. Pharmacia, 700 Chesterfield Parkway North, St. Louis, Missouri 63198 [1]. Ecogen Inc., 2000 Cabot Boulevard West, Langhorne, Pennsylvania 19047 [2]

12:30 Lunch

Thursday, 14:00-16:00

University Central Archway Passage
POSTER SESSION II

BACTERIA

BP29. Selection of chitinolytic strains of *Bacillus thuringiensis.* J. Eleazar Barboza-Corona[1], J. Carlos Contreras, Rocio Velázquez-Robledo[1], Mayela Bautista-Justo[1] and Jorge E. Ibarra[2]. [1]Instituto de Ciencias Agrícolas, Universidad de Guanajuato, Apdo. Postal 311, 36500 Irapuato, Gto., México. [2]Departamento de Biotecnología y Bioquímica, CINVESTAV-IPN, Apdo. Postal 629, Irapuato, Gto., Méx.

BP30. Monitoring non-target effects of Bt-corn plantation in Germany. Detlef Bartsch[1], Wolfgang Burgermeister[2], Bernd Freier[2], Bernd Hommel[2], Gustav-Adolf Langenbruch[2], Danila Liebe[2], Thomas Meise[2], Thomas Mücher[1], Martina Ross-Nickoll[1], Christiane Saeglitz[1], Gregor Schmitz[1], Ingolf Schuphan[1]. [1]Aachen University of Technology - RWTH, 52056 Aachen, Germany [2]Federal Research Centre for Agriculture an Forestry (BBA), Braunschweig, Germany

BP31. Large-scale screening for novel *cry* genes by hybridisation. Cheryl Beard, Charani Ranasinghe and Ray Akhurst. CSIRO Entomology, Black Mountain Laboratories, Acton, ACT, 2614, Australia.

BP32. A novel *Bacillus thuringiensis* delta-endotoxin Cry1 hybrid protein with high activity against Colorado potato beetle. Samir Naimov,[1] Mieke Weemen-Hendriks,[1] Elena Ouzounova,[2] Stefan Dukiandjiev,[2] Dirk Bosch,[1] and Ruud A. de Maagd[1]. [1]Business Unit Cell Cybernetics, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands. [2]Department of Plant Physiology and Molecular Biology, University of Plovdiv, 24 Tsar Assen Street, Plovdiv, Bulgaria

BP33. A *Bacillus thuringiensis* collection isolated from Costa Rican natural ecosystems: Potential source of novel insecticidal crystal proteins. Glen Arrieta[1], César Rodríguez[1], Ana Víquez[1], Rebeca Mora[1], Ana M. Espinoza[1,2]. Centro de Investigación en Biología Molecular y Celular[1],Ciudad de la Investigación. Universidad de Costa Rica, San José, Costa Rica. Escuela de Fitotecnia[2], Facultad de Agronomía, Universidad de Costa Rica, San José, Costa Rica.

BP34. Improvement of the HPLC analysis of beta-exotoxin : down to 0.3 µg/ml in culture supernatants. Michel Gohar[1] and Stéphane Perchat[2]. INRA, station de recherche de lutte biologique, La Minière, 78285 Guyancourt Cedex, France[1,2] Aventis Crop Science[1], Jozef Plateaustraat 22, B-9000 GENT, Belgium

BP35. Characterization of scFv antibodies that interfere Cry1Ab receptor interaction. Isabel GÓMEZ, Jorge Sánchez, Alejandra Bravo and Mario Soberón. Instituto de Biotecnología Universidad Nacional Autónoma de México, Apartado Postal 510-3 Cuernavaca 62250, Morelos, México **(STUDENT POSTER)**

BP36. Partial characterization of midgut proteases of non-target Lepidoptera relevant to *Bacillus thuringiensis* sensitivity. Mike Grove[1], Brenda Oppert[2], Kris Hartzer[2], and Heidi Appel[1]. Department of Entomology, The Pennsylvania State University, University Park, PA 16802. 2. USDA-ARS Grain Marketing and Production Research Center 1515 College Ave., Manhattan, KS 66502.

BP37. Identification of *Bacillus thuringiensis* virulence genes by signature-tagged mutagenesis (STM). Daniel M. GUTTMANN and David J. Ellar. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge CB2 1GA, UK. **(STUDENT POSTER)**

BP38. Biochemical mechanisms of resistance to Bt toxins in *Plodia interpunctella.* Salvador Herrero[1], Brenda Oppert[2] and Juan Ferré[1]. [1]Departament de Genètica, Universitat de València, Dr Moliner 50, 46100 Burjassot (València), Spain. [2]USDA Agricultural Research Service, Grain Marketing and Production Research Center, 1515 College Ave., Manhattan, KS 66502. **(STUDENT POSTER).**

BP39. Potentiation of *Bacillus thuringiensis* toxins by a metabolite from *Bacillus pumilus.* Desmond R. Jimenez, Denise C. Manker, and Lori J. Lehman. AgraQuest Inc. 1530 Drew Avenue, Davis, CA 95616

BP40. Mechanism for high levels of resistance against *Bacillus thuringiensis* δ-endotoxins in *Heliothis virescens.* Juan L. Jurat-Fuentes[1], Fred L. Gould[2], and Michael J. Adang[1]. [1]Department of Entomology, University of Georgia, Athens, GA, 30602, USA; [2]Department of Entomology, North Carolina State University, Raleigh, NC, 27607, USA. **(STUDENT POSTER).**

BP41. Nematocide Activity of *Bacillus thuringiensis* towards *Meloidogyne incognita.* Vladimir E. Likhovidov, Vasily S. Perepechko, Larisa I. Volodina, Elena K. Savostianova. State Research Center for Applied Microbiology, Obolensk, Serpukhov region, Moscow area, 142279, Russia.

BP42. Production and evaluation of cultures of *Bacillus thuringiensis* (Berl.) with effect on *Polyphagotarsonemus latus* (Banks) (Acarina: Tarsonemidae). María Elena Márquez Gutiérrez, Orietta Fernández-Larrea, Lérida Almaguel. Plant Health Research Institute Street 110 #514 e/5ta and 5ta F. Playa, Ciudad Habana. Cuba

BP43. Intramolecular cleavage of Cry1Ab by midgut proteases. Raúl Miranda and Alejandra Bravo. Instituto de Biotecnología. UNAM. Apdo. Postal 510-3, Cuernavaca 62250, Morelos, México. **(STUDENT POSTER)**

BP44. Expression of mosquito active toxin genes by a Colombian native strain of *Asticaccaulis excentricus.* Magally Romero, Flor M. Gil, and Sergio Orduz. Biotechnology and Biológical Control Unit, Corporación para Investigaciones Biológicas, Apartado Aéreo 7378, Medellín, Colombia

BP45. A Theoretical Model of the Tridimensional Structure of *Bacillus thuringiensis* subsp. *medellin* Cry 11Bb Toxin Deduced by Homology Modelling. Pablo Gutierrez[1*] Oscar Alzate[2] and Sergio Orduz[1]. [1] Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas, CIB. Medellin, Colombia, [*] Current address, Department of Biochemistry, McGill University, Montreal, Canada, [2] Biochemistry Department, The Ohio State University, 484 w. 12th. Ave., OH. 43210

BP46. Characterization of a *Bacillus thuringiensis* strain active against *Epilachna varivestis* (Coleoptera:Coccinellidae). Guadalupe Peña and Alejandra Bravo. Instituto de Biotecnología-UNAM. Apartado postal 510-3, Cuernavaca, Mor., México. C P 62250. **(STUDENT POSTER)**

BP47. Analysis of the Activity of Cry1Ab1 and Cry1Ac1 toxins on the Insect Cell Line CF1 and isolated midgut cells from *Manduca sexta*. Mauricio Realpe and Alejandra Bravo. Departmento de Microbiologia Molecular, Instituto de Biotecnología. A.P. 510-3. Cuernavaca, C.P. 62250. Morelos, Mexico. **(STUDENT POSTER)**

BP48. Evaluation of toxic activity of native and collection strains of *Bacillus thuringiensis* against larvae of the sugarcane borer *Diatrae*a sp. Ninfa M. Rosas-García[1], Hiram Medrano-Roldán[2], Katiushka Arévalo-Niño[1], Benito Pereyra-Alférez[1] and Luis J. Galán-Wong [1]. [1]Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, U.A.N.L., San Nicolás de los Garza, N.L. México. C.P. 66450, [2] Departamento de Biotecnología, ITD, Durango, Durango. México.

BP49. Persistence of the *B.t.* CryIA(c) Protein from Cotton in the Soil. D. A. Streett. USDA, ARS, Southern Insect Management Research Unit Stoneville, MS, USA 38776

BP50. Effect of the alpha-helix 4 mutations, N135Q, on the properties of the Cry1Ac1 and Cry1AB5 toxins. Natalie J. Tigue, Juliette Jacoby & David J. Ellar. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge, CB2 1GA. **(STUDENT POSTER)**

BP51. *Bacillus thuringiensis* and *Beauveria bassiana* based formulation as an alternative for the control of *Spodoptera frugierda*. URIBE D, Aponte L, Martinez W, Grosso V and J Cerón. Instituto de Biotecnología, Universidad Nacional de Colombia, A.A. 14-490, Santafe de Bogotá, Colombia

BP52. Cloning and sequencing of BTR-CAD, a gypsy moth midgut protein related to a putative receptor for the insecticidal toxins of *Bacillus thuringiensis*. Karen J. Garner and Algimantas P. Valaitis. USDA Forest Service, Northeastern Research Station Delaware, OH 43015

BP53. Cloning and Subcloning of the *cyt* Gene of *Bacillus thuringensis subsp.israelsis TN-189*. Li Yubing, Chen Yuehua, Ren Gaixin. (Department of Microbiology, Nankai University, Tianjin, 300071, P.R.China)

BP54. Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. J. Z. Zhao[1] H. L. Collins[1] J. D. Tang[1] J. Cao[2] E. D. Earle[2], R. T. Roush[3] S. Herrero[4] B. Escriche[4] J. Ferré[4] A. M. Shelton[1]. [1]Dept. of Entomology, Cornell University, NYSAES. Geneva, NY 14456; [2]Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853; [3]Dept. of Crop Protection, Waite Institute, South Australia 5064, Australia; [4]Dep. Genética, Universitat de Valencia, 46100-Burjassot (Valencia), Spain

BP55. Protein-Protein Interactions May Reveal New Roles for a Novel Insect Protein. Meibao Zhuang[1], Hyun-Ku Lee[2], Sarjeet S. Gill[3]. Environmental Toxicology Graduate Program[1,3] & Department of Cell Biology and Neuroscience[3], University of California, Riverside, CA 92521, The Scripps Research Institute[2], 10550 N. Torrey Pines Road, La Jolla, CA 92037. **(STUDENT POSTER)**

BP56. Specific binding and pore formation activity of Cry3A toxin in membranes isolated from *Leptinotarsa decemlineata* and *Tenebrio molitor*. Inmaculada Garcia-Robles2, Jorge Sanchez1, Carolina Rausell2, Amparo C. Martinez-Ramirez2, Ruud de Maagd3, M. Dolores Real2 and Alejandra Bravo1. 1 Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Ap. Postal 510-3 Cuernavaca 62250 Morelos, Mexico. 2 Dep. Genetica, Universidad de Valencia, Dr. Moliner 50, 46100-Burjassot, Valencia, Espana. 3 Plant Research International, Wageningen, Netherlands.

BP57. *Serratia* spp and other Enterobacteriaceae active against *Phyllophaga* spp (Coleoptera: Melolonthidae) larvae in Mexico. Villalobos, F.J.[1], Ramírez-Gama, R.M.[2], Calderón. M.A.[2], Hernández, L.[2], Tenango, J.L.[2] Nuñez-Valdez, M.E.[3]. Facultad de Ciencias Agropecuarias, UAEM[1], Facultad de Química, UNAM[2], Instituto de Biotecnología, UNAM[3].

BP58. *Bacillus thuringiensis* isolates from host plant leaves, guts and fecal pellets of caterpillars: A case study of *Bt* in natural ecosystems César Rodríguez[1], Ana Sittenfeld[1], Daniel Janzen[3] and Ana M. Espinoza[1,2] Centro de Investigación en Biología Celular y Molecular[1], Universidad de Costa Rica, Ciudad de la Investigación, San José, Costa Rica. Escuela de Fitotecnia[2], Facultad de Agronomía, Universidad de Costa Rica. San José, Costa Rica. Department of Biology[3], University of Pennsylvania, Philadelphia, USA

BP59. Assigning functional roles for Cry1Ac domains in its toxicity to *Manduca sexta*. James Pearce, Bill James, Ray Akhurst and David J. Ellar. Department of Biochemistry, University of Cambridge, UK. **(STUDENT POSTER)**

BP60. Analysis of â-Exotoxin type strains of *Bacillus thuringiensis* and in selected insecticidal strains. Carmen Sara Hernández[1], Isabelle Larget-Thiéry[2] and Juan Ferré[1]. [1] Departament de Genètica, Universitat de València. Spain. [2] Laboratoire des Bactéries et Champignons Entomopathogènes, Institut Pasteur. France.

16:00 Coffee break

Thursday, 16:30-17:30
Room Pasteur

BACTERIA V - CONTRIBUTED PAPERS.
Chair: Trevor A. Jackson

16:30. Evidence of a new family of insecticidal proteins from similarity between *Serratia* and *Photorhabdus* gene products. Mark R.H. Hurst[1,2], Travis R. Glare[1] Trevor A. Jackson and Clive W. Ronson[2]. [1]Biocontrol and Biosecurity, AgResearch, PO Box 60, Lincoln, New Zealand. [2]Department of Microbiology, University of Otago, PO Box 56, Dunedin, New Zealand

16:45. Dynamics of *Serratia* spp. pathogenic to the New Zealand grass grub, *Costelytra zealandica*. Maureen O'Callaghan, Trevor A. Jackson, Sandra D. Young and Steven Dodd. Biocontrol and Biosecurity, AgResearch, PO Box 60, Lincoln, New Zealand

17:00. The ecology distribution of *Bacillus thuringiensis* and *cry* gene diversity in China. Jin-hong Wang, Wei-hui Wu, Yue-hua Chen, Gai-xin Ren. Department of microbiology, College of Life sciences, NanKai University, Tianjin, P.R.China 300071

17:15. Variability in tolerance to *Bacillus thuringiensis* insecticidal crystal proteins between standard susceptible laboratory strains of diamondback moth. J. González-Cabrera[1], S. Herrero[1], B. Escriche[1], A.H. Sayyed[2], D.J. Wright[2], S. Meyer[3], Y.B. Liu[3], B.E. Tabashnik[3], and J. Ferré[1]. [1] Dep. Genética, Fac. CC. Biológicas, Universitat de Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia) SPAIN. [2] Dep. Biology, Imperial College of Science, Silwood Park, Ascot, Berkshire SL5 7PY, U.K. [3] Dep. Entomology, University of Arizona, Tucson, AZ 85721, U.S.A.

Thursday, 17:30-18:30
Room Pasteur
Business meeting Bacteria

Thursday, 8:30-10:00
Room Steinhaus
8:30. **MICROSPORIDIA WORKSHOP**
Chair: James J. Becnel and Leellen Solter.

10:00 Coffee break

Thursday, 10:30-12:15
Room Steinhaus
VIRUSES IV - CONTRIBUTED PAPERS.
Chair: Max Bergoin

10:30. Complementation of a gp64null mutation in AcMNPV with the Vesicular Stomatitis Virus G protein. J. Mangor[1], S. A. Monsma[2], and G. W. Blissard[1]. [1]Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, [2]Novagen, Inc., 601 Science Drive, Madison, WI 53711

10:45. Expansion of the *Autographa californica* nucleopolyhedrovirus host range to *Spodoptera littoralis*. Liqun Lu, Quansheng Du and Nor Chejanovsky. Department of Entomology, Inst. of Plant Protection, The Volcani Center, POB 6, Bet Dagan, Israel

11:00. Comparative genomics and baculovirus phylogeny. Elisabeth Herniou, Teresa Luque, Ruth Finch*, Doreen Winstanley*, David O'Reilly. Department of Biology, Imperial College, London SW7 2AZ, UK. * Horticultural Research International, Wellesbourne, Warwickshire CV35 9EF, UK. **(STUDENT PAPER)**

11:15. A Host Midgut Cell-Binding Envelope Protein of *Spodoptera litura* Nucleopolyhedrovirus. Wenfu Mao[1], Xiaoguang Ouyang[1], Yi Pang[1],Jiang Zhong[1,2] and Deming Su[1,2]. [1]State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P. R. China. [2]Virology Research Unit, Fudan University, Shanghai 200433, P. R. China

11:30. Laboratory and field evaluation of genetically modified *Helicoverpa armigera* single-nucleocapsid nucleopolyhedroviruses (HaSNPV) in cotton. Xiulian Sun[1,2], Xinwen Chen[1,2] Zhongxin Zhang[1] Felix J.J.A. Bianchi[2], Hualin Wang[1,] Huiying Peng[1,2], Just

M. Vlak[2] and Zhihong Hu[1]. Joint-Laboratory of Invertebrate Virology[1], Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, PR China, and Laboratory of Virology[2], Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

11:45. Isolation of a *Spodoptera exigua* baculovirus recombinant with improved biological activity against the beet army worm. Xiaojiang Dai[1,2], Monique M. van Oers[1], Nina N. Joosten[1], Wilfred F.J. IJkel[1], József P. Hajós[1], Douwe Zuidema[1], Yi Pang[2] and Just M. Vlak[1]. Laboratory of Virology[1], Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, and State Key Laboratory for Biocontrol[2] and Institute of Entomology, Zhongshan University, Guangzhou 510275, P.R. China

12:00. Efficacy of the *Helicoverpa armigera* nuclearpolyhedrovirus (HaNPV) against *H. armigera* Hübner (Lepidoptera: Noctuidae) on citrus. Sean D. Moore[1], Timothy M. Pittaway[1], Jeremy G. Fourie[1] and Gustav Bouwer[2]. [1]Outspan Citrus Centre, P.O. Box 12531, Centrahil 6006, Port Elizabeth, South Africa; [2]Department of Microbiology, University of the Witwatersrand, Johannesburg 2050, South Africa

12:15 Lunch

Thursday, 14:00-16:00
University Central Archway Passage
POSTER SESSION II

VIRUS

VP19. Localisation of LEF-2 in *Ac*NPV-infected cells and in stably-transformed insect cell lines. O. ARGAUD[1], D. Durantel[1], A. Patmanidi[1], R.D. Possee[2] and L.A. King[1]. [1]Oxford Brookes University, School of Biological and Molecular Sciences, Gypsy Lane Campus, Oxford, OX3 0BP, UK. [2]NERC-CEH, Oxford, OX1 3SR, UK

VP20. Induction of apoptosis by a polydnavirus gene in insect cells: mode of expression. Sassan Asgari. Department of Applied and Molecular Ecology, Waite Campus, Adelaide University, Glen Osmond SA 5064, Australia

VP21. Molecular cloning and sequence analysis of the *Anticarsia gemmatalis* MNPV *p74* gene. M. N. Belaich[1]; P. N. Perrat[1]; A. Sciocco-Cap[2]; V. Romanowski[1,3]; M. E. Lozano[1] and P. D. Ghiringhelli[1]. [1]Departamento de Ciencia y Tecnología - CEI, Universidad Nacional de Quilmes, Saenz Peña 180, B1876BXD-Bernal; [2]IMYZA, INTA-Castelar, [3]IBBM, Fac. Cs. Exactas, UNLP, Argentina. **(STUDENT POSTER)**

VP22. Population dynamics of the Indian meal moth *Plodia interpunctella* infected with two viruses. Rebecca Benmayor[1, 2], Steven M. Sait[2], Jenny Cory[1] and Caroline Griffiths[1]. NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR[1] Population Biology Research Group, Department of Environmental and Evolutionary Biology, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK[2]. **(STUDENT POSTER)**

VP23. Predicted interaction domains between AcMNPV P143 (helicase) and LEF3 (single stranded DNA binding protein). E.B. Carstens, T. Behra, K. Neumann, A. Taha and M. Garrett. Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, K7L 3N6

VP24. Spatial distribution of pathogen explains non-linear LdNPV transmission. Vince D'Amico[1], Joseph Elkinton[2], John Podgwaite[1]. [1]USDA Forest Service, Hamden, CT 06514. [2]University of Massachusetts, Amherst MA 01003

VP25. Prolongation of the UV-persistence of nucleopolyhedroviruses by the Lignin Derivative product. El-Salamouny S., El-Sheikh M.A.K., Elnagar S. and *Huber J. Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Cairo, Egypt and *Institute for Biological Control (BBA), Heinrichstr.243 64287 Darmstadt, Germany.

VP26. Stably transformed cell lines from *Trichoplusia ni* with baculovirus p35 or SV40 T antigen genes produce high levels of AcMNPV and recombinant proteins. P. Wang[1], G. Li[1], J. Garcia[1], Helge Zieler[2], and R. R. Granados[1]. [1]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853, and [2]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

VP27. Localization of host range factor 1 (hrf-1) protein in Ld652Y cells infected with recombinant AcNPV bearing hrf-1 gene. Motoko Ikeda[1], Wade A. Williams[2] and Suzanne M. Thiem[2,3]. [1]Graduate School of Bioagricultural Sciences Nagoya University, Chikusa, Nagoya 464-8601, Japan; Department of Entomology[3] & Microbiology[2] Michigan State University, East Lansing, Michigan 48824, USA

VP28. Effects of host age and virus dosage on the yield of a nucleopolyhedrovirus in larvae of *Adoxophyes honmai* (Lepidoptera: Tortricidae). Takayoshi Ishii, Madoka Nakai, and Yasuhisa Kunimi. Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan. **(STUDENT POSTER)**

VP29. Replication of Hz-2V in insect tissues. Christopher P. Rallis[1],Woojin Kim[1], Sarah Carpenter[1,2] and John P. Burand[1,2]. Departments of Entomology[1] & Microbiology[2]. University of Massachusetts-Amherst, Amherst, Massachusetts 01003

VP30. Expression of viral and cellular PCNAs in Sf9 cells infected with *Autographa californica* nucleopolyhedrovirus. Satoko Iwahori, Motoko Ikeda and Michihiro Kobayashi. Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

VP31. Multiplex PCR and quality control of *Epap*GV production. M. A. Manzán[1]; E. M. Aljinovic[3]; A. Sciocco-Cap[1,2]; P. D. Ghiringhelli[3] and V. Romanowski[1,3]. [1]IBBM, Facultad de Ciencias. Exactas, UNLP, Calle 49 y 115, 1900-La Plata; [2]IMYZA, INTA-Castelar, [3]Dto. de C. y Tecnol. - CEI, UNQ, Argentina. **(STUDENT POSTER)**

VP32. Baculovirus Infection at the cellular and organismal level: bio-imaging of viral proteins using fluorescent proteins and probes. A.L. Patmanidi(1,2), C.J.Thomas (1), G. Saville(1), R.D.Possee (2), and L.A. King (1). (1) School of biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP. (2)NERC, Centre for Ecology and Hydrology, Oxford OX1 3SR. **(STUDENT POSTER)**

VP33. *Cydia pomonella* granulovirus: Lethal time of neonate codling moth varies with temperature. Eva Fritsch[1], Karin Undorf-Spahn[1], Susanne B. Steineke[2], Jürg Huber[1] and Johannes A. Jehle[2]. [1] Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Pest Control, Heinrichstr. 243, 64287 Darmstadt, Germany [2] State Education and Research Centre Neustadt/Weinstr, Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany

VP34. Molecular characterization of *Adoxophyes orana* granulovirus. Sally Wormleaton and Doreen Winstanley. Department of Entomological Sciences, Horticulture Research International, Wellesborne, Warwick, CV35 9 EF. **(STUDENT POSTER)**

VP35. Euprosterna elaeasa *virus: a member of a new group of insect RNA viruses.* Jean-Louis Zeddam(1), Fiona Pringle(2), Karl Gordon(3), Vernon Ward(2) and Terry Hanzlik(3). 1: IRD, 213, rue La Fayette, 75483 Paris Cedex 10 (France) 2: Department of Microbiology, Otago University, P. O. Box 56, Dunedin (New Zealand) 3: CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601 (Australia)

VP36. Characterization of three temperature-sensitive mutants of *Autographa californica* nucleopolyhedrovirus defective in budded virus production and polyhedra formation. Makiko Sakurai, Yoshitaka Sano, Tsuguo Matsumoto, and Yoshifumi Hashimoto.

Department of Applied Biology, Faculty of Textile Science Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan. **(STUDENT POSTER)**

VP37. Cloning and Expression of the *gp37* Gene of *Spodoptera liture* Nucleopolyhedrovirus and its Implication in the Virus Infection Chongbi Li[1], Zhaohui Li[1], Ping Zhang[1], Yi Pang[1] and Deming Su[1,] [1]State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P. R. China [2]Virology Research Unit, Fudan University, Shanghai 200433, P. R. China **(STUDENT POSTER)**

16:00 Coffee break

Thursday, 16:30-18:30
Room Steinhaus
SYMPOSIUM: *Hexapoda Aegis*: Current Knowledge of Insect Defense Mechanisms.
Chair: Jeff Lord and Leellen Solter

16:30. Introduction. (L. Solter)

16:35. Agglutinins and reactive oxygen and nitrogen intermediates as determinants of infection of *Rhodnius prolixus* by trypanosomes. Norman Ratcliffe[1], Miranda Whitten[1], Patricia Azambuja[2], Suzete Gomes[2] and Eloi Garcia[2]. [1]School of Biological Sciences, University of Wales Swansea, South Wales, UK. [2]Department of Biochemistry and Molecular Biology, Fiocruz, Rio de Janeiro, Brazil.

17:00. *In Vivo* Cell Phenotypes of Insect Mycopathogens: Cells Invisible to the Host Cellular Defenses. D.G. Boucias. Department of Entomology and Nematology. Gainesville, Florida 32601

17:25. The role of opsonins in cellular immune reactions of insects. Peter Goetz[1], Alexandra Graebert[1], Marc Niere[1], Chris Weise[2] and Ute Wernig-Pohl[1]. [1]Institute for Zoology, Free University of Berlin, D-14195 Berlin, Germany. [2]Institute for Biochemistry, Free University of Berlin, D-14195 Berlin, Germany

17:50. Antimicrobial peptides: a key component of the humoral immune response in insects. P. Bulet. Institute of Cellular and Molecular Biology, UPR 9022 CNRS. 15, Rue René Descartes, 67084 Strasbourg cedex, France

18:15. Conclusion (J. Lord)

Thursday, 8:30-12:00
Room Bassi
MICROBIAL CONTROL - SYMPOSIUM: Microbial Pesticides: Uptake and use in Developing Countries.
Chair: David Grzywacz

8:30. Delivery of Biocontrol Technologies to IPM Farmers: Opportunities and Constraints. Nina Jenkins, Belinda Luke, Janny Vos, Stephanie Williamson, B Ali, D. Raj and Gary Hill. CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berks, SL5 7TA, UK

9:00. Microbial insecticides in Africa, current use and future prospects. Andy J Cherry[1], Nguya K. Maniania[2], George Oduor[3]. [1]International Institute of Tropical Agriculture, 08 BP 0932, Cotonou, Benin; [2]The International Centre of Insect Physiology and Ecology, PO Box 30772, Nairobi, Kenya; [3]CAB International, Africa Regional Centre, PO Box 633, Village Market, Nairobi, Kenya.

9:30. Microbial pesticides - making them count. David Dent. CABI Bioscience, Silwood Park, Buckhurst Road, Ascot SL5 7TA

10:00 Coffee break

10:30. Promotion and development of viral biopesticides in India and Thailand: Lessons for the promotion of microbial control products in developing countries. David Grzywacz[1] , Uthai Ketunuti,[2] and R Jebamoni Rabindra[3]. [1:] Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK. [2:] Department of Agriculture, Chatuchak, Bangkok 10900, Thailand [3:] Department of Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

11:00. Bioinsecticide Marketing Aspects in Mexico. H. Medrano-Roldán [1] and C. García-Gutiérrez [2] . [1] Instituto Tecnológico de Durango, Felipe Pescador 1830. [2] CIIDIR-IPN Unidad Durango, Sigma s/n Fracc. 20 de Nov. II 34220 Durango, Mex.

11:30. The use of a baculovirus (*Phthorimaea Operculella* granulosis virus) in rustic storage facilities, to manage the potato tuber moth: Case studies. Abdelaziz Lagnaoui. International Potato Center (CIP), Apartado 1558, Lima 12 – Peru.

12:00 Lunch

Thursday, 14:00-16:00
University Central Archway Passage
POSTER SESSION II

FUNGI

FP14. Comparison of the Scotch tape method to washing or imprinting leaves for quantifying fungal spore deposition on leaf surfaces. Scott Costa, Vladimir Gouli and Bruce Parker. Entomology Research Laboratory, Department of Plant and Soil Science, University of Vermont, Burlington, Vermont 05405

FP15. Epizootiology of the mite-pathogenic fungus *Neozygites floridana* in the cassava green mite in north-eastern Brazil. Sam L. Elliot[1], Gilberto J. de Moraes[2] and John D. Mumford[3]. [1]Section Population Biology, Univ. Amsterdam, Postbus 94084, 1090 GB Amsterdam. [2]Dept[2] Zoologia, Escola Superior de Agricultura, ESALQ/USP, 13418-900 Piracicaba, SP, Brazil. [3] T H Huxley School of Environment, Earth Sciences and Engineering, Imperial College, London SW7 1NA.

FP16. The efficacy of nematophagous fungi against potato cyst nematodes in field soil. Helen Jacobs[1,2], Simon N. Gray[2] and David H. Crump[1]. [1]Department of Entomology & Nematology, IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, UK. [2] Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., UK.

FP17. Ultrastructural observations on the transovarial transmission of a yeast-like symbiote in the brown planthopper, *Nilaparvata lugens*. Dor-Jih Cheng and Roger F. Hou. Department of Entomology, National Chung Hsing University, Taichung, Taiwan 402, ROC

FP18. Physiological characterization of *Verticillium lecanii* (Moniliales : Moniliaceae) and preliminary investigation on its virulence to the silverleaf whitefly, *Bemisia argentifolii* (Homoptera:Aleyrodidae). Chu-Hsu Lin[1], Wen-Feng Hsiao[2], and Roger, F, Hou[1]. Dept. of Entomology, National Chung-Hsing University, Taichung, Taiwan 400 Dept. of Biological Resources, National Chiayi University, Chiayi, Taiwan 60083

FP19. Screening of stink bug infectious entomopathogenic fungi. Fumio Ihara[1], Katsuhiko Yaginuma[2], Norio Kobayashi[3], Koji Mishiro[1], and Takeru Sato[1]. National Institute of Fruit Tree Science, 2-1 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan[1] & 92 Nabeyashiki, Shimo-kuriyagawa, Morioka, Iwate 020-0123, Japan[2]. Horticultural Institute, Ibaraki Agricultural Center, Sekijo, Makabe, Ibaraki 308-0125, Japan[3].

FP20. Occurrence of *Batkoa* sp. and *Furia* sp. on spittlebugs pests of pasture in eastern São Paulo State, Brazil. Luis G. Leite[1,2,4], Sérgio B. Alves[3], Hélio M. Takada[1], Antonio Batista Filho[1] and Donald W. Roberts[2]. Instituto Biológico, Centro Experimental do Instituto Biológico, C.P. 70, Campinas, SP, 13001-970, Brazil[1], Department of Biology, Utah State University, Logan, UT, 84322-5305, USA[2], Escola Superior de Agricultura Luiz de Queiróz, Universidade de São Paulo, Departamento de Entomologia e Fitopatologia, C.P. 9, Piracicaba, SP, 13418-900, Brazil[3].

FP21. *Beauveria bassiana* and *Metarhizium anisoplae* Isolations Virulence Against *Phyllophaga crinita* (Coleoptera: Melolonthidae) Larvae in Laboratory. Garcia-Martinez, M.[1], M. Najera-Rincon[2], Robert L. Crocker[1], V. Hernandez-Velazquez[3], and L. A. Rodriguez del Bosque[4]. [1]Texas Agricultural Experimental Station, Texas A&M University Research & Extension Center, 17360 Coit Road, Dallas, Texas, U.S.A. [2]National Research Center for Sustainable Production, [3]National Reference Center for Biological Control, [4]North East Regional Research Center.

FP22. Prevalences of fungal pathogens of cereal aphids in wheat under dryland and irrigated conditions in South Africa. Justin L. Hatting[1], Tadeusz J. Poprawski[2], and Ray M. Miller[3]. [1]South African Agricultural Research Council, Small Grain Institute, Private Bag X29, Bethlehem 9701, South Africa; [2]USDA-ARS Beneficial Insects Research Unit and Texas A&M Agric. Exper. Station, 2413 East Hwy 83, Weslaco, TX 78596; [3]Univ. of Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa.

FP23. Evaluation of several supports for the air-drying method of blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) produced in two different liquid media. Carlos F. Sandoval-Coronado[1], Hugo A. Luna-Olvera[1], Katiushka Arévalo-Niño[1], Mark A. Jackson[2], Tadeusz j. Poprawski[3] and Luis J. Galán-Wong[1]. [1] Dept. de Microbiología, Fac. de Ciencias Biológicas, UANL, Cd. Universitaria. A. P. 414. San Nicolás de los Garza, N. L., México. 66450. [2] USDA-ARS-NCAUR, 1815 N University St. Peoria, IL, USA. 61604-3999. [3] USDA-ARS-Biological Control of Pests Research Unit, 2415 East Highway 83, Weslaco, TX. 78596.

FP24. Characterisation of native entomophthoralean fungi associated with *Plutella xylostella* (Lepidoptera: Plutellidae) in the Bajio region, Guanajuato, Mexico. José Luis Velasco-Silva[1], Raquel Alatorre-Rosas[1], Judith K. Pell[2] and Ariel Guzman Franco[1]. [1]Instituto de Fitosanidad, Colegio de Postgraduados, Km 36.5 Carretera Mexico-Texcoco, CP56230 Montecillo, Edo. México. [2]Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK. **(STUDENT POSTER)**

FP25. Effects of UV-B irradiance on conidia and germinants of the entomopathogenic Hyphomycete *Metarhizium anisopliae.* Gilberto U. L. Braga[1,3], Stephan D. Flint[2], Anne J. Anderson[1] and Donald W. Roberts[1]. Department of Biology [1] & Department of Rangeland Resources and Ecology Center[2]. Utah State University, Logan, Utah 84322-5305

FP26. Preliminary laboratory biotests of *Beauveria bassiana* against *Leptinotarsa decemlineata* overwintering adults Ana-Maria Andrei and G. Galani Research Institute for Plant Protection, 71592, Bucuresti, Romania

PROTOZOA

PP6. *Nosema* disease of the encyrtid parasitoid *Tachinaephagus zealandicus.* Christopher J. Geden[1], Maria Ferreira de Almeida[2], James J. Becnel[1] and Carl K. Boohene[1]. USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA 32604[1]; and Departmento de Parasitiologia, University of Campinas, CP 6109, Campinas, Brazil, CEP 13083-97[2]

PP7. Spatial distribution and prevalence of trypanosomatids in natural populations of the stream-dwelling gerrid *Aquarius remigis.* Kata Gurski and Mercedes A. Ebbert. Department of Zoology, Miami University, Oxford, OH 45056 USA. **(STUDENT POSTER)**

PP8. Susceptibility of *Aedes aegypti* and *Aedes albopictus* larvae to single and dual infections of the gregarines *Ascogregarina culicis* and *Ascogregarina taiwanensis.* Filiberto Reyes-Villanueva[(1)] , James J. Becnel[(2)] , and Jerry F. Butler[(1), (1)] University of Florida, Entomol. & Nematol. Dept., Bldg. 970, Hull Road, PO Box 110620, Gainesville, FL 32611. [(2)] Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, 1700 S.W. 23rd Drive, Gainesville, FL 32608. **(STUDENT POSTER)**

PP9. Does pebrine exist in Brazilian silkworm farms? Rie Teramoto[1], Mihoko Hashiguchi[1], Chisa Yasunaga-Aoki[1], Takeshi Kawarabata[1], and Hidehiro Tomimaru[2]. [1] Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan [2] Fiacao De Seda Bratac S/A, Caixa Postal 39-Cep, 17690-000 Bastos-Est, Sao Paulo, Brazil. **(STUDENT POSTER)**

PP10. Preliminary results on the occurrence of microsporidia in the pine bark beetles *Tomicus piniperda* and *Tomicus minor* (Coleoptera, Scolytidae). B. Kohlmayr*[1], R. Wegensteiner*[1], J. Weiser*[2] and Z. Zizka*[3]. Institute of Forest Entomology, Univ. BOKU-Vienna*[1]. Institute of Entomology, CAV Ceske Budejovice*[2]. Institute of Microbioloy, CAV Prague*[3]. **(STUDENT POSTER)**

GENERAL

GP1. Development of aseptic rearing system for the brown-winged green bug on semi-artificial diets. Koji Mishiro, Fumio Ihara and Takeru Sato. National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305-8605, Japan

GP2. The discovery of late male-killing in the oriental tea tortrix, *Homona magnanima* (Lepidoptera: Tortricidae). Sayaka Morimoto, Madoka Nakai, and Yasuhisa Kunimi. Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan. **(STUDENT POSTER)**

GP3. Infectious diseases of the water invertebrates from rivers and lakes on territory bordered with north part of the Black Sea. Elena Oleinikova. Moldavian Research Institute for Horticulture, Chisinau, Moldova, 2019

GP4. Evaluation of the clotting response of the hemolymph of cochineal (*Dactylopius sp.*) and its predator (*Laetilia coccidívora*). González López L.[1], Alba Murillo M.[1], García-Gil M.F.[1-2], Lanz Mendoza H.[4], Rojas Martínez A.[1], del Río Dueñas I[2], Hernández-Hernández F.C.[3].Universidad Simón Bolivar[1], CICATA-IPN[2], CINVESTAV-IPN[3], CISEI-INSP[4]

16:00 Coffee break

Thursday, 16:30-18:00
Room Bassi
MICROBIAL CONTROL I - CONTRIBUTED PAPERS.
Chair: Wendy Gelernter

16:30. Dimethylsulfoxide as an enhancer of bacterial formulations. Margarita V. Shternshis[1] and Vladimir V. Gouli[2]. Department of Plant Protection,[1] Novosibirsk State Agrarian University, Russia 630039 and Entomology Research Laboratory[2] University of Vermont, Burlington, Vermont 05401-3404 USA

16:45. Efficacy of bitoxibacillin, a formulation based on *b*- and *d*-toxins of *Bacillus thuringiensis*, for control of suctorial soybean pests. Vladimir Lichovidov, Natalia Lichovidova. State Research Center for Applied Microbiology, Obolensk, Serpukhov region, Moscow area, 142279, Russia. Vladimir Gouli. Entomology Research Laboratory, University of Vermont, Burlington, VT 05405-3400

17:00. Formulations based on *Bacillus thuringiensis* as effective means for control of beet webworm - *Pyrausta sticticalis* L. on soybean plantations. Natalia Lichovidova, Vladimir Lichovidov. State Research Center for Applied Microbiology, Obolensk, Serpukhov region Moscow area, 142279, Russia. Vladimir Gouli, Entomology Research Laboratory, University of Vermont, P.O. Box 53400, Burlington, VT 05405-3400

17:15. Evaluation of the insecticidal properties of baculoviruses for their effectiveness as biological control agents. F.J.J.A. Bianchi[1,2], W. van der Werf[1], R. Rabbinge[1] and J.M. Vlak[2]. Laboratories of [1]Theoretical Production Ecology and [2]Virology, Wageningen University, The Netherlands. **(STUDENT PAPER)**

17:30. Storage of insect pathogenic nematodes of the genus *Heterorhabditis* at different pH-values and the influence on selected quality characteristics. Kerstin Jung. Federal Biological Research Centre, Institut for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany

17:45. Mortality factors of the wintering codling moth - *Carpocapsa pomonella* in Uzbekistan. Erkin A. Abdullaev and Vladislav V. Gulii. Department of Zoology, University of Samarkand 15 University Blvd, Samarkand, Uzbekistan, 703004

FRIDAY

Friday, 8:15-13:00
Auditorium
BACTERIA - SYMPOSIUM IV: Recent Advances in the Development and Use of Bacteria for the Control of Insects of Public Health Importance.
Chair: Mir S. Mulla

8:15. Entomopathogenic bacteria for the control of mosquitoes and black-flies in Brazil. Maria Helena Neves Lobo Silva-Filha and Lêda Regis. Department of Entomology, Centro de Pesquisas Aggeu Magalhães-Fundaçã Oswaldo. Cruze, Av. Moraes Rêgo s/n, Cidade Universitária, Recife-PE 7472 Brazil 50670-420

8:40. Recent developments in the control of dipterans in Germany. Norbert Becker. German Mosquito Control Association (KABS). Ludwigstr. 99, 67165 Waldsee, Germany

9:05. The role of *Bacillus sphaericus* in vector control programs. Steve Krause. Valent BioSciences Corporation, 870 technology Way, Libertyville, Il 60048, USA

9:30. Standardizing black fly bioassay techniques in the laboratory and field. W. W. Gray[1], R. A. Fusco[2], and Ray Noblet[1]. [1]Department of Entomology, University of Georgia, Athens, Georgia 30602. [2]Valent BioSciences, Mifflintown, Pennsylvania 17059 USA

10:00. Coffee Break

10:30. Strategies for mass production of mosquitocidal strains of *Bacillus thuringiensis* in pilot plant. A. González, A. Restrepo, E. Habeych, and S. Orduz. Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas (CIB). A. A. 7378, Medellín, Colombia

10:55. Resistance in larvae of *Culex pipiens* complex mosquitoes to *B. sphaericus:* Mechanisms, genetics and management. C. Nielsen-LeRoux[1], J. F. Charles[1], M. H. Silva-Filha[2], L. Regis[2], C. M. F. Oliveira[2]. C. Chevillon[3], N. Pasteur[3], G. F. Pei[4], Q. X. Cai[4], and Z. M. Yuan[4]. [1]Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France; [2] FIOCRUZ Centre de Pesquisas Aggeu Malgalhães P.O., 7472, 50670-420 Recife, PE, Brazil; [3] University of Montpellier II, 34095 Montpellier, France; [4] Wuhan Institute of Virology, Wuhan, 430071, China.

11:20. Activity of *Bacillus thuringiensis* toxins against *Bacillus sphaericus* resistant mosquitoes. S. Poopathi, T. R. Mani, G. Baskaran, and Lalitha Kabilan. Centre for Research in Medical Entomology (Indian Council of Medical Research). 4 Sarojini Street, Chinnachokkikulam, Madurai-625-002, South India

11:45. Markedly improved bacterial insecticides for vectors by combining Bti and Bs proteins. B. A. Federici[1,2,3], D. K. Bideshi[1,2], H. W. Park[1], M. C. Wirth[1], W. E. Walton[1], and J. J. Johnson[1]. [1]Deparatment of Entomology and Interdepartmental Graduate Programs in [2]Genetics & [3]Microbiology, University of California, Riverside, CA 92521 USA

12:10. Field efficacy of *Bacillus thuringiensis israelensis* (Bti) against aquatic midges of public health importance. M. S. Mulla. Department of Entomology, University of California, Riverside, CA 92521 USA

12:35. Mosquito Control with Bti: Ecology and Economy. Peter Luethy[1] and Nicola Patocchi[2]. [1]Institute for Microbiology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland[2]. [2]Fondazione Bolle di Magadino, Casa comunale. CH-6573 Magadino, 1Switzerland

13:00 Lunch

Friday, 8:15-10:00
Room Steinhaus
MICROBIAL CONTROL II - CONTRIBUTED PAPERS.
Chair: Amos Navon

8:15 Simple and rapid method for estimating microbial propagules on plants after a application of microbial formulations. Vladimir Gouli, Bruce Parker and Svetlana Gouli. Entomology Research Laboratory, University of Vermont, P.O.Box 53400, Burlington, VT 05405-3400

8:30. Can plants use entomopathogens as bodyguards? Sam L. Elliot[1]*, Maurice W. Sabelis[1], Arne Janssen[1], Leo P.S. van der Geest[1], Ellen A.M. Beerling[1,2] and Joanne Fransen[2]. [1]Section Population Biology, Univ. of Amsterdam, Postbus 94084, 1090 GB Amsterdam, The Netherlands. [2]Research Station for Floriculture and Glasshouse Vegetables, Linnaeuslaan 2a, 1431 JV Aalsmeer, The Netherlands.

8:45. Electronic measurements of insect feeding effects caused by biopesticides. A. Navon[1], V. Alchanatis[2], J. Grinshpun[2], I. Glazer[1]. [1]Institute of Plant Protection, [2]Institute of Agricultural Engineering, ARO The Volcani Center, POB 6, Bet Dagan 50250, Israel.

9:00. Bioassay of the Toxicity of *Bacillus thuringiensis* Against Plant-parasitic Nematode *Meloidogyne incognita.* Liu Bin, Sun Ming, Yu Ziniu. College of Life Science & Technology, Huazhong Agricultural University, Key Laboratory of Agri-Microbiology, Ministry of Agriculture, P .R. China Wuhan 430070

9:15. Shelf-life of *Anagrapha falcifera* nuclear polyhedrosis virus (*Af*MNPV) microcapsular lignin-based formulations. Patricia Tamez-Guerra*[1], Michael R. McGuire[2], and Robert W. Behle[2]. [1]Dep. de Microbiología e Inmunología, Fac. de Ciencias Biológicas, UANL, AP 46-F, San Nicolás de los Garza, N. L., México. [2]Bioactive Agents Research Unit, NCAUR-USDA-ARS. 1815 N. University St., Peoria, IL USA. 61604.

9:30. Studies in Mexico of *Hirsutella thompsonii* Fisher for use as mycoacaricide. José Luis Rosas-Acevedo[1,5], Audel Sánchez-Infante[2], Ana Yolanda Rosas-Acevedo[2], Raquel Alatorre-Rosas[3] Salvador Guzmán-González[4] & Laura Sampedro-Rosas[1]. [1]Centro de Desarrollo Regional-UEPI. [2]Preparatoria No. 13. Universidad Autónoma de Guerrero. Apdo. Postal 612 Acapulco, Gro. [3]Colegio de Posgraduados [4]Facultad de Ciencias Biológicas y Agropecuarias. [5]Doctorado en Biotecnología. Universidad de Colima. Apdo. Postal 10. Tecomán, Col. 28100.

9:45. DrCP1, a putative developmental cysteine protease in Crucifer root maggot (*Delia radicum*): Target for inactivation? Dwayne Hegedus[1], Michael O'Grady[1], Mahmood Chamankhah[1], Doug Baldwin[1,2], Lorraine Braun[2], and Martin Erlandson[2]. Department of Molecular Genetics[1] & Ecological Crop Protection[2]. Agriculture and Agri-Food Canada, Saskatoon, SK, Canada, S7N 0X2.

10:00 Coffee break

Friday 10:30-11:45
Room Steinhaus
MICROSPORIDIA II - CONTRIBUTED PAPERS.
Chair: Andreas Linde

10:30. Comparative pathogenicity of three *Nosema* species from Europe infecting the gypsy moth, *Lymantria dispar*. Leah S. Bauer[1], Deborah L. Miller[1] and Leellen F. Solter[2]. USDA Forest Service, North Central Research Station, Department of Entomology and Center for Integrated Plant Systems, Michigan State University, E. Lansing, MI USA 48823[1]. Illinois Natural History Survey, Champaign, IL USA 61820[2]

10:45. *Thelohania solenopsae* (Microsporida: Thelohaniidae) impact on imported fire ant populations at two locations in Texas. Tamara J. Cook[1], Jerry L. Cook[1], and S. Bradleigh Vinson[2]. Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341[1] and Department of Entomology, Texas A&M University, College Station, TX 77843[2]

11:00. Prevalence and incidence of microsporidia in *Ips typographus* (Col., Scolytidae). Rudolf Wegensteiner*[1] and Jaroslav Weiser*[2]. *[1] Institute of Forest Entomology, Forest Pathology and Forest Protection, University of Natural Resources-BOKU-Vienna, Austria; *[2] Insect Pathology, Institute of Entomology, Academy of Sciences, Ceske Budejovice, Czech Republic.

11:15. Physiological aspects of host-parasitoid-microsporidia interactions. Gernot Hoch[1], Michael W. Henn[2], and Axel Schopf[1]. [1] Institute of Forest Entomology, Forest Pathology and Forest Protection, Universität für Bodenkultur, Hasenauerstr. 38, A-1190 Vienna, Austria; [2] Institute of Applied Zoology, Technical University Munich, Am Hochanger 13, D-85354 Freising, Germany

11:30. Microsporidia and other pathogens in associated spruce bark beetles (Col., Scolytidae). U. Händel *[1], R. Wegensteiner *[1], E. Führer *[1] and J. Weiser *[2]. *[1] Institute of Forest Entomology, Forest Pathology and Forest Protection, Univ.-BOKU-Vienna, Austria; *[2] Institute of Entomology, Academy of Sciences, Ceske Budejovice, Czech Republic

11:45 Lunch

Friday, 8:30-9:50
Room Bassi
CROSS-DIVISION SYMPOSIUM: Diseases of non-insecta.
Chair: Harry Kaya

8:30. Microsporidia: protozoan pathogens of mites. Susan Bjørnson. Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Agassiz, British Columbia, Canada

8:50. Host specificity and virulence in the nematode hyperparasite *Pasteuria penetans*: the role of fibronectin. Keith G. Davies and Sharad Mohan, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ

9:10. Surface proteins on stagings of non-insect microsporidians. Earl Weidner. Department of Biology, Louisiana State University. Baton Rouge, Louisiana 70803

9:30. Nematicidal effects of entomopathogenic nematodes and their symbiotic bacteria on plant-parasitic nematodes. Parwinder Grewal, Department of Entomology, Ohio State University, Wooster, OH 44691

9:50. Closing remarks (H. Kaya)

10:00 Coffee break

Friday, 10:30-12:15
Room Bassi
FUNGI - SYMPOSIUM: Development of entomopathogenic fungi as biocontrol agents in Mexico and Central America.
Chair: Raquel Alatorre-Rosas, Judith K. Pell, Hugo Arredondo

10:30. Entomopathogenic fungi in Mexico: regulation, mass production and application. H. C. Arredondo-Bernal and V. M. Hernández-Velázquez. Centro Nacional de Referencia de Control Biológico DGSV-CONASAG. Km. 1.5 Carretera Tecomán - Estación FFCC, A. P. 133. C. P. 28120 Tecomán, Colima, Mexico

10:45. Production and application of entomopathogenic and antagonistic fungi in Cuba. Mercedes Lujan Macias, O. Fernández Larrea, Esperanza Rijo and Ofelia Milán. Instituto de Investigaciones de Sanidad Vegetal. Calle 110 Nº 514. Playa, Ciudad Habana, Cuba

11:00. Propagule production in submerged culture of the entomopathogenic Hyphomycete, *Paecilomyces fumosoroseus*. Zenguo He,* Héctor Cárdenas-Cota, Pablo Fernández-Sumano y Mayra de la Torre. Departamento de Biotecnología y Bioingeniería. CINVESTAV-IPN. Apdo. Postal 14-740, 07000 México D.F. *Centro de Ciencias de Sinaloa. Ave. Apdo. Postal 1889, Culiacán, Sin.

11:15. Solid fermentation of entomopathogenic fungi. Juan Esteban Barranco Florido, and Gerardo Saucedo.

11:30. Biochemical and Molecular characterization of native strains and the development of autonomous replicating vector of the entomopathogenic fungus *Metarhizium anisopliae.* Angélica González Hernández, Eduardo Calderón Alanís, Leslie Román Reyes, Claudia Morales Hernández, Eduardo Salazar Solis*, Félix Gutiérrez Corona and Juan Carlos Torres Guzmán. Instituto de Investigación en Biología Experimental de la Facultad de Química, *Instituto de Ciencias Agrícolas, Universidad de Guanajuato. AP187, Noria Alta s/n, 36050 Guanajuato, Gto. México.

11:45. The effect of relative humidity (RH) and degree days (GDD) on the rate of development of whiteflies and entomopathogenic fungi. Margarito Ortiz-Caton, Raquel Alatorre Rosas, Fernando Tamayo Mejia. Institute of Fitosanidad, Colegio de Postgraduados. Km. 36.5 Carretera México- Texcoco. CP 56230. Montecillo Edo of Mexico.

12:00. Commercial development of entomopathogenic fungi in Mexico and Central America. Cliff Bradley

12:15 Lunch

ABSTRACTS

Please note: These abstracts should not be considered as publications and may not be cited without the author's permission.

CONTRIBUTED PAPER - Thursday, 17:45 (Microbial Control I)

Mortality factors of the wintering codling moth - *Carpocapsa pomonella* in Uzbekistan

Erkin A. Abdullaev and Vladislav V. Gulii

Department of Zoology, University of Samarkand
15 University Blvd, Samarkand, Uzbekistan, 703004

The codling moth is the most important pest of the fruit-trees in Uzbekistan. This pest damages the principal fruit plants including, first of all, apple, pear, quince, apricot, peach and pomegranate. In the latitude of southern Uzbekistan, there are from 3 to 4 generations of the codling moth each season. Wintering is very important and vulnerable period in the life history of this pest. The analysis of numbers and estimation of physiological conditions of the codling moth during wintering can gave the information for prognosis of the potential harmful of this pest. It is very important especially for the early fruits, because quantity of the first pest generation depends on the wintering numbers of larvae. From this point of view, there is practical interest to estimate the significance of entomopathogenic microorganisms as mortality factor for the wintering codling moth.

In our research the insects were collected in fall period and during spring before the larvae change inside their cocoons to a stage of pupa. About 2000 insects were collected. The pathological material was analyzed by means direct light microscopy (phase contrast method) and beside that, this material was used for isolation of entomopathogenic microorganisms on the special media (M. Goettel, G. Inglis, 1997. In: "Manual of techniques in insect pathology", 213-250 pp.). It was established that in the fall period mortality level of larvae was fluctuating from 2% to 5%. The larvae mortality after wintering was increased up to 80-85%. Autumnal and spring-time pathological material contained *Nosema carpocapsae* and *Pleistophora carpocapsae* spores in 1.0-1.5 % cases. The springtime material had the fungal propagules. Saprophytic, phytopathogenic and entomopathogenic fungi including *Penicillium sp., Spicaria sp., Pythium artrotrogus, Fusarium sp., Paecilomyces fumoso-roseum,* and *Beauveria bassiana* were isolated on the different type of media. Fungus *B. bassiana* was isolated in the majority of cases. We believe that this fungus is the principal biotic factor regulating number of larvae during the winter period. This situation can be explain the specific climatic conditions, including changeable temperatures, thaws and early-dews. The fungus B. bassiana has very good perspectives for the biological regulation of codling moth in Uzbekistan during autumnal and springtime periods.

SYMPOSIUM - Tuesday, 9:35 (Nematodes)

Nematode biodiversity: parasitism and speciation

Byron J. Adams, Khuong B. Nguyen and Heather L. Smith.

Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

Although nematodes are arguably the most abundant metazoans on earth, estimates of species diversity range from less than 50,000 to over 100,000,000. The wide discrepancy in estimates of nematode biodiversity reflects the dearth of taxonomic attention historically allocated to this large clade of animals. The majority of nematode species are thought to be freeliving microbivores, but taxonomically the best-characterized species tend to be parasites of agronomic importance. Hypothetical factors that should promote nematode species-richness include their small body size, small geographic and ecological range requirements, facultative utilization of males, accelerated rates of DNA substitution, and other so-called "key innovations". Alternatively, species-richness may simply be an artifact of variation in speciation and extinction probabilities. By comparing cladogenesis among sister groups of nematodes, we examine the mode and origin of parasitism and test trends of species richness with an emphasis on identifying features that are correlated with insect parasitism.

SYMPOSIUM I - Monday, 14:20 (Bacteria)

Bacillus thuringiensis Cry1 toxin interactions with susceptible and resistant *Heliothis virescens*

M.J. Adang[1,2], J.L. Jurat-Fuentes[1], D. Banks[2] and F. Gould[3]

[1]Departments of Entomology, and [2]Biochemistry and Molecular Biology, University of Georgia, Athens, GA, 30602. [3]Department of Entomology, North Carolina State University. Raleigh, NC, 27695.

Heliothis virescens, an important pest of cotton in the U.S., is effectively controlled by cotton expressing *Bacillus thuringiensis* (*Bt*) Cry1Ac. The usage of *Bt* cotton increases the probability that larvae will become resistant. Also, cross-resistance to other Cry proteins is a disconcerting possibility. For example, *H. virescens* selected against Cry1Ac in the laboratory are cross-resistant to Cry1Aa, Cry1Ab and Cry1F.

By characterizing toxin binding in *Bt* susceptible and resistant insects, we hope to reveal mechanisms underlying cross-resistance. The accepted model for Cry1A binding in *H. virescens* consists of 3 receptor populations. Receptor A binds Cry1Aa, Cry1Ab and Cry1Ac; receptor B binds Cry1Ab, and receptor C binds Cry1Ac. We predicted that because Cry1Ac-resistant *H. virescens* are cross-resistant to Cry1Fa and Cry1Ja, those toxins should recognize receptor A. We showed this to be the case.

The properties of *Bt* toxin receptors are an area of continuing investigation and some disagreement. Receptor A was identified as a 170-kDa aminopeptidase by Luo et al. (1997). Oltean et al. (1999) presented evidence that a 130-kDa aminopeptidase should be considered receptor A. Three Cry1A toxins; Cry1Fa and Cry1Ja each recognized 170-kDa and 115-kDa aminopeptidases. Do these results mean that receptor A is comprised of distinct aminopeptidases that facilitate toxin permeation of membranes?

H. virescens train (YHD2) has remained under selection for Cry1Ac resistance since Lee et al. (1995) characterized this strain with respect to Cry1A binding. YHD2 resistance to Cry1A toxins was explained by the loss of Cry1Aa binding, while binding of Cry1Ab and Cry1Ac remained unchanged. Presently, Cry1Aa, Cry1Ab, and Cry1Ac do not bind to BBMV from YHD2. We found no reduction in Cry1A binding proteins. *H. virescens* can adapt to *Bt* toxins by altering the target site for a toxin resulting in cross-resistance, but the mechanism by which this is accomplished remains to be determined.

Lee et al. 1995. *Appl.Environ.Microbiol.* 61:3836-3842.

Luo et al. 1997. *Insect Biochem.Mol.Biol.* 27: 735-743.

Oltean et al. 1999. *Appl. Environ.Microbiol.* 65:4760-4766, 2000.

POSTER FP13 - Tuesday (Fungi)

Study of the alcohol dehydrogenase activity in *Metarhizium anisopliae*.

Callejas Negrete Olga Alicia, Angélica González and Juan Carlos Torres Guzmán.

Instituto de investigación en Biología Experimental. Facultad de Química. Universidad de Guanajuato. Guanajuato, Gto. México. Email: torguz@monitor.quijote.ugto.mx.

Current widely publicized problems with synthetic chemical insecticides have given rise to a sense of urgency in the development of biological control agents as supplements or alternatives to these chemicals (1). Insect pathogenic fungi, and in particular *Metarhizium anisopliae*, have great potential for use in pest control (2). *M. anisopliae* invades their hosts directly through the insect exoskeleton or cuticle. Under aerobic conditions, the conidia germinate on the host surface and differentiate to form a specialized structure called apresorio. The infection hypha penetrates through the host cuticle by a combination of mechanical pressure and enzymatic degradation (3). This invasion process occurs in a low O_2 pressure where the fermentative metabolism could be important to support the growth during the process. In our laboratory we are interested in the study of the importance of the fermentative process in the pathogencity of *M. anisopliae*, particularly studying the alcohol dehydrogenase activity.

M. anisopliae was grown in aerobic conditions with glucose or glycerol as sole carbon source, and in micro aerobic conditions on rich medium. The NAD-dependent alcohol dehydrogenase activity was

detected (spectrophotometrically and by zymograms). The results shown that the specific activity is higher in micro aerobic conditions compared with aerobic conditions, with the maximum specific activity at 120 h. Zimogram analysis indicated the existence of two activity bands in micro aerobic conditions whereas the cells grown in presence of oxygen and grow in different carbon source (glucose or glycerol), revealed the existence of a single activity band. By the polymerase chain reaction we isolated the internal fragment of the gene ADH1 of *M. anisopliae*, with high homology with the *Saccharomyces cerevisiae* ADH1. The isolation of complete gene is in process.

1. R. St Leger, L. Joshi, M. J. Bidochka, D. W. Roberts, *Proc Natl Acad Sci U S A* **93**, 6349-54 (1996).
2. I. C. Paterson, A. K. Charnley, R. M. Cooper, J. M. Clarkson, *Microbiology* **140**, 3153-9 (1994).
3. A. K. Charnley, in *Invertebrate Microbial Interactions (British Mycological Society Symposium 6)* A. D. M. R. D. W. H. W. J.M. Anderson, Ed. (Cambridge University Press, London, 1984) pp. 229-270.

POSTER FP11 - Tuesday (Fungi)

Blastospore growth and infectivity by injection into fall armyworm: a comparison of two strains of the entomopathogenic fungus *Paecilomyces fumosoroseus*

Jennifer A. Altre[1] and John D. Vandenberg[2]

[1]Department of Entomology, Cornell University, and
[2]USDA Agricultural Research Service, US Plant Soil & Nutrition Lab, Tower Road, Ithaca, NY 14853

We previously found that aerial conidia of *Paecilomyces fumosoroseus* ARSEF strain 1576 are less infective than those of ARSEF strain 4461 when applied to the cuticle of diamondback moth larvae. Strain 1576 does not attach to, germinate on, or penetrate through cuticle as readily as strain 4461. Clearly, strain 1576 is less successful in overcoming the cuticle as a barrier to infection. Is strain 1576 also less successful in the host hemolymph? In this study, strain 1576 was less infective than strain 4461 when blastospores (the spore type formed in liquid environments like hemolymph) were injected into fall armyworm larvae. Strain 1576 demonstrated relatively reduced blastospore growth in vitro: 1) the mean time was longer for strain 1576 than for strain 4461; 2) the mean dry weight of blastospores produced was less for strain 1576 than for strain 4461; and 3) mean blastospore length was significantly smaller for strain 1576 than for strain 4461. Strain 1576 also demonstrated relatively reduced blastospore growth in vivo. We conclude that the lower infectivity of strain 1576 against lepidopteran larvae is due not only to reduced ability to overcome the cuticle barrier, but also to reduced growth in the host hemolymph. This may cause strain 1576 to be more easily overcome by hemocyte defenses.

SYMPOSIUM III - Tuesday, 16:30 (Bacteria)

Site-directed Spin Labeling of Cry1Aa and Cry1Ab for Analysis of Membrane Insertion into Manduca sexta BBMV

Oscar Alzate[1,2] and Donald H. Dean[2]

Pontificia Bolivariana University, Medellin, Colombia[1] and Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210-1292[2]

Domain I of the Bacillus thuringiensis Cry1Aa and Cry1Ab d-endotoxins has been targeted to study the structural topology assumed by this toxin in the membrane-bound state on Manduca sexta midgut. Site directed mutagenesis was used to introduce single cysteine mutations into the wild type proteins. Circular dichroism spectroscopy and protease analysis were used to select those mutant toxins that were stable and did not have structural changes in their 3D conformation. Electron paramagnetic resonance (EPR) spectroscopy reveals that all the amino acids tested are able to bind the MTS spin label under non-denaturalizing conditions. Further analysis using power saturation recovery in the absence and presence of either O2 or NiEDDA shows that both a-helices 5 and 7, enter the membrane when analyzed in brush border membrane vesicles. An E.P.R. spectral change in mutant S170R1-1Ab (a buried residue in a-helix 5) indicates that this amino acid residue flips from a protein environment into a lipid environment when it binds the membrane, while the power saturation of amino acid residue E235R1-1Ab (a buried residue in a-helix 7) shows a membrane location that suggest large structural changes. Voltage clamping experiments with toxin labeled either with the, methanethiosulfonate spin-label (MTS-SL) or N-ethylmaleimide (NEM) on whole midgut tissue reveal that some of the amino acids in a-helix 5 affect either, the insertion rate of the toxin into the membrane or the ion transport ability. The use of protein protection analysis from protease activity, assayed both by EPR and SDS-PAGE shows that most of the protein is protected suggesting that either virtually the whole protein inserts into the membrane, or there are interactions with other elements of the brush border membrane that need to be accounted for. The preliminary data do not support any of the current models that account for the membrane bound state of these toxins.

POSTER FP26 - Thursday (Fungi)

Preliminary laboratory biotests of *Beauveria bassiana* against *Leptinotarsa decemlineata* overwintering adults

Ana-Maria Andrei and G. Galani

Research Institute for Plant Protection, 71592, Bucuresti, Romania

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, is the most damaging insect pest of potatoes in Romunia. We conducted laboratory experiments to evaluate the effect of different *Beauveria bassiana* conidia formulations against the adult from second generation, that overwinter in the soil. The fungal material was applied directly to the soil in the form of fungally infected grams (1×10^9 conidia/g soil) and infected cadavres of *L. decemlineata* ($1{,}4 \times 10^9$ conidia/g soil), respectively in suspension of conidia ($12{,}8 \times 10^8$ conidia/g soil) and powder ($1{,}3 \times l0^8$ conidia/g soil). In our experiments the amount of infective material was not necessarily related with the insecticidal activity of fungal formulation and we find that the efficacy of the applications is correlated with the conidia repartition in the soil profile; the quantity and the quality of the substratum that each type of formulation provide for *B. bassiana* multiplication, spreading and persistence in the soil.

STUDENT POSTER BP1- Tuesday (Bacteria)

Binding sites for the Cry1Ac Insecticidal Crystal Protein of *Bacillus thuringiensis* in *Helicoverpa armigera* (Lepidoptera: Noctuidae)

C. Angelucci[1,2], R.A. Akhurst[1]

[1]CSIRO Division of Entomology; [2]Faculty of Science, Australian National University, Canberra, Australia

Insecticidal transgenic cotton expressing the *cry1Ac* gene of *Bacillus thuringiensis* is a key component of the strategy adopted in Australia to control *Helicoverpa* spp., the major cotton pests. The propensity for *Helicoverpa armigera* to develop resistance to synthetic insecticides and the development of resistance to Cry1Ac by other species prompted the Australian cotton industry to support research into Cry1Ac-resistance in *H. armigera*. Resistance to Cry1Ac in a laboratory-selected line was found to be associated with a change in Cry1Ac binding to the high affinity site (Akhurst, R. and James, W., pers. comm.).

We are seeking to identify the functional binding sites for Cry1Ac in *H. armigera*. N-terminal sequence from a putative aminopeptidase N (120kDa) purified from *H. armigera* (Liao, C. and Akhurst, R., pers. comm.) was used to clone the ApN gene, which was subsequently sequenced. The *H. armigera* gene has about 84% homology to the 170 and 130 kDa ApNs which have been isolated as Cry1Ac binding sites in *Heliothis virescens* (Oltean et al., 1999). A further four Cry1Ac-binding proteins have been purified by affinity chromatography.

SYMPOSIUM II - Tuesday, 8:55 (Bacteria)

***Bacillus thuringiensis* conjugation under environmental conditions**

Olivia Marcia Nagy Arantes

Bio/CCB, Universidade Estadual de Londrina
86051-970, Londrina, Brazil

arantes@uel.br

Genetic exchange of toxin genes in environmental conditions is an important area to understand the ecological role of *B. thuringiensis*.The structural organization of the *cry* genes associated with transposable elements, the presence of several *cry* genes in the same strain, and the presence of a same *cry* gene in different strains suggests that gene transfer can contribute for the broad diversity of *B. thuringiensis*. The widespread occurrence of large self- transmissible plasmids in *B. thuringiensis* strains suggests that conjugation may be an important way of plasmid dissemination in *Bacillus* populations in nature. *B. thuringiensis* conjugation is well described in broth conditions and is used to construct *B. thuringiensis* strains carrying new combinations of *cry* genes and these strains are used as bioinsecticide. In the environment the conditions for *B. thuringiensis* conjugation requires the presence of early vegetative cells. In order to study conjugation in environmental conditions, a plasmid carrying an insecticidal crystal protein gene was genetically marked with a gene conferring resistance to erythromycin. The conjugative transfer of this plasmid between *B. thuringiensis* strains was detected in non-amended sterile soil. Plasmid transfer occurs in vegetative cells before the onset of sporulation. After 24 hours of incubation, about 30% of viable cells were recovered as spores. The asporogenic strain (*B. thuringiensis* var. *thuringiensis* 407-0A) was able to conjugate as recipient strain, even though this strain was no longer recovered after 8 days of incubation. Dead insect larvae can provide suitable conditions for germination and growth of *B. thuringiensis*. High conjugation frequency has been shown in infected insect larvae.

STUDENT PAPER - Tuesday, 8:45 (Viruses II)

Impact of transposon TCp3.2 and TCl4.7 integration on *Cydia pomonella* granulovirus

Hugo M. Arends and Johannes A. Jehle

State Education and Research Centre Neustadt/Weinstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany

The horizontal escape of host insect transposons into the genome of infecting baculoviruses is a rare event which occurs both in cell cultures and natural infections of insect larvae. Previously we have shown that Tc1-like transposons TCp3.2 and TCl4.7 are integrated into the genome of *Cydia pomonella* granulovirus (CpGV-M) generating the mutants MCp4 and MCp5, respectively. Transposon integration may cause genetic disorder and have an impact on the molecular function of the genes adjacent to the integration site and, hence, on the biological activity of the recipient virus.

Biological fitness and a possible selective advantage of a virus can be based on the replication rate of the viruses or the amount of produced virus offspring. In order to analyse the biological fitness of the transposon harboring virus mutants MCp4 and MCp5 compared to CpGV-M, competition experiments were performed. *C. pomonella* larvae were co-infected with different ratios of MCp4 and CpGV-M, and MCp5 and CpGV-M, respectively. The composition of the virus offspring of these mixed infections was determined by quantifiying genotype specific restriction bands. Our experiments showed that CpGV-M had a propagation advantage over the transposon containing viruses.

POSTER VP19 - Thursday (Viruses)

Localisation of LEF-2 in *Ac*NPV-infected cells and in stably-transformed insect cell lines.

O. Argaud[1], D. Durantel[1], A. Patmanidi[1], R.D. Possee[2] and L.A. King[1]

[1] Oxford Brookes University, School of Biological and Molecular Sciences, Gypsy Lane Campus, Oxford, OX3 0BP, UK
[2] NERC-CEH, Oxford, OX1 3SR, UK

The baculovirus late expression factor 2 (LEF-2) is involved in viral DNA replication and late gene expression. To study its role in late gene expression we have stably expressed wild type *Ac*NPV *lef*-2 and several modified *lef*-2 genes in Sf9 cells under control of the *ie-1* (immediate early 1) gene promoter. The selection of stable transfected cell lines was carried out using cell resistance against neomycin or zeocin provided by the expression vectors (respectively pIE-1KaNeo or pIZT/V5/His). We obtained a number of different cell lines: a cell line expressing wild type *Ac*NPV LEF-2; cell lines expressing LEF-2 tagged with HA and cPRO which will be used in co-immunoprecipitation experiments to detect LEF-2 interactions with viral or cellular proteins; and a cell line expressing LEF-2 mutated at the putative phosphorylation locus. The latter will be used to determine the phosphorylation role in LEF-2 function and localisation.

In order to localise LEF-2 in Sf9 cells we also used cells infected with a recombinant virus expressing LEF-2 tagged with C-MYC. Confocal microscopy using a C-MYC antibody showed that staining was only found in the nucleus of infected cells in an area consistent with the virogenic stroma at both early and late times post infection. Cells were also infected with Acoverlef-2, a virus that overexpresses a non-tagged LEF-2 protein. The results of double staining of the nucleus with propidium iodide and of LEF-2 by indirect immunofluorescence were consistent with the C-MYC tagged LEF-2 data and confirmed the nuclear localisation of LEF-2.

SYMPOSIUM III - Tuesday, 16:30 (Bacteria)

Studies on the status of *the Bacillus thuringiensis* Cry1A toxins in *Manduca sexta* larval membranes

Arthur Aronson, Lan Wu and Manoj Kumar

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

Following the reversible binding of the Cry1Ab3 or Cry1Ac1 toxins to larval midgut membranes, there is an irreversible insertion of all or part of the toxin into the larval membrane. Ion channels are formed and these are believed to constitute the basis for toxicity. We have investigated the insertion and extent of aggregation of these toxins in vesicles (BBMV) prepared from the midguts of fifth instar larvae of *Manduca sexta*. Following binding, there was protection of all of the toxin molecule from digestion by protease K except for the amino terminal end which is helix α1 in domain I. Since most of the toxin molecule in or on the larval membrane was inaccessible to protease K, the extent of penetration onto the cytosolic side was examined by preloading BBMV with protease K prior to mixing with toxin. There was no evidence of digestion of the toxin in such preloaded vesicles nor alteration of the aggregation or ability to form ion channels (as described below). Most of the toxin must be within the plane of the membrane and function in such an environment. This conclusion is consistent with the results of mutagenesis of the loop connecting helices α4 and α5 in domain I. On the basis of mutagenic and other studies, these two helices, which constitute a very hydrophobic region of the toxin, are the most likely to form the ion channels. If so, the loop connecting them could project onto the cytoplasmic face of larval midgut cells and contribute to toxin function by interacting with a membrane or cytoplasmic factor. None of several mutations in this region, however, resulted in reduced toxicity implying that it is the helices within the membrane rather than the loops which are important. Ion channel formation implies some extent of aggregation of these toxin molecules in the membrane. Conditions were found which permitted extraction of the wild type Cry1Ac1 and Cry1Ab3 toxins from BBMV as ca 120 and 200kDa aggregates. This aggregation was not due to binding to the aminopeptidas N receptor but interactions with other membrane proteins have not been excluded. The sizes of the aggregates are consistent with the formation of toxin dimers and trimers in BBMV. There is a functional correlation between the formation of these aggregates and activity since several inactive mutant Cry1Ac1 toxins with alterations within helix α5 or between helices α2 and α3 of domain I did not aggregate in BBMV's. Interestingly, inactive Cry1Ac1 toxins due to mutations within helix α4 did form aggregates but not functional ion channels as determined by light scattering. The aggregation results suggest different functions for these two helices, most likely in the formation or function of ion channels, although other or additional mechanisms of toxin action have not been excluded.

SYMPOSIUM - Friday, 10:30 (Fungi)

Entomopathogenic fungi in Mexico: regulation, mass production and application

H. C. Arredondo-Bernal and V. M. Hernández-Velázquez

Centro Nacional de Referencia de Control Biológico DGSV-CONASAG.
Km. 1.5 Carretera Tecomán - Estación FFCC, A. P. 133
C. P. 28120 Tecomán, Colima, Mexico

In Mexico, official dispositions for biological control agents importation, include entomopathogenic fungi, are contents in the articles 101 and 102 of the Plant Health Standard and including the following information: name and address from importer; name and address from exporter; scientific name of the biological control agent; source place of the culture; host to rear or culture material; use of the biological control agent; place country where will use biological control agent; pest target; number biological control agent for release by hectare and cost; origin and biological purity certificate.

On process acceptation, there is a official mexican standard about importation and mobilization biological control agents that including more requirement. Respect quality control, Mexico is elaboration a proposal that including quality control evaluation about entomopathogenic fungi mass producing.

On the other hand, rice in polypropylene plastic bags is the most widely used means of producing *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*. Yields of fungus spores vary from 2.5×10^{11} to 5×10^{11} conidia/bag with 250 gr of substrate. In 1991, mass production and large-scale application of *B. bassiana* for control of coffee berry borer *Hypothenemus hampei*, *M. anisopliae* for control of *Diaphania hyalinata* and *Aschersonia aleyrodes* for control *Dialeurodes citrifoli*, were begun in Oaxaca and Colima. In 1995, more than 50 ton of rice and fungus spores of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* were produced to control *H. hampeii*, *Aenolamia* sp. and *Bemisia* spp. In other example, during 1994-99, in Tres Valles, Veracruz, were carried out a project for production of *M. anisopliae* for control of sugarcane froghopper *Aenolamia* spp., in 1994 the fungus were produced and appliqued in 510 ha, in 1995 3 253, in 1996 19833, in 1997 26 000 and in 1998 more than 50 000 ha. The mass production of a pathogen requires rigorous quality control; during production, the conidial concentration, conidial viability, purity and virulence are carried out. The goal is the production of indigenous strains of fungal pathogens in labor-intensive model.

POSTER BP33 - Thursday (Bacteria)

A *Bacillus thuringiensis* collection isolated from Costa Rican natural ecosystems: Potential source of novel insecticidal crystal proteins

Glen Arrieta[1], César Rodríguez[1], Ana Víquez[1], Rebeca Mora[1], Ana M. Espinoza[1,2]

Centro de Investigación en Biología Molecular y Celular[1],Ciudad de la Investigación. Universidad de Costa Rica, San José, Costa Rica.
Escuela de Fitotecnia[2], Facultad de Agronomía, Universidad de Costa Rica,
San José, Costa Rica.

In the past, screening of diverse environmental samples has yielded *Bacillus thuringiensis* (*Bt*) isolates that have proven to be toxic to some species in the orders Hymenoptera, Homoptera, Orthoptera, Mallophaga and Nematoda. Further research is necessary to increase the genetic diversity of strains, if the range of insects affected by δ-endotoxins is to be extended. Costa Rican insect diversity is close to 360.000 species and there is a coevolutionary relationship among insects and the *Bt* strains that infect them. Thus, prospection of *B. thuringiensis* strains in rich and diverse ecosystems is promising for the search of novel insecticidal crystal proteins (ICP).

Our *Bt* collection consists of a total of 419 strains, half of them collected in nine protected areas of Costa Rica that include sixteen different life zones. Two hundred nine strains were isolated from environmental samples such as soil, leaf litter, fresh leaves and from the guts of insects found in natural ecosystems. The other 210 strains were isolated from agricultural fields (wild and cultivated species of Cruciferae) and their surroundings. A high diversity of crystals was observed in terms of morphology, size and number in the strains collected from natural ecosystems. Large bipyramidal, small irregular, large and small spherical, cubical, cylindrical and large rectangular are the shapes of the crystals observed in these strains by means of light microscopy or scanning electron microscopy when necessary. Furthermore, several of these strains show from two to four different crystal morphologies in the same cell. On the other hand, the analysis of the strains isolated from agricultural ecosystems revealed mainly bipyramidal or oval crystal morphologies, reflecting the homogeneity of strains derived from a habitat with high insect densities but a limited diversity. This collection is now being characterized using PCR general primers for the main *cry* gene families. In preliminary experiments, 40 strains, 25 isolated from natural ecosystems and 15 from agricultural ecosystems, were screened for the presence of *cry*1 genes. Eigthy five percent of the strains from environmental ecosystems did not amplify with the general *cry*1 primers. In contrast, all the strains from agricultural ecosystems contained *cry*1 genes. *Bt* strains derived from live and dead homopterans are being isolated and will be tested in bioassays against *Tagosodes orizicolus* (Homoptera:Delphacidae), an important pest of rice tropical Latin America. A high frequency of rare δ-endotoxins, combinations of less frequently observed *cry* genes or even the discovery of novel genes is expected from this *Bt* prospection program.

POSTER VP20 - Thursday (Viruses)

Induction of apoptosis by a polydnavirus gene in insect cells: mode of expression

Sassan Asgari

Department of Applied and Molecular Ecology, Waite Campus, Adelaide University, Glen Osmond SA 5064, Australia

Symbiotic polydnaviruses (PDVs) are associated with the ovaries of certain groups of hymenopteran parasitoid wasps belonging to the families braconidae and ichneumonidae. Replication of the viruses occurs in specialized calyx cells in the ovaries initiating sometime during the pupation and continuing in the female adult wasps. PDVs accumulate in the oviduct and are injected into the host body cavity together with the parasitoid egg. The viruses do not replicate in the host but viral transcripts are detected after a few hours following parasitization. Viral transcripts are found in almost all tissues in host caterpillars. This leads to host immune suppression and developmental arrest by interfering with the immune system, the endocrine system and other physiological functions.

Cotesia rubecula PDV (CrPDV) genes are expressed in the host caterpillars, *Pieris rapae*, over a short period of time from 4 h to 12 h after parasitisation. One of the genes, CrV1, is abundantly expressed in host hemocytes and inactivates cells by destabilizing the cytoskeleton. Whereas a calyx fluid protein (Crp32) provides an initial protection for the parasitoid egg against the host immune responses, PDV genes provide a transient protection by actively suppressing the host immune system during a critical period of larval emergence from the egg shell. Since transient inactivation of immune cells is a unique feature of CrV1, we wanted to examine its mode of expression further. Purified CrV1 from baculovirus-expressing cells causes cytoskeleton inactivation in hemocytes after injection into caterpillars. This suggests that CrV1 may not act in cells that synthesize the protein, but in cells that internalize the protein from hemolymph. This is supported by the presence of a signal sequence in the coding DNA, suggesting secretion of CrV1 into the hemolymph.

To understand the mode of action of CrV1 we examined the importance of CrV1 uptake by transfecting an insect cell line (Sf21) with a plasmid expressing CrV1 without a signal peptide. Under these conditions, CrV1 is synthesized in the cytoplasm of transfected cells instead of being secreted. Surprisingly, this leads to apoptosis instead of transient inactivation of actin cytoskeleton.

WORKSHOP I - Thursday, 8:15 (Bacteria)

Environmental, physical and biological factors affecting gene flow from hybrid corn to teosinte

Baltazar M. Baltazar[1], Salvador V. Luna[1] , Jesus M. Figueroa[1], Rodolfo M.Gomez[1], Rod Townsend[2], and John. B. Schoper[2]

Hibridos Pioneer de Mexico S.A. de C.V.[1], Carr. Guadalajara - Morelia Km. 21 #8601, Nicolas R. Casillas - Mpio. Tlajomulco Jalisco, CP 45635 Pioneer Hi-Bred International, Inc.[2], P.O. Box 1000, Johnston, IA, USA 50131-1000.

Considerable research has been conducted on the effects of gene flow from improved varieties of maize into the ancient farming areas of Mexico. Most of this research has been focused on conventionally derived, improved varieties and their impact on the germplasm diversity in farmer's fields. The concern of those maize genetic resources is the loss of germplasm diversity in farmer's fields in the ancient areas as new improved varieties are developed. More recently, concern has also developed about the potential flow of transgenes from commercial varieties into landraces and wild relatives of maize. Two important vectors of introgression are seed and pollen. Exchange of commercial seed among farmers obtained from previous plantings can result in the transferal of a transgene into a particular ecosystem. This process has been slowed by the regulatory officials in Mexico declaring a moratorium on the movement of seed containing transgenes into Mexico. Pollen control and management has begun to be investigated as a method for researchers to limit transgene flow in Mexico yet allows field research to continue. Results indicated that routine-breeding activities could be conducted with transgenic maize without danger of pollen dissemination and gene escape if transgenic plants were used as females and detasseled prior to anthesis. As might be expected, results also indicated that it is more difficult to control pollen dissemination if transgenic plants are used as the male. Isolation distance and maize pollen biology have been also investigated and used as a tool for providing controlled hybridization in maize. Data obtained from these studies, combined with experience have resulted in the practice of using 200 m to isolate adjacent plantings of maize. In addition, it was determined that pollen viability is lost after one hour pollen grains are released from the tassel, indicating that maize and teosinte have to be in close proximity for pollination to occur. Experiments are currently underway to determine amount of gene flow between maize and teosinte, which could confirm or deny gene exchange from hybrid corn to wild teosinte populations.

STUDENT POSTER BP28 - Tuesday (Bacteria)

Bacillus thuringiensis Cry1Ac and Cry1Fa d-endotoxins share aminopeptidase binding proteins in *Heliothis virescens*

David J. Banks[1], Juan L. Jurat-Fuentes[2] and Michael J. Adang[1,2]

[1] Department of Biochemistry and Molecular Biology, University of Georgia, Athens GA, 30602, US
[2] Department of Entomology, University of Georgia, Athens GA, 30602

The Cry1A toxins and Cry1Fa share high sequence homology in domain II (Tabashnik *et al.*, 1996), which has been shown to be involved in toxin binding and activity (Rajamohan *et al.* 1996a, 1996b). Based on this sequence homology, and since cross-resistance between Cry1Ac and Cry1Fa has been described in the *H. virescens* Cry1Ac resistant YHD2 strain (Gould *et al.* 1995), we predicted that Cry1Ac and Cry1Fa would share binding proteins in *H. virescens*. Cry1Fa binding and toxicity are inactivated with iodination (Luo *et al*. 1999a). Therefore, we used affinity chromatography with native Cry1Ac and Cry1Fa as well as heterologous competition ligand blots with I^{125} -labeled Cry1Ac to compare Cry1Ac and Cry1Fa *H virescens* binding proteins.

Affinity chromatography with immobilized Cry1Ac and Cry1Fa both identified 110-kDa, 120-kDa and 170-kDa binding proteins from *Heliothis virescens* brush border membrane vesicles (BBMV). Biotinylated Cry1Fa recognized the Cry1Ac eluted binding proteins in a ligand blot, and heterologous competition ligand blot assays with I^{125}-labeled Cry1Ac showed that both Cry1Ac and Cry1Fa, but not Cry1Ea, competed for these molecules, indicating specific interaction. Western blot analysis showed that the 110-kDa, 120-kDa and 170-kDa binding proteins are recognized by anti-aminopeptidase N (ApN) antibody. While the 120-kDa and 170-kDa binding proteins have been previously reported as ApNs, the 110-kDa molecule has been described, but not identified. N-terminal sequencing of the 110-kDa binding protein did not reveal an ApN consensus sequence. However, V8 protease digestion of the 110-kDa binding protein and amino acid sequence of a 32-kDa fragment revealed a 100% homologous sequence with *Helicoverpa punctigera* aminopeptidase 2. These results show that Cry1Ac and Cry1Fa interact specifically with shared 110-kDa, 120-kDa and 170-kDa ApNs from *H. virescens* BBMV.

CONTRIBUTED PAPER - Monday, 11:15 (Viruses I)

Genomic and transcriptional organization of *Cp*DNV, a mosquito densovirus with an ambisense genome

E. Baquerizo, F.X. Jousset, A. M. Abd-Alla, F. Cousserans, and M. Bergoin

Unité de Virologie Moléculaire, Laboratoire de Pathologie Comparée, Université Montpellier II, 34095 Montpellier, France

We have recently isolated from a laboratory strain of the mosquito *Culex pipiens* a typical densonucleosis virus (Jousset *et al*., 2000. *Virus Res*. **67**, 11-16.). This virus, named *Cp*DNV, replicates in the nucleus, has an icosahedral symmetry, its capsid is 25 nm in diameter and contains 4 structural polypeptides with MW of 90, 64, 57, and 12 kDa, and the viral genome is a single-stranded linear DNA of about 6 kb equimolecularily encapsidated as plus and minus strands. By cloning the 4 *Hin*DIII restriction fragments of the double-stranded viral DNA a 5708 nt sequence corresponding to the almost complete *Cp*DNV genome was obtained.

This genome consists of an internal unique sequence flanked by long (over 300 nt) inverted terminal repeats. Unlike the monosense genome of the two previously reported DNVs of mosquitoes, the *Aae*DNV and the *Aal*DNV belonging to the genus brevidensovirus, both strands of the *Cp*DNV genome have coding sequences. The 5' half of one strand contains the largest open reading frame (ORF1) and its N-terminal amino-acid (aa) sequence has the highly conserved motif YKYLGPNS of vertebrate and some insect parvovirus (genus Densovirus) VP1 capsid polypeptide. On the complementary strand, three major ORFs: ORF2, ORF 2' and ORF3, and a minor ORF, ORF3' occupy the 5' half of the molecule. The aa sequences of ORF2 and ORF 2' share homologies with the N-terminal and C-terminal regions of vertebrate and insect parvovirus NS1 polypeptide, respectively. These homologies include the highly conserved initiator protein and helicase superfamily III motifs. Similarly, the aa sequences of ORF3 and ORF3' are homologous to the N-terminal and C-terminal regions of NS2 polypeptide of DNVs belonging to the genus Densovirus.

Two major promoters have been identified. Upstream of ORF2 the P18 promoter regulates the transcription of NS genes. A 1.8 kb mRNA transcribed from this promoter is spliced of a short (53 nt) intronic sequence thus allowing to put in frame ORF2 and ORF2' and ORF3 and ORF3', respectively. The expression of structural polypeptides is under the control of the P11 promoter located upstream of ORF1, on the complementary strand. This promoter regulates the transcription of a 2.2 kb mRNA encoding the 4 structural polypeptides.

By its overall organization and the modalites of expression of its genome, this mosquito DNV differs significantly from brevidensoviruses and appears to be more closely related to the members of the genus Densovirus

POSTER BP29 - Thursday (Bacteria)

Selection of chitinolytic strains of *Bacillus thuringiensis*

J. Eleazar Barboza-Corona[1], J. Carlos Contreras, Rocio Velázquez-Robledo[1], Mayela Bautista-Justo[1] and Jorge E. Ibarra[2]

[1]Instituto de Ciencias Agrícolas, Universidad de Guanajuato, Apdo. Postal 311, 36500 Irapuato, Gto., México. [2]Departamento de Biotecnología y Bioquímica, CINVESTAV-IPN, Apdo. Postal 629, Irapuato, Gto., Méx.

A series of studies using insecticidal Cry proteins from *Bacillus thuringiensis* in combination with heterologous chitinases (from *Serratia marcescens*, *Beauveria bassiana*, and *B. circulans*), have shown that these enzymes may increase the insecticidal activity of *B. thuringiensis*. Although the *B. thuringiensis* chitinases have been barely studied, the potential of using these enzymes and the δ-endotoxin synergistically still remains to be proven, especially because these chitinases are original from the same bacterial species. As a first step in a long-term study of endogenous chitinases of *B. thuringiensis*, aimed to improve its

insecticidal activity, a *B. thuringiensis* collection of Mexican origin was screened by their ability to degrade colloidal chitin within a broad pH range (5 to 12). Three strains were selected based on their ability to degrade chitin under alkaline conditions, similar to those normally found in the midgut of insects susceptible to *B. thuringiensis*. In spite of this preliminary selection, low levels of chitinolytic activity were found when quantified by the reduction of 3,5-dinitrosalicylic acid with the *N*-acetyl-D-glucosamine released from degraded chitin. Different chitinase activities were discriminated by their reactivity to specific fluorescent analogs to chitin derivatives. Endochitinase and chitobiosidase activities were revealed by PAGE *in situ* detection. Results indicate that *B. thuringiensis* chitinases are poorly expressed and genetic manipulation is required to test its potential as a synergistic agent. Cloning of a chitinase gene from *B. thuringiensis* is in progress.

POSTER BP30 - Thursday (Bacteria)

Monitoring non-target effects of Bt-corn plantation in Germany

Detlef Bartsch[1], Wolfgang Burgermeister[2], Bernd Freier[2], Bernd Hommel[2], Gustav-Adolf Langenbruch[2], Danila Liebe[2], Thomas Meise[2], Thomas Mücher[1], Martina Ross-Nickoll[1], Christiane Saeglitz[1], Gregor Schmitz[1], Ingolf Schuphan[1]

[1]Aachen University of Technology - RWTH, 52056 Aachen, Germany
[2]Federal Research Centre for Agriculture an Forestry (BBA), Braunschweig, Germany

Transgenic *Bacillus thuringiensis* (Bt) toxin expressing corn (Bt176 Novartis) was commercialised EU wide in 1997 and is expected to be used after the still pending admission of new cultivars. An additional cultivar (Mon 810 Monsanto) was commercialised in 1998. The target pest species European corn borer (ECB, *Ostrinia nubilalis*) is common in Germany as E- and Z-strain, but only the Z-strain normally causes damage in corn. The ECB is controlled by several chemical, biological and mechanical measures. Accompanying the introduction of transgenic Bt-corn, a monitoring research project was established and funded by the German Ministry of Education and Research (BMBF). The project is expected to cover its first full vegetation period in 2000. The project addresses:

Measurement of the baseline susceptibility of ECB against Bt corn

Discovery of ECB strains with a reduced susceptibility to Bt toxins at an early stage

Detection of occurrence and frequency of resistance alleles in ECB populations

Evaluation of the genetic differentiation of ECB populations by RAPD-PCR and AFLP.

Estimation of gene flow between E- and Z-strains

Toxicological tests with non-target organisms: Collembola, Lepidoptera, soil living microorganisms

Surveying non-target organisms in the field, with special regard on Lepidoptera, Coleoptera, Araneae

WORKSHOP I - Thursday, 10:30 (Bacteria)

The role of gene flow in biosafety research on transgenic plants

Detlef Bartsch and Thomas Mücher

Chair of Biology V, Aachen University of Technology – RWTH, 52056 Aachen, Germany

Scientific based risk assessment for Genetically Engineered Plants (GEP) is based both on likelihood of hazard occurring and hazard identification. Since 12 of 13 of the world's most important food crops hybridise with wild or weedy relatives in some part of their agricultural distribution, biosafety research should focus on consequences of gene flow especially for wild plant relatives. In general, the ecological impact of any novel genotype will depend primarily on the plant's expressed phenotype and its ecological interaction with the plant's environment. We assess how current biosafety research matches the following five appropriate components: (a) relevant biological characteristics of the target plant species including crossable relatives, (b) phenotypic characteristics of the transgenic plant, (c) the time period addressed by the study, (d) number or demography of habitats examined, and (f) range of scientific questions addressed in the study. Important questions of biosafety research addressed (1) gene flow probability to wild relatives of the cultivar, (2) the ecological performance of hybrids between the cultivar and his wild plant relatives, (3) the inclusion of ecological advantage for the transgenic trait in the experiment, (4) the measurement of competitiveness, if one complete plant generation (seed to seed) cannot be addressed (5) the measurement of fitness, and (6) the examination or monitoring of potential non-target effects. We need more valuable data on the ecological performance of a given GEP. There should be an expanded experimental evaluation of genetically modified organisms for studying the ecological behavior of both the crop and wild plant hybrids under field conditions. Transgenic plants are considered more risky if they contain a trait that confers a large fitness advantage in natural situations like pathogen resistance, herbivore resistance, and abiotic stress tolerance (e.g. salt or freeze resistance). Because biosafety research is a resource-intensive process, we will have to concentrate on appropriate experiments and target the ecologically "riskier" organisms.

CONTRIBUTED PAPER - Friday, 10:30 (Microsporidia II)

Comparative pathogenicity of three Nosema species from Europe infecting the gypsy moth, *Lymantria dispar*

Leah S. Bauer[1], Deborah L. Miller[1] and Leellen F. Solter[2]

USDA Forest Service, North Central Research Station, Department of Entomology and Center for Integrated Plant Systems, Michigan State University, E. Lansing, MI USA 48823[1]
Illinois Natural History Survey, Champaign, IL USA 61820[2]

Gypsy moth populations throughout Europe are known to harbor several different species of microsporidia. Comparative laboratory bioassays were performed with *Nosema portugal*, *Nosema sp*. from Romania, and *Nosema sp.* from Levishte, Bulgaria using *per os* inoculations of second-instar gypsy moth larvae. All three species were highly infective and virulent, with median infective and lethal doses ranging from 6 to 7 spores/larva. Larval death took place 31.1, 36.1, and 40.7 days after inoculation with *Nosema sp*. from Romania, *Nosema sp.* from Bulgaria, and *N. portugal*, respectively. The number of infected larvae reaching the adult moth stage was greatest for *N. portugal* (3.2%), followed by *Nosema sp.* from Romania (2.8%), and least for *Nosema sp.* from Bulgaria (0.4%). In all cases, surviving adults were predominantly female. The female moths were mated with healthy males, allowed to oviposit, the eggs were diapaused, and allowed to eclose. To date, we have determined that both *N. portugal* and *Nosema* sp. from Bulgaria are transovarially transmitted. Previous studies with *N. portugal* revealed that virulence is significantly less when transmission is transovarial vs. *per os*, resulting in greater numbers of infected larvae reaching the adult stage.

CONTRIBUTED PAPER - Thursday, 12:15 (Bacteria IV)

Cry proteins from *Bacillus thuringiensis* toxic to the cotton boll weevil

Jim Baum[1], Greg Brown[1], Bill Donovan[2], Elysia Joyce[1], Victor Kabuye[1], Anne-Marie Mettus[2], Fred Moshiri[1], and Saku Sivasupramaniam[1]

Pharmacia, 700 Chesterfield Parkway North, St. Louis, Missouri 63198 [1]
Ecogen Inc., 2000 Cabot Boulevard West, Langhorne, Pennsylvania 19047 [2]

The boll weevil *Anthonomus grandis* Boheman is a major pest of cotton in the New World. We have identified two distinct classes of crystal proteins from *Bacillus thuringiensis* that have significant toxicity towards this pest, exemplified by the Cry proteins ET33/ET34 and ET70. ET33, also known as Cry23Aa1, belongs to a diverse family of crystal proteins and comprises a binary toxin with ET34. ET70 represents a unique class of crystal proteins that shares limited sequence similarity with chitinases derived from *Clostridium paraputrifucum* and exhibits toxicity towards the western corn rootworm. The discovery and characterization of these coleopteran toxins will be reviewed.

POSTER BP31 - Thursday (Bacteria)

Large-scale screening for novel *cry* genes by hybridisation.

Cheryl Beard, Charani Ranasinghe and Ray Akhurst.

CSIRO Entomology, Black Mountain Laboratories, Acton, ACT, 2614, Australia.

CSIRO Entomology is searching for novel *cry* genes with toxicity towards the cotton bollworm, *Helicoverpa armigera*, a major pest of field crops in Australia and Asia. Part of this search has involved the construction and screening of gene libraries from Australian isolates of *Bacillus thuringiensis*. To increase the rate of screening and to improve efficiency of detection, we have developed a screening strategy that utilises a mixture of *cry* gene sequences, from a range of *cry* families, as a hybridisation probe. Using low stringency washing conditions, we have been able to detect novel genes with relatively low DNA sequence similarity (60-65%) to known genes. We have compared probes that contain either the partial or complete coding sequences of ten different *cry* genes. The relative effectiveness of these two probes in identifying novel toxin genes will be presented. We have found the use of hybridisation with a cocktail of *cry* sequences to be a fast and efficient way of identifying library clones containing novel *cry* genes.

SYMPOSIUM IV - Friday, 8:40 (Bacteria)

Recent developments in the control of dipterans in Germany

Norbert Becker

German Mosquito Control Association (KABS)
Ludwigstr. 99, 67165 Waldsee, Germany

For almost two decades, *Bacillus thuringiensis isaaelensis* (Bti) has been used as an environ- mentally safe control agent against nuisance and vector mosquitoes as well as other nematoceran Diptera. The protection of humans from nuisance and vector dipterans as well as the demand for the conservation of biodiversity and protection of naural resources has emphasized the use o target specific tools and appropriate control strategies which can be intergrated by the use of biorational control measures against dipterans. However, higher costs of microbials compared to conventional insecticides can prohibit the wide spread use of these control atents despite their enormous environmental compatibility and safety. The development of new tailor-made inexpensive and effective formulations against major nuisance and vector dipterans, which take into account the specific feeding behaviour of the target species and the most suitable mode of application help to overcome these barriers in the use of microbial control agents. Apart from a self-produced Bti-sand granules which have been used successfully in our program for more than a decade, recently new target specific formulations have been developed. These are: a) Icybac granules a formulation of ice grains which contain as an active ingredient the new Vectobac WDG product and b) Culinex tablets, a fizzy type of tablet which is based on Bti and *B. sphaericus*. The ice granules can easily be mass produced in a nitrogen based production unit and easily applied against mosquitoes and in a modified formulation and at higher dosages also against chironomid larvae. Thus the costs per hectare compared with Bti-sand granules could be reduced by about 50%. The sporefree fizzy tablets are tailor-made for wide-spread use and cost-effective control of container breeding mosquitoes particularly with the help of community participation.

CONTRIBUTED PAPER - Tuesday, 11:15 (Viruses III)

Evidence for budded virions in a new baculovirus from the mosquito *Culex nigripalpus*

James J. Becnel, Susan White, Bettina Moser, Tokuo Fukuda and Margaret J. Rotstein

Center for Medical, Agricultural and Veterinary Entomology. U. S. Department of Agriculture, Agricultural Research Service, Gainesville, Florida 32604

Most Nucleopolyhedroviruses (NPVs) and Granuloviruses (GVs) have an initial colonization phase of replication in the nuclei of midgut epithelial cells where budded virions (BV) are produced that spread the virus to other tissues in the body. The mechanisms for the spread of baculoviruses that are found only in midgut tissues are not known. Insect baculoviruses restricted to the midgut epithelium are NPVs from Hymenoptera, Thysanura, Trichoptera, and Diptera and one species of GV (HbGV) from *Harrisina brillians*. A new Dipteran baculovirus isolated from the larval stages of the mosquito *Culex nigripalpus* specifically infects and develops occlusion bodies in midgut epithelial cells. We have studied the early phases of virogenesis and found evidence that lateral spread of this mosquito baculovirus within the midgut occurred by means of BV. Non-enveloped nucleocapsids were released from infected nuclei into the cytoplasm of midgut epithelial cells by budding through the nuclear envelope. These nucleocapsids exited the nucleus either singly or in groups forming transport vesicles in the process. The membranes of these transport vesicles (formed by the membranes of the nuclear envelope) break down and release the naked nucleocapsids into the cytoplasm. This process is similar to the lateral transmission mechanisms established for NPVs and GVs that spread from the midgut to other tissues and may help resolve these mechanisms for baculoviruses restricted to the nuclei of midgut epithelium.

Microsporidia Workshop, Thursday 8:30

"Methods for sampling and diagnosis of microsporidia from field populations of insects"

James J. Becnel[1] (Convenor)

Participants:
James J. Becnel[1] and Leellen Solter[2]

[1]Center for Medical, Agricultural and Veterinary Entomology. U. S. Department of Agriculture, Agricultural Research Service, Gainesville, Florida 32604
[2] Illinois Natural History Survey, Champaign, Illinois, 61820

The Division of Microsporidia will conduct an informal workshop on techniques used to systematically sample insects from terrestrial and aquatic systems. Distinctly different methods are required when studying microsporidia from insects in terrestrial and aquatic systems. The discussions will focus on strategies and approaches that have proven useful for isolation, identification and preservation of microsporidia as part of ecological surveys examining the impact of diseases on natural populations of insects. This workshop is anticipated to be of benefit to students and/or new researchers in the field of microsporidiology.

STUDENT POSTER VP21 - Thursday (Viruses)

Molecular cloning and sequence analysis of the *Anticarsia gemmatalis* MNPV *p74* gene

M. N. Belaich[1]; P. N. Perrat[1]; A. Sciocco-Cap[2]; V. Romanowski[1,3]; M. E. Lozano[1] and P. D. Ghiringhelli[1].

[1]Departamento de Ciencia y Tecnología - CEI, Universidad Nacional de Quilmes, Saenz Peña 180, B1876BXD-Bernal; [2]IMYZA, INTA-Castelar,
[3]IBBM, Fac. Cs. Exactas, UNLP, Argentina
(e-mail: mbelaich@unq.edu.ar or pdg@unq.edu.ar)

The velvetbean caterpillar, *Anticarsia gemmatalis* (Lep. Noctuidae), is a major pest of soybean crops in Argentina, Brazil and other countries. An isolate of a nucleopolyhedrovirus of *A. gemmatalis* is currently used as a microbial insecticide in more than 1.0 million hectares annually in Brazil. However, the low speed of action (6-8 days after feeding) limits its application. This problem could be addressed using recombinant DNA methodologies to introduce genetic modifications. On the other hand, the release in the environment of genetically modified viruses, with their intact replication capacity casts certain doubts about the potential risk, even when these risks are not too different from to those associated with the deliberate release of *wild type* baculoviruses. If the potential risk is defined as the time of exposure to a danger, the biosafety in the use of recombinant baculoviruses can be increased by means of the addition of genetic modifications that limit the natural spread in the environment. To this end, diverse strategies have been developed, based on the use of variants defective in the polyhedrin gene co-occluded with *wt* variants or pre-occluded, or based on other strategies related with the infection or

with the production and progeny release to the environment. As an alternative strategy to increase the biosafety we propose to develop a cellular line of *Anticarsia gemmatalis* genetically modified to express the *p74* gene of *Ag*MNPV. In conjunction, with this cell line we plan to use *p74*$^-$ recombinant baculoviruses. Since the *p74* locus will be deleted from the viral genome, there will be no possibility to generate fully infective occlusion body of recombinants and the progeny will be homogeneous. As a first step, we designed a pair of conserved primers, based on a multiple alignment of known sequences of *p74* genes of *Ac*MNPV, *Bm*MNPV, *Cf*MNPV, *Ld*MNPV, *Op*MNPV and *Xc-nc*GV. With these primers we amplified fragments of differente sizes using DNAs of two distinct isolates of *Ag*MNPV (Brazil and Argentina) and of other baculoviruses as templates. Using the ~1200 bp PCR fragment as homologous probe in a Southern blot of genomic *Ag*MNPV DNA we identified the restriction fragment containing the complete ORF. After cloning and sequencing the complete locus, the information of *Ag*MNPV *p74* gene was compared with the homologous sequences of other baculovirus. We used this sequence in phylogenetic studies to assess the relatedness of *Ag*MNPV with other members of the Baculoviridae familiy.

STUDENT POSTER VP22 - Thursday (Viruses)

Population dynamics of the Indian meal moth *Plodia interpunctella* infected with two viruses

Rebecca Benmayor[1,2], Steven M. Sait[2], Jenny Cory[1] and Caroline Griffiths[1]

NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR[1]
Population Biology Research Group, Department of Environmental and Evolutionary Biology, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK[2]

Insect host-pathogen interactions are of importance when evaluating the potential of pathogens as biological control agents and help to understand the role that disease may play in regulating populations. However, few long-term empirical studies have looked at the interaction of a host and two pathogens due to the difficulties of studying these interactions in the field and the time required. *P. interpunctella* is a serious economic pest of stored products that is predominantly found in temperate climates. *P. interpunctella* is an ideal system to study multispecies interactions, having many generations per year and susceptibility to baculoviruses. Additionally, it can be studied in a microcosm where environmental conditions and other biotic factors may be controlled. The baculoviruses *Plodia interpunctella* granulosis virus (*Pi*GV) and *Ephestia cautella* nucleopolyhedrosis virus (*Ec*NPV) have been shown to be infect *P. interpunctella* larvae. Experimental populations of *P. interpunctella* containing PiGV are compared and contrasted with those containing mixed infections with both baculoviruses. Host densities and sex ratio were monitored to determine long-term changes in sex ratio and population size. Infected larvae were also recorded to determine the dynamics of the pathogen populations. The experimental populations are still ongoing to establish the long-term effects of both single and mixed infections in the host and pathogen populations.

Key words: *Plodia interpunctella*, baculovirus, population dynamics, mixed infections.

WORKSHOP I - Thursday, 8:55 (Bacteria)

Opportunities and challenges of developing and testing Bt maize in developing countries

David Bergvinson[1] and David Hoisington[2]

[1]Maize Program and [2]Applied Biotechnology Center
International Maize and Wheat Improvement Center (CIMMYT)
Apdo. Postal 6-641, 06600 México, D.F., México

The International Maize and Wheat Improvement Center (CIMMYT) believes that the development of transgenic maize is a valuable component in meeting the food security needs of clients in developing countries. As such, CIMMYT is assisting National Agricultural Research Systems (NARS) to develop and evaluate insect resistant maize that combines conventional host-plant resistance with Bt genes as a means of decreasing maize losses due to insect pests. CIMMYT has transformed tropical maize using biolistics with *cry1Ac*, *cryIAb*, *cryIB*, *cryIAb-1B* and *cryIE* gene constructs with an emphasis on genes provided by public institutions in which joint agreements for release are in place. Following a forum held in 1995 to discuss the testing of transgenic maize in Mexico, CIMMYT has conducted small-scale field trials of Bt maize in Mexico in close collaboration with the National Biosafety Committee and SAGAR. CIMMYT has also started to look at the ecological impact of this technology by studying insect resistance to Bt maize under biosafety greenhouse conditions. Research has shown no breakdown of Bt resistance to two tropical stem borer species, *Diatraea grandiosella* and *D. saccharalis*; however, after 18 cycles of selection both populations have shown an increase in pupal weight and survival when exposed to Bt plants for 48h. A project with CIRAD in France has determined the complementarity of the available Bt genes in targeting receptors in tropical American stem borers; information critical in developing appropriate insect resistance management strategies. Under a new partnership (the Insect Resistant Maize for Africa - IRMA project), CIMMYT and the Kenyan Agricultural Research Institute are working together to develop transgenic maizes that only contain the Bt of interest with necessary control sequences; to combine Bt with host-plant resistance; to assess the environmental, social and economic impacts of insect resistance maize; to establish management and monitoring strategies to minimize the environmental impact of Bt maize in tropical ecologies; to provide training and educational opportunities for all stakeholders in biotechnology; and to document the entire process so that all lessons learned can be made available to other developing countries. CIMMYT will continue to work on GMOs only with the full support and involvement of our NARS partners to make this technology and associated biosafety and monitoring tools available to developing countries in Africa, Asia and Latin America.

CONTRIBUTED PAPER - Monday, 11:45 (Bacteria I)

The toxin-coding plasmid of *Bacillus thuringiensis* subsp. *israelensis* is related to the major virulence plasmid of *Bacillus anthracis*

Colin Berry[1], Andrew F. Jones[1], Eitan Ben-Dov[2] and Arieh Zaritsky[2]

[1]Cardiff School of Biosciences, Cardiff University, Wales, UK
[2]Department of Life Sciences, Ben-Gurion University of the Negev, Israel

Bacillus cereus, *Bacillus thuringiensis* (Bt) and *Bacillus anthracis* (Ba) are known to be very closely related and are often termed the *B. cereus* group or *B. cereus sensu lato*. In this view, all of the above 'species' are considered instead as different subspecies of *Bacillus cereus*. The major distinguishing feature for the classification of individual isolates as Bt or Ba is the production of characteristic virulence factors: insecticidal toxins in the case of Bt and the mammalian-active anthrax toxins in the case of Ba. The three toxins of the latter are encoded on a 182 kb megaplasmid, pXO1, the entire sequence of which has recently been determined (Okinaka *et al.*, J. Bacteriol. **181**: 6509-6515, 1999). Similarly, most strains of Bt encode their Cry and Cyt toxins on large extrachromosomal plasmids. The Bt *israelensis* (Bti) ~137 kb megaplasmid (pBtoxis), which encodes all the four Cry and two Cyt toxins known in this subspecies, has been restriction mapped and partially subcloned into *E. coli* (Ben-Dov *et al.*, Plasmid **42**: 186-191, 1999). Sequencing some of these subclones has revealed approximately 3 kb of highly significant homology (90% identity) with regions of pXO1 (these homologies are distinct from the similarities between the Ba IS element sequences and those of Bt that were noted by Okinaka). Furthermore, PCR reactions, using primers based on one of the pXO1–like regions we have identified, have shown that homologous sequences exist in at least 20 other Bt subspecies, including other mosquitocidal strains (Bt *jegathesan*, Bt *medellin*), strains pathogenic to lepidoptera and in the house fly-toxic *Bacillus cereus moritai*. Hence, not only are the 'species' Ba and Bt very closely related but also the major virulence plasmids from both groups share significant regions of homology. These observations suggest that a single ancestral plasmid has diverged by the acquisition of different pathogenicity islands encoding toxins active against either mammalian or insect hosts.

STUDENT PAPER - Thursday, 17:15 (Microbial Control I)

Evaluation of the insecticidal properties of baculoviruses for their effectiveness as biological control agents

F.J.J.A. Bianchi[1,2], W. van der Werf[1], R. Rabbinge[1] and J.M. Vlak[2]

Laboratories of [1]Theoretical Production Ecology and [2]Virology, Wageningen University, The Netherlands.

A validated simulation model for the epidemiology of baculoviruses was used to evaluate the relative importance of biological properties of baculoviruses, such as speed of action, infectivity and inactivation on their effectiveness as biological control agents. The model applies to a population of beet armyworm, *Spodoptera exigua*, feeding on greenhouse chrysanthemums that are sprayed with baculoviruses. The model simulates the dynamics of the baculovirus-insect system and integrates available knowledge of the *S. exigua* bionomics, plant growth, spray deposition, virus infectivity, virus speed of action and inactivation and the pathways of virus transmission. The model was used to generate scenario studies for baculoviruses with varying speed of action, infectivity and inactivation rates. The relative importance of these baculovirus characteristics will be discussed. This knowledge may be used to direct future genetic modification strategies to obtain baculoviruses with optimal insecticidal characteristics.

POSTER VP1 - Tuesday (Viruses)

Molecular cloning and sequence analysis of the *immediate early 1* gene of *Anticarsia gemmatalis* MNPV

M.F. Bilen[1]; M.G. Pilloff[1]; B. Morais Ribeiro[2]; V. Romanowski[1, 3]; M.E. Lozano[1] and P.D. Ghiringhelli[1].

[1]Departamento de Ciencia y Tecnología - CEI, Universidad Nacional de Quilmes, Saenz Peña 180, B1876BXD-Bernal; [2]Dto. de Biología Celular, Universidade de Brasilia, [3]IBBM, Facultad de Ciencias Exactas, UNLP, Argentina
(e-mail: mbilen@unq.edu.ar or pdg@unq.edu.ar)

Anticarsia gemmatalis (Lep. Noctuidae), is a major pest of soybean in South America. An indigenous nucleopolyhedrovirus (AgMNPV) isolated in Brazil proved to be a feasible microbial control agent. This baculovirus is currently used in more than one million hectares annually in Brazil. However, the low speed of action (6-8 days after feeding) limits its wider acceptance. The pathogenicity could be altered using recombinant DNA techniques to introduce genetic modifications, such as the insertion of foreign genes coding for toxins, insect hormones, etc. and/or the deletion of specific viral genes. The strategies for the expression of foreign genes could involve early and late promoters. Several early genes have been characterized in other baculoviruses; most of them are associated with the regulation of late and very late genes (e.g. *ie1*, *ie2*, etc). The possible use of the *ie1* promoter to drive the expression of foreign genes is an interesting approach that has been successful in other baculovirus-insect systems. We identified the genomic restriction fragment containing the *ie1*, cloned and sequenced the locus of this virus. A 2396 bp *Bgl*II/*Xho*I fragment of the *Ag*MNPV genome contains the 1707 nucleotides long *ie1* ORF and regulatory upstream and downstream regions. The IE1 protein has 63% amino acid identity (77% homology) with the IE1 of *Orgyia pseudotsugata* and 65% amino acid identity (75% homology) with the IE1 of *Choristoneura fumiferana*. On the other hand, we used the nucleotide sequence to locate conserved regulatory signals in non-coding regions and to conduct phylogenetic studies with the corresponding sequences of other baculoviruses.

This initial cloning and characterization will enable the further development of transfer vectors for the expression of foreign genes under the control of the *ie1* promoter.

CONTRIBUTED PAPER - Friday, 9:00 (Microbial Control II)

Bioassay of the Toxicity of *Bacillus thuringiensis* Against Plant-parasitic Nematode *Meloidogyne incognita*

Liu Bin Sun Ming Yu Ziniu

College of Life Science & Technology, Huazhong Agricultural University, Key Laboratory of Agri-Microbiology, Ministry of Agriculture, P .R. China Wuhan 430070

The study on bioassay method was conducted and the bioassay standardization procedure of *Bacillus thuringiensis* against plant-parasitic nematode was settled. B.t. strain 032 toxic to plant-parasitic nematode screened by our laboratory and the most damaging plant-parasitic nematode *Meloidogyne incognita* in our country were used as the materials. The spore-crystal mixture of B.t. strain 032 was solubilized by alkaline solution, then the soluble protein supernatant was dialyzed and then served as the samples of bioassay. The *Meloidogyne incongta* was reproduced purely in greenhouse (temperture: 25±1°,light: 12-14Hrs/days) by planting tomato. After 40 days, the eggs were collected from the roots and then were hatched in plates. The new hatched second-stage juveniles were used as tested nematodes. 50μl prepared samples of protein and 40 second-stage juveniles were added to each well of the microtitre plates. The mortality was examined by dissecting microscope and was assessed by prodding with a dull probe. Those did not respond to prodding were considered moribund. On the condition of 7 days of incubation at 25°, pH9.0, 3 times replications and 40 juveniles for each dilution, the bioassay results can be obtained reproducibly and precisely. The 95% confidence limit (CL) ratios (max:min) were less than 2 and the coefficient of variation of LC_{50} values was 0.085(<0.20) after 10 times of bioassays were conducted.. So this bioassay procedure can be served as a bioassay standardization procedure of *Bacillus thuringiensis* product against plant-parasitic nematode.

CROSS-DIVISION SYMPOSIUM - Friday, 8:30

Microsporidia: protozoan pathogens of mites.

Susan Bjørnson

Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Agassiz, British Columbia, Canada

Microsporidia, the most common protozoan pathogens of insects, infect both beneficial and pest arthropods. These spore-forming protozoa can produce either acute or chronic disease in their hosts. Acute microsporidiosis may result in premature death, whereas chronic infections are thought to be responsible for the regulation of some insect populations. The first report of a microsporidium in mites was by Weiser (1956) of *Nosema steinhausi* in the phytophaguous mite *Tryophagus noxius* Zakhvatkin. Reports of microsporidia in other terrestrial and aquatic mites soon followed. Early studies of microsporidioses in mites often included a description of the pathogen accompanied by a list of host tissues that were infected. Over the past decade, microsporidia have been detected in mass-reared predatory mites that are used for pest control in integrated pest management systems. Microsporidia are known to infect the spider mite predator *Phytoseiulus persimilis* Athias-Henriot, and *Neoseiulus cucumeris* (formerly *Amblyseius*) Oudemans and *Amblyseius barkeri* Hughes, predators of western flower thrips and onion thrips, respectively. The detection of microsporidia in mites of economic importance has prompted a closer look into other aspects of the predator-pathogen relationship, including the effects of microsporidia on life history characteristics of the host, the prevalence of pathogens in commercial mass-rearings, and the means by which these pathogens are transmitted. Reports of microsporidia and concerns regarding predator performance have raised questions regarding the quality of commercially produced biological control agents and has generated a growing interest in quality control of mass-produced mites.

SYMPOSIUM - Tuesday, 8:20 (Nematodes)

Bacterial symbionts and correlation with entomopathogenic nematode taxonomy

Noël Boemare

Laboratoire de Pathologie comparée, UMR CNRS-INRA n° 5087, CP 101,
University of Montpellier II F-34095 Montpellier CEDEX 5, France

A polyphasic approach, including many phenotypic tests, ribotyping, sequencing analysis of 16S rRNA genes, and DNA-DNA hybridizations

with determination of the ΔTm, is required to characterize strains of *Xenorhabdus* and *Photorhabdus*. Thus, for *Photorhabdus* two new species have been recently reported. *P. temperata*, encompasses symbionts from *H. megidis* (including the Palaeartic and Nearctic strains), and *H. zealandica*. *P. asymbiotica*, contains the previous clinical strains described as non symbiotic for EPNs. Several subspecies are recognized. *P. luminescens* subsp. *luminescens* contains the symbionts of *H. bacteriophora* (subgroup Brecon), *P. luminescens* subsp. *akhurstii* the symbionts of *H. indica*, *P. luminescens* subsp. *laumondii* the symbionts of *H. bacteriophora* (subgroup HP88), and *P. temperata* subsp. *temperata* the symbionts of the Palaearctic group of *H. megidis*. For *P. temperata*, other sub-species will be described shortly when additional strains are collected from *H. zealandica* and from the uncertain subgroup NC1 of *H. bacteriophora* (= *heliothidis*).

Apart from the five described *Xenorhabdus* species (*X. beddingii*, *X. bovienii*, *X. japonica*, *X. nematophila*, and *X. poinarii*), the lack of "sister" bacterial strains, freshly isolated from several samples of the same species of *Steinernema*, limits the definition of other *Xenorhabdus* species. New bacterial species are quite obvious for the symbionts of *S. riobrave* and *S. scapterisci* and they will be defined shortly. *X. bovienii* is the symbiotic species of *S. affine*, *S. feltiae*, *S. kraussei* and *S. intermedium*, although a specific 16S rDNA genotype has been recognized for the symbionts of each nematode. Recently new data provided evidence that the symbiont of *S. cubanum* belongs to *X. poinarii*. From the phylogenetic relatedness of *S. cubanum* and *S. glaseri*, which is supported by morphological and genotypic similarities, presumably the divergence of these two species is relatively recent.

Consequently, when these results are compared with the taxonomic data of the host nematodes, a close relatedness of the two taxonomic structures is noticed, and the phenomenon of co-speciation between bacterium and nematode genera is shown. It is remarkable to notice such a co-speciation between both partners, even if today among the described bacterial species some of them share several nematode species. When such exceptions happen, the nematode species are closely related.

STUDENT POSTER BP3 - Tuesday (Bacteria)

A new procedure and method of analysis to evaluate the performance of liquid formulations of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) in streams or rivers.

Mario Boisvert[1] and Jacques Boisvert[1]

Département de Chimie-Biologie[1], Université du Québec à Trois-Rivières,
Trois-Rivières (Québec, Canada), G9A 5H7.

Many field tests have shown *Bacillus thuringiensis* var. *israelensis* (*Bti*) to be an effective simuliid larvicide. However, literature indicated that the evaluation and comparison of *Bti* based formulations when tested in streams or rivers was difficult to establish. Until today, most field trials were done in different rivers (different discharge, river profile, water temperature, suspended matter, larval species, etc.), thus rendering the evaluation of the performance of liquid formulations of *Bti* very arbitrary or even impossible. A new field procedure is proposed to evaluate the performance of liquid formulations of *Bti* in a same lotic environment and under similar conditions. The method based on a system of gutters located on the bank of a stream, showed very good reproducibility of the percentage of mortality recorded at the different stations over a two-year field trial, proving the efficacy of the system. Because either a single formulation or different formulations can be tested repeatedly in the same portion of a stream, this system could permit a more accurate evaluation of the performance of a *Bti* formulation or a much better comparison between different formulations. The use of the probit model (using comparison of slopes and intercepts) gives reliable statistical value for the analysis of the results. In the summer of 1998 and 1999, tests were performed with two different commercial formulations of *Bti*, Teknar HP-D and Vectobac 1200L, in a stream characterized by its high density of black fly larvae. To make sure to get similar conditions for the experiments (water temperature and discharge), treatments were done on consecutive days in May (water temperature : 15-16°C) and June (water temperature : 20-22°C). Results of May showed that there was no significant difference between the carry of the two products. In June, the carry of the Vectobac was significantly greater than the Teknar HP-D in warmer temperature. Inert ingredients present in the formulations could explain the difference, although the hyporheic zone and the presence of periphyton could also play a role in explaining the difference in carry. For the first time, our procedure allowed the evaluation of the performance and comparison of different *Bti* formulations in a same portion of a stream and under similar abiotic and biotic conditions. Moreover, this system is not expensive and can be moved easily on the same or to another stream or river.

STUDENT POSTER FP1 - Tuesday (Fungi)

A laboratory trial demonstrating that *Erynia neoaphidis* is able to overwinter in aphid cadavers at low relative humidity.

Tony Bonner[1], Simon Gray[1] and Judith Pell[2].

[1]Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., LU1 3JU, U.K., tony.bonner@luton.ac.uk.
[2]Entomology and Nematology Dept., IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, U.K.

The aphid pathogenic fungus *Erynia neoaphidis* does not appear to produce zygospores and may overwinter in aphid cadavers attached to above ground plant material where the relative humidity is lowest. This study shows that *E. neoaphidis* within dried aphid cadavers is able to survive simulated winter temperatures at low relative humidity (RH) for several months.

Dried, mycosed cadavers of the pea aphid *Acyrthosiphon pisum* were kept at relative humidities of 100%, 90%, 50% and 20% in sterile 96 well ELISA plates. To achieve the desired RH, glycerol solutions were placed in 16 wells of each plate. The remainder of the wells contained the mycosed cadavers. The plates were then sealed and incubated in an alternating temperature regime of 8°C for twelve hours and –1°C for twelve hours, over a period of 24 weeks. These temperatures were chosen as representative of the mean day and night temperatures for the period of November to March in the southern UK.

Cadavers (ten replicates per treatment) were sampled at three-week intervals and placed on a sporulation monitor developed for the quantification of the production of conidia by entomophthoralean fungi. Sporulation was monitored for 168 hours and the number and size of conidia produced was measured with the aid of an image analyzer. Cadavers kept at 100% RH and 90% RH initially produced many more conidia than those kept at 50% RH and 20% RH, but ceased to produce conidia after only 6 weeks storage. The number of conidia produced by cadavers maintained at 20% RH remained constant throughout the experiment, whereas conidia production from cadavers kept at 50% RH gradually declined over the duration of the experiment, falling below that from cadavers maintained at 20% RH after 9 weeks' storage. There was no significant inter- or intra-treatment difference in conidia volume for any of the treatments used.

STUDENT PAPER - Tuesday, 17:00 (Protozoa I)

The use of heat and drug therapy for the management of *Nosema* disease in *Muscidifurax raptor* (Hymenoptera: Pteromalidae).

Carl K. Boohene[1], Christopher J. Geden[2] and James J. Becnel[2]

Department of Entomology & Nematology, University of Florida, Gainesville, Florida USA[1]; and USDA – ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA[2].

Muscidifurax raptor, a pupal parasitoid of house flies and other muscoid flies, has been found to be infected with the microsporidian pathogen, *Nosema muscidifuracis*. The infection causes a chronic disease in adult parasitoids resulting in reduced fecundity and longevity. Studies were carried out to evaluate the effect of heat and drug therapy to cure colonies of the disease. For heat therapy we used heat shock treatments as well as continuous rearing at elevated temperatures. Infected *M. raptor* were allowed to oviposit in *Musca domestica* pupae for 24 hours at a host parasite ratio of 5:1. Groups of 400 pupae for each treatment were then subjected to heat shock at the parasitoid's egg stage for 1, 3, 5, and 7 hours at 40°C and 45°C, and also at 50°C for 15, 30, 45, and 60 minutes at high humidity. About 80% of emerged progeny were cured when treated at 50°C for 45 minutes while 60 minutes exposure at this temperature resulted in over 95% cure. Limited cure was obtained from

the treatments at 45°C, while there was negligible cure at the 40°C treatments. Continuous rearing of infected *M. raptor* colonies at 32°C was effective in reducing the infection rate by the second generation, although the sex ratio was distorted towards more males. A 3% solution of the drugs, albendazole and rifampicin were fed to infected adult *M. raptor*, after which the parasitoids were given hosts for oviposition at successive intervals of 2,4,6,and 8 days post exposure. Progeny from the third exposures showed a significant reduction in the infection rate for both drugs. There was negligible reduction in the infection rate from the first and second exposures. The above strategies can be utilized to cure infected colonies and could be incorporated into mass production systems to manage *Nosema* disease of *M. raptor.*

POSTER PP4 - Tuesday (Protozoa)

Biological and Pathological Studies on the *Helicosporidium* spp.

D. Boucias, J. Becnel*, G. White*, C. Stokes, A. Tartar, and B. Adams

Department of Entomology and NematologyUniversity of Florida, Gainesville, Florida, 32611, USA.
*USDA/ARS Center for Medical, Agricultural and Veterinary Entomology
P.O. Box 14565,Gainesville, Fl 32604

Helicosporidium spp. are unicellular organisms that have been reported to cause disease in various invertebrate hosts including insects, mites, cladocerans, and trematodes. These organisms are defined by the production of a unique cyst stage measuring approximately 6.0 microns in diameter with a spore wall that encloses three ovoid nurse cells and a single elongate filamentous cell. We have isolated a *Helicosporidium* sp.from the blackfly *Simulium jonesis* Stone & Snoddy (Diptera:Simuliiidae). An analysis of sequences of the ribosomal (5.8S, 18S, and 28S) and nuclear (actin and tubulin) genes demonstrated that this *Helicosporidium* sp. may represent a very unique lineage in eucaryote evolution. Interestingly, this organism was always positioned between protozoa and green algae. *In vivo* studies have demonstrated that various lepidopterans and dipterans, including *Manduca sexta, Galleria mellonella, Helicoverpa zea, Anopheles albimanus, An. quadrimaculatus, Musca domestica* and *Aedes taeniorhynchus* larvae support helicosporidial development. Light microscopy of challenged *Manduca sexta* larva revealed that within hours post ingestion the cysts bound to the peritrophic membrane. *In vitro* studies have shown that the midgut fluid readily induced the dehiscement of the cysts resulting in the release of the filamentous cells. Within three days vegetative stages may be detected in *M. sexta.* Within the haemocoel vegetative development was exocellular. The insect haemocytes at this stage produced pseudopodia and elicited a normal spreading behavior. Within 10-14 days the haemolymph was filled with progeny cysts. Initial *in vitro* studies demonstrated that dihisced cysts replicated in imaginal disc and conventional cell lines. Epicellular growth in wells inoculated with $5x10^3$ and $4x10^4$ cysts produced a 52 and 30-fold increase, respectively, in cyst numbers. Recently, a series of media, Czapeks Dox + yeast extract (CDY), CDY + insect extract, Lees, Vogels, TC100, TC100+10% FBS, and Sabouraud dextrose (SD) broth have been tested for their ability to support the replication of this organism. All of these media supported vegetative development but none of these produced abundant levels of mature cysts. RFLP analysis confirmed that the *in vitro* produced *Helicosporidium* cells were identical to the *in vivo* cells. These experiments demonstrated clearly that the *Helicosporidium* is not an obligate intracellular parasite and does not require living cells to support vegetative development.

SYMPOSIUM - Thursday, 17:00 (Insect Immunity)

***In Vivo* Cell Phenotypes of Insect Mycopathogens: Cells Invisible to the Host Cellular Defenses**

D.G. Boucias

Department of Entomology and Nematology
Gainesville, Florida 32601
email dgb@gnv.ifas.ufl.edu

Insect fungi gain ingress through the cuticle barrier and upon reaching the nutrient-rich hemocoel replicate exocellularly. Within the hemocoel, these fungal cells must cope with the insect cellular defense response that is designed to recognize and neutralize non-self. Over the past ten years our lab has determined that insect mycopathogens produce *in vivo* phenotypes that use a combination of strategies to survive the host defense reaction. The primary strategy is the modification of surface composition that allows the *in vivo* cells to evade recognition. The protoplast-producing entomophthorans were one of the first groups of fungi demonstrated to evade the host cell defense reaction. Within the hemocoel these fungi synthesize a set of inhibitors that turn off various wall synthetase systems. The resulting protoplasts, lacking the chitin and glucan cell wall elicitors, are not recognized as non-self. Similarly, the common hyphomycete *Beauveria bassiana* produces an *in vivo* phenotype that lacks a formal wall and the galactomannan surface components that are targets for various insect opsonins. More recently, we have shown that the *Nomuraea rileyi* and *Paecilomyces fumosoroseus* produce a walled-form with surface epitopes that mimic insect basement membrane components. For example, monoclonal antibodies raised against *Nomuraea* hyphal bodies specifically cross-reacted with basement membrane proteins. Additionally, mouse polyclonal antibody preparations, produced against *Spodoptera exigua* (beet armyworm) larval hemolymph components and against cell wall surfaces of *N. rileyi*, cross-reacted with both insect and fungal substrates. For example, the hemolymph antibody bound to hemocytes and vice versa, and both antibodies cross-reacted to the insect fat body basement membrane (extracellular matrix=ECM) and to *N. rileyi* and *Beauveria bassiana* cell wall surfaces (ECM). Likewise, the anti-fungal antibodies cross-reacted with *S. exigua* hemolymph and hemocytes, especially the granules that may contain ECM components, and with fat body basement membrane. These cross-reactivities were specific as indicated by negative controls in the microscopy and Western blotting assays. Parallel labeling experiments using ConA demonstrated that the reactive epitopes contain mannose; however, none of the antibodies bound to mannose residues of non-entomopathogenic *Candida albicans* or *Saccharomyces cerevisiae* yeast cells. Thus, these cross-reactivities indicate that the host mimicry expressed by insect mycopathogens allows for replication in the presence of phagocytic cells and thus represent a crucial pathogenic determinant.

CONTRIBUTED PAPER - Thursday, 11:30 (Bacteria IV)

Gene flow in the European Corn Borer: implications for the sustainability of transgenic insecticidal maize

Denis Bourguet [1], Marie Thérèse Bethenod[1] and Frédérique Viard[2]

Unité de Recherches de Lutte Biologique, INRA La Minière, 78285 Guyancourt, France[1], Laboratoire de Génétique et d'Evolution des populations végétales, Université Lille I, 59 655 Villeneuve d'Ascq, France[2].

Strategies proposed for delaying resistance to *Bacillus thuringiens*is toxins expressed by transgenic maize require intense gene flow between individuals that grew on transgenic and on normal (referred to as refuges) plants. To investigate gene flow in the European corn borer, *Ostrinia nubilalis* (Hübner), the genetic variability at 29 sites sampled on maize was studied by comparing allozyme frequencies at 6 polymorphic loci. Almost no deviations from Hardy-Weinberg expectations occurred, and a high stability of allelic distribution was found among samples collected in the same site over two to three different generations, indicating a high stability of the genetic structure over time. The overall genetic differentiation was low whatever the geographical scale, suggesting a high and homogeneous gene flow. The European corn borer, (Hübner), may also be found on several host plants that may act as natural refuges. The genetic variability of samples collected on sagebrush (*Artemisia sp.*), hop (*Humulus lupulus* L.) and maize (*Zea mays* L.) was studied by using the same allozyme polymorphic loci. We found again a high level of gene flow within and between samples collected on the same host plant. The level of gene flow between sagebrush and hop insect samples appears to be sufficiently high for these populations to be considered a single genetic panmictic unit. Conversely, the samples collected on maize were genetically different from those collected on sagebrush and hop. Three of the six loci considered displayed greater between-host plant than within-host plant differentiation in comparisons of the group of samples collected on sagebrush or hop with the group of samples collected on maize. This indicates either that there is a genetic isolation of the insects feeding on maize or that there is host plant divergent selection at these

three loci or at linked loci. These results have important implications for potential sustainability of transgenic insecticidal maize.

STUDENT POSTER FP25 – Thursday (Fungi)

Effects of UV-B irradiance on conidia and germinants of the entomopathogenic Hyphomycete *Metarhizium anisopliae*

Gilberto U. L. Braga[1,3], Stephan D. Flint[2], Anne J. Anderson[1] and Donald W. Roberts[1]

Department of Biology[1] & Department of Rangeland Resources and Ecology Center[2]
Utah State University, Logan, Utah 84322-5305

The effects of two irradiance levels (920 and 1200 mW m^{-2} weighted irradiance) on conidia and germinants were investigated in one strain of *Metarhizium taii*, one of *M. flavoviridae*, two of *M. album* and 26 of *M. anisopliae*. For our location (41.5° N latitude, 1.5 Km elevation), a model (Fiscus & Booker, 1998) shows the low irradiance level approximating noon sunlight on 20 April and the high irradiance level simulating a 30 % ozone depletion on this date. From a different perspective, the high irradiance level could be considered to simulate current noon sunlight on 1 July at our location. Conidia in aqueous suspensions were placed onto potato-dextrose agar + 0.1 % of yeast extract (PDAY) and exposed to the two irradiance levels for 1, 2, 4 and 6 h. The effects of irradiation were determined both by monitoring germination and by counting the number of colony forming units (CFUs) in the different treatments in relation to non-irradiated controls. After 2h of exposure, all strains at both irradiance levels decreased in viability. A 30 % increase (from 920 to 1200 mW m^{-2}) in irradiance caused a significant decrease in culturability after all periods of exposure for all strains. Culturability after 2 h of exposure (920 mW m^{-2}) ranged from 93.3 ± 5.4 % (*M. anisopliae*; ARSEF 324) to 0.2 ± 0.0 % (*M. album*; ARSEF 1840). After 4 h exposure, culturability ranged from 82.5 ± 5.4 % (*M. anisopliae*; ARSEF 324) to 0.0 *% (M. anisopliae*; ARSEF 1432, 4570 and 5626 and *M. album*; ARSEF 1840). For the irradiance of 1200 mWm^{-2}, variation ranged from 88.9 ± 6.6 % (*M. anisopliae* ARSEF 324) to 0.0 % (*M. anisopliae*; ARSEF 5626 and *M. album*; ARSEF 1840) after 2 h exposure, and from 44.8 ± 10.3 % (*M. anisopliae*; ARSEF 324) to 0.0 % (*M. anisopliae*; ARSEF 1095, 1044, 925, 1055, 1432, 3609, 4570, 1187, 2213, 5626, 4343, 444, 1545, 2341 and 4295 and *M. album*; ARSEF 1840 and 1942) after 4 h exposure. We also investigated the dose-time dependency in a reciprocity study. Reciprocity was not observed when conidia in suspension and germinants in different stages of the germinative process were exposed to the 17.28 kJ m^{-2} dose of both irradiances. Although non-reciprocity was observerd in all situations, its magnitude varied as a function of the metabolic state and/or the cell cycle phase in which the conidia were at the time of exposure. The least difference between the effects of the two irradiances was observed when non-germinated conidia in suspension were exposed, and the greatest difference was observed when the conidia were exposed during an advanced germination phase.

[3]Fellowship received by Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (FAPESP). Research supported in part by grants from NRI/CREES and Utah State Mineral Lease funds.

CONTRIBUTED PAPER - Thursday, 11:45 (Bacteria IV)

Response of bertha armyworm to transgenic canola expressing Bt toxins

Lorraine Braun[1], Elke Diederichsen[2], and Martin Erlandson[1]

[1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan Canada S7N 0X2;
[2]Plant Genetic Systems N.V., Josef Plateaustraat 22, 9000 Gent, Belgium

The bertha armyworm, *Mamestra configurata*, is a sporadic pest of canola in western Canada. Commercial formulations of *Bacillus thuringiensis* var. *kurstaki* (Btk) give highly variable control of bertha armyworm (BAW): in an assessment of 61 isolates from 10 different varieties of Bt, Trottier *et al.* (1988, J. Invert. Pathol 51:242-249) reported the median lethal dose for Btk in 3rd instar BAW larvae was 15,400 IU/ml of diet, one of the highest reported for Noctuids. Several of the strains, including *aizawai, kenyae*, and *entomocidus* were more potent than HD-1, yet in greenhouse and small scale field trails they have all shown poor control of BAW. Morris *et al*. (1996, J. Econ. Ent. 89:359-365) reported that the *B. t.* var. *aizawai* strain HD-133 was the most toxic for BAW larvae (LC_{50} of 244 ìg/ml of diet for3rd instar larvae), and subsequently Masson *et al.* (1998, Appl. Environ. Microbiol. 64: 4782_4788) found the toxic activity of HD-133 was associated equally with Cry1Ab and Cry1C gene products.

Preliminary screening of additional Bt toxins identified Cry9C as having good activity against BAW. Transgenic canola expressing two Bt -endotoxins, Cry1Ab and Cry9C, either singly or in combination, were tested against BAW. A series of 7 T1 lines of canola expressing Bt toxins were assayed against BAW armyworm with 5 to 10 plants tested in 2 replicate assays. Plants expressing Cry9C showed good toxicity against BAW: Cry9C expression levels at 0.25 to 1.10% of total leaf protein provided 100% protection against second instar larvae; Cry9C expression levels at 0.13 to 2.13% of total leaf protein reduced feeding by 4th instar BAW by 68%. A protocol for testing resistance to BAW feeding on transgenic plants was developed based on plant bagging experiments in the greenhouse. The results of the greenhouse tests showed good protection of canola plants over 14 day feeding trials. The plant bagging protocol was extended to field trials with transgenic canola. Approximately 36 lines of transgenic canola expressing Bt were tested against 1st, 2nd and 4th instar BAW. There was good correlation between Bt -endotoxin expression and protection from BAW feeding damage.

POSTER BP4 - Tuesday (Bacteria)

Ecological risk of transgenic insect resistance under Canadian field conditions

Lorraine Braun[1], Anne Légère[2], Peter Mason[3], Neal Stewart Jr.[4], and Suzanne Warwick[3]

[1]AAFC, 107 Science Place, Saskatoon SK Canada S7N 0X2;
[2]AAFC, 2560 boulevard Hochelaga, Sainte-Foy PQ Canada G1V 2J3;
[3]AAFC-ECORC, Ottawa ON Canada K1A OC6;
[4]Dept. Biol. U.N.C. Greensboro NC 27402-6174, USA

The Research Branch of Agriculture and Agri-Food Canada (AAFC) has teamed up with the Canadian Food Inspection Agency (CFIA), the Pest Management Regulatory Agency (PMRA) and Environment Canada (EC) to address the need for relevant data to support Canadian risk assessment and risk management decisions for insecticidal transgenes. Currently, no information is available to predict the spread and persistence of insecticidal transgenes in crop and related weed populations. In collaboration with Dr. Neal Stewart from the University of North Carolina at Greensboro, we are developing protocols and generating data to assess ecological effects of *Bacillus thuringiensis* (Bt) transgenes under Canadian field conditions. Stewart's group developed a unique fluorescent marker to monitor gene flow from transgenic crops to related wild plant species in the field. This technology is being evaluated for use in risk assessment of transgenic crops in Canada.

We plan to investigate the movement of insecticidal and "in vivo marker" transgenes (Bt crylAc and green fluorescent protein) from canola (*Brassica napus*) into two closely related weeds, bird rape (*B. rapa*) and wild radish (*Raphanus raphanistrum*) via hybridization and introgression. The fitness of a Bt insecticidal transgene conferring resistance to insect attack in the crop and weeds will be evaluated. Diamondback moth (DBM), *Plutella xylostella*, will be used to assess ecological risks related to development of insect resistance to such transgenes under Canadian field conditions. We hypothesize that the spread of Bt transgenes to weeds will accelerate resistance development through continuous exposure of diamondback moth to Bt, reducing the effectiveness of Bt and Bt transgenic plants for DBM control. We will develop probability estimates for the likelihood of insecticidal transgenes to become incorporated into surrounding weed communities and their survival in the presence and absence of selection pressures. This 3-year project will generate much of the data needed for the risk assessment and management evaluation required by the regulatory process ruling the release of transgenic crops in Canada.

STUDENT POSTER NP2 - Tuesday (Nematodes)

Gene regulation and expression of haemolytic/cytolytic activity from the insect pathogenic bacterium *Xenorhabdus nematophilus*

Brillard J., Boemare N., Bréhelin M. and Givaudan A.

Laboratoire de Pathologie comparée, Université Montpellier II - INRA - CNRS UMR5087, 34095 Montpellier Cedex 5, France.

Bacterial haemolysins are often considered to be virulence factors and many of them are able to lyse leukocytes. This activity may enhance the survival of the bacterium by escaping the immune response of the host. The entomopathogenic bacterium *Xenorhabdus nematophilus* displays a haemolytic activity. A *X. nematophilus flhD*-null mutant that was lacking the motility and a full extracellular haemolysis was previously shown to be attenuated for virulence in insect when compared to the wild-type strain.

We examined the *X. nematophilus* production of different extracellular cytolytic activities during bacterial broth-growth. The cytolytic activities observed on sheep erythrocytes, rabbit erythrocytes and on immunocompetent cells from the Lepidoptera *Spodoptera littoralis* were growth phase dependent. We confirmed that the *flhD*-null mutant was unable to produce sheep erythrocyte cytotoxicity, but it was still able to produce a cytolytic activity on rabbit erythrocytes and insect hemocytes. Therefore, we studied the haemolytic activity of *X. nematophilus* by a genetic approach. We found that a cloned prophage locus from *X. nematophilus* F1 induced haemolytic activity on sheep blood agar plate in *Escherichia coli*. Sequencing of this prophage locus revealed homology with various phagic proteins, but no significant homology with other known haemolysins was detected. The *E. coli* K12 laboratory strain carries a cryptic haemolysin gene *clyA* (*sheA*) which is under the control of various transcriptional regulators. Though, we investigated to see if this prophage locus should be a positive regulator of the cryptic haemolysin gene *clyA*. Any haemolytic activity was observed when cloning the prophage locus in a *clyA* defective mutant. This result showed that the prophage locus of *X. nematophilus* was a positive regulator which acted directly or undirectly on the cryptic haemolysin gene *clyA*. Work is currently underway to find a *clyA* homologous gene in *X. nematophilus*.

CONTRIBUTED PAPER - Monday, 14:45 (Fungi II)

Efficacy of *Beauveria bassiana* for control of *Lygus lineolaris* and the interaction with a *Peristenus* parasitoid

A. Bruce Broadbent

Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre,
1391 Sandford St., London, Ontario, Canada, N5V 4T3

Lygus lineolaris, the tarnished plant bug, is a major pest on many agricultural crops in Canada and is an excellent candidate for biological control. A strain of the entomopathogenic fungus, *Beauveria bassiana*, field-collected from a *L. lineolaris* host was cultured and put into conidial suspension [4 x 10^7 viable conidia/ml] + 0.005% Tween 80 and applied by airbrush sprayer to *Lygus* nymphs and adults in laboratory bioassays. Mortality [and subsequent mycosis] of *Lygus* was checked daily from day 1 to 8 except for days 2 and 3 and compared to the controls for each test. Adults were significantly more susceptible to *Beauveria* than the nymphs of *Lygus*. Typically, mortality of *Lygus* nymphs and adults was first observed at 4 to 5 days after treatment and cumulative mortality reached 80-90% for treated adults and 30-50% for treated nymphs by day 8 at 24^{0}C and 90% RH. Initial tests of the interaction of *Beauveria* with the parasitic wasp, *Peristenus stygicus*, or with *Lygus* nymphs parasitized by this wasp, will be discussed.

CONTRIBUTED PAPER - Monday, 11:45 (Fungi I)

Effect of *in vitro* -passage of *Beauveria bassiana* on virulence to silverleaf whitefly

Michael Brownbridge[1], Scott Costa[1] and Stefan Jaronski[2]

[1]Entomology Research Laboratory, University of Vermont, Burlington, Vermont 05405 and [2]Mycotech Corp., Butte, Montana 59702

There appears to be considerable variation in the effects of repeated *in vitro* subculture on the pathogenic or morphological characteristics of entomogenous fungi. Differences may be observed between fungal species and isolates of the same species. The purpose of the current studies was to assess effects of repeated *in vitro* transfer of *Beauveria bassiana* GHA (726) strain on its pathogenicity for silverleaf whitefly, *Bemisia argentifolii*. This is a commercialized strain, so this information is important to prevent attenuation of the fungus in routine laboratory transfers prior to large-scale fermentation and ensure that product virulence is maintained. The series was initiated with isolating the fungus from artificially infected *Galleria mellonella* larvae; thereafter, the fungus was sequentially subcultured by conidial transfer on full-strength Sabouraud dextrose agar supplemented with 0.1% yeast extract. Conidia were harvested by physically scraping 21-d old cultures after passaging 1, 5, 10 and 15 times in vitro and were dried at 25-28º C over Drierite®. Conidial powders were stored at –20º C until assay. Viability of each test batch was >95%. Virulence was assessed against neonate and 2-d old whitefly nymphs, using two test concentrations ($2.5x10^6$ and $4.0x10^7$ viable conidia/ml 0.02% Silwet). Suspensions were applied using a Potter spray tower and whitefly mortality and infection rates determined 6, 10 and 14 d after spraying. *In vitro* passaging of *B. bassiana* did not appear to significantly ($P>0.05$) influence the levels of whitefly mortality and infection obtained. No significant differences were detected in mortality and infection levels between fungi passaged 1, 5, 10 or 15 times *in vitro*. As would be expected, percentage mortality and infection were significantly greater ($P<0.0001$) in treatments where the highest conidial concentrations were used. Significantly higher ($P<0.0001$) mortality and infection rates were observed in 2-d old nymphs compared to those treated as neonates. Additional studies to quantify effects of continued passaging on growth, conidiation rate, viability, and virulence are needed, but the results indicate that the genetic factors controlling pathogenicity in *B. bassiana* 726 are stable enough to facilitate limited transfer and culture on artificial media without rendering the isolate avirulent.

CONTRIBUTED PAPER - Tuesday, 8:45 (Fungi III)

Persistence of fungal conidia on different varieties of poinsettia and tomato

Michael Brownbridge, William Reid and Alek Adamowicz

Entomology Research Laboratory, University of Vermont, Burlington, Vermont 05405, USA

There is a paucity of information available about the influence of host-plants on insect infection with entomopathogenic fungi. Several volatiles and exudates produced at the leaf surface are known to have fungistatic or fungicidal effects on plant pathogens, and presumably play a role in protecting the plant against infection. However, if these materials negatively impact conidial survival and insect infection with entomogenous fungi, pest control will be adversely affected. Using silverleaf whitefly (*Bemisia argentifolii*) as a model test insect, we are currently investigating the role of the host plant in the success or failure in a fungal-based control program. Using different varieties of poinsettia and tomato, two economically important hosts, the first stage in this assessment has been to evaluate conidial survival on leaf surfaces over time, to determine whether viability is affected by leaf-surface products. Four poinsettia varieties are being used in the studies: Lilo, Freedom, Supjibi and Peterstar; all appear to be equally susceptible to whitleflies but have different genetic characteristics. Four tomato varieties have also been included in the trials: Trust, Buffalo, BHN A-Bt (Bt transgenic) and BHN A (non-transgenic 'parent'). All fungi used in the trials have been shown to be highly pathogenic to whiteflies in laboratory and greenhouse tests and include formulated *Beauveria bassiana* (BotaniGard™ WP and ES), as well as strains of *B. bassiana*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* produced on laboratory media. Lower leaf surfaces are sprayed with conidial suspensions (ca. 2.10^7 conidia/ml) prepared in 0.02% Silwet to promote leaf wetting and coverage and plants are held in a production greenhouse at 25-28º C. Conidial viability is assessed using the leaf-press technique 0, 2, 4, 8 and 16 d after spraying. Persistence was similar on all poinsettia varieties, for all fungal strains tested and the viability of conidia recovered declined gradually over time from >95% on d 0, to approx. 85% by d 16. Preliminary data on persistence of *B. bassiana* on tomato leaves suggest that conidial survival is much poorer, falling to approx. 30% by d 16. Experiments are continuing to quantify

effects for other fungal species and formulations. These will be followed by a study of host-plant effects on insect infection. This information is essential to the appropriate and successful use of fungi in plant protection.

SYMPOSIUM - Thursday, 17:50 (Insect Immunity)

Antimicrobial peptides: a key component of the humoral immune response in insects

P. Bulet

Institute of Cellular and Molecular Biology, UPR 9022 CNRS
15, Rue René Descartes, 67084 Strasbourg cedex, France
Email: P.Bulet@ibmc.u-strasbg.fr

One factor contributing to the enormous success, that insects have exhibited through evolutionary time, is their ability to mount a rapid and effective response against infectious microorganisms. The current view is that the antimicrobial host defense of insects is multifaceted process, which involves the activation of proteolytic cascades, reactions by hemocytes, and the rapid synthesis of antimicrobial peptides. Since the original discovery of cecropins in the diapausing pupae of the lepidopteran *Hyalophora cecropia*, in the early 1980s, by Hans Boman and collaborators, approximately 200 immune peptides from insects have been characterized. These peptides are (i) rapidly synthesized by the fat body, certain hemocytes and epithelia, (ii) easily stored, and (iii) promptly available after infection. Most antimicrobial peptides from insects are cationic. They have structures that are predominantly either α-helical (cecropin family) or open-ended cyclic mixed α-helical/β-sheet (insect defensins, insect antifungal peptides). Some have simply β-sheet structures (thanatin and androctonin). A number of other antimicrobial peptides exist, often with poorly defined or described structures. These peptides are rich in certain amino acids such as proline (apidaecins, abaecins, drosocin, etc..), glycine (attacin, coleoptericin, etc...) or proline and glycine residues (diptericin). The cDNAs for representative members of the antimicrobial peptides from insects have been cloned. In most cases, these peptides were found to be synthesized as precursor molecules. They consist of a conventional hydrophobic single peptide, a prosequence upstream or downstream the mature sequence. A common feature of the gene encoding antimicrobial peptides from insects is that they are silent under normal conditions. Upon experimental immune challenge, all genes are rapidly and strongly induced in the fat body. These peptides, released into the hemolymph, rapidly kill bacteria after contact with the micro-organism, at concentrations of 0.1-10 µM. The main site of action of the peptides is the cytoplasmic membrane where they can bind to form pore or channels or simply act as detergent-like through a "carpet" or a "barrel-stave" mechanism.

Gene-encoded antimicrobial peptides are now clearly established as key players in both plant and animal defense systems. In addition, along with this has come a renewed awareness of the potential therapeutic applications of these peptides.

STUDENT PAPER - Tuesday, 9:00 (Viruses II)

A model of Nucleopolyhedrovirus population genetics applied to co-occlusion

Jim Bull, Charles Godfray and David O'Reilly

Department of Biology, Sir Alexander Fleming Building, Imperial College, South Kensington, London, SW7 2AZ, UK

A model of Nucleopolyhedrovirus (NPV) genetics had been developed and applied to co-occlusion dynamics. Data from the serial passage of recombinant and wild-type NPVs in a co-occlusion scenario have been obtained in order to estimate key parameters in this model. Co-occlusion is the incorporation of wild-type and mutant virus in the same occlusion body. This can be employed as a strategy to deliver genetically modified viruses as insecticides in a way that minimises their persistence in the environment. It may also serve as a method by which naturally occurring mutant forms of NPVs can remain as a stable sub-section of the virus population. The model predicts that the persistence of the modified virus depends most critically on the number of viruses that infect an occlusion body-producing nucleus, the number of occlusion bodies typically ingested by an insect, and the number of viruses that cross the gut wall. The only unspecified term in this model is the distribution of virus particles that infect cell nuclei within the host tissues. In order to assess this parameter, a recombinant strain of Autographa californica nucleopolyhedrovirus (AcMNPV) was constructed with a deletion of its polyhedrin gene, rendering it incapable of producing occlusion bodies (occlusion-negative). This was co-occluded with wild-type AcMNPV and used to infect fifth instar *Trichoplusia ni* larvae. The fate of the two genotypes was monitored over serial passage through several rounds of infection and the data obtained were compared to the predictions of the model. Levels of the occlusion-negative virus genome remained consistently detectable after several rounds of infection, suggesting a high frequency of multiple infection of cells within the insect host.

SYMPOSIUM - Tuesday, 9:10 (Nematodes)

Molecular genetics of EPN: current status and future developments.

Ann M. Burnell

Institute of Bioengineering and Agroecology, Department of Biology, National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland.

Much of the focus in EPN genetics has been on applied aspects relating to strain improvements for biocontrol applications. Techniques of classical genetics – mutagenesis, hybridisation and artificial selection have been successfully used in such genetic improvement programs. By contrast, the techniques of molecular genetics have not been widely applied to EPN, except in the area of molecular diagnostics and in studies of molecular phylogeny. EPN belong to the same family as *Caenorhabditis elegans* whose genome has been fully sequenced and annotated. In principal, the tools which have been developed for *C. elegans* (e.g. RNAi, transposon mutagenesis, genetic transformation, EST analysis etc.) could be developed and/or applied to studies on EPN but, in practise, such technology transfer has been rare. EPN could be excellent models in which to study a range of fundamental problems relating to symbiosis and animal parasitism. Like *C. elegans*, their genome is small, they can be cultured *in vitro* or *in vivo*, they are extremely prolific and have a short life cycle. Hopefully we are entering a new phase in EPN research which the tools of molecular genetics will be increasingly used to address a wider range of questions. The knowledge gained from this approach should ensure that EPN will become even more effective biopesticides and should also ensure that EPN and their symbionts gain prominence as unique and intrinsically interesting biological systems.

POSTER VP23 - Thursday (Viruses)

Predicted interaction domains between AcMNPV P143 (helicase) and LEF3 (single stranded DNA binding protein)

E.B. Carstens, T. Behra, K. Neumann, A. Taha and M. Garrett

Department of Microbiology and Immunology,
Queen's University, Kingston, Ontario, K7L 3N6

P143 is one of the AcMNPV genes essential for viral DNA replication. Based on the characterization of a ts mutant defective in DNA replication, we predicted that P143 was a multi-functional protein essential for viral DNA replication, regulation of late viral protein synthesis, and modification of host gene expression. Based on our analysis of the amino acid sequence, we hypothesized that P143 carried motifs characteristic of proteins having DNA unwinding (helicase) activity. This activity has recently been confirmed. However, the helicase motifs of P143 actually form a small portion of the 1221 amino acid polypeptide so we have been studying other activities in order to understand the exact role that P143 plays during viral DNA replication. P143 interacts with a viral single stranded DNA binding protein (LEF3) and this interaction is necessary for the nuclear localization of P143. We have begun to investigate the domains of P143 that are necessary for this interaction with LEF-3. A number of point- and deletion-mutant P143 proteins were expressed alone or in the presence of LEF-3 in transfected insect cells and the intracellular localization of P143 and LEF3 were determined by immunofluorescence. The results suggest that point mutations within the helicase motifs that interfere with DNA replication do not affect P143--LEF3 interaction. Other point mutations outside the helicase motifs that caused defectives in DNA replication also do not affect P143-LEF3 interactions. However,

deletion mutant analysis suggests that a LEF3 interacting domain is located in a region between P143 amino acids 767 and 866. In addition, the species specificity of P143-LEF3 interactions are being investigated by expressing heterologous combinations of P143 and LEF3 from two different species of baculovirus, AcMNPV and CfMNPV. Preliminary results suggest an aberrant interaction between P143 and LEF3 from heterologous baculovirus species.

SYMPOSIUM - Friday, 11:45 (Fungi)

The effect of relative humidity (RH) and degree days (GDD) on the rate of development of whiteflies and entomopathogenic fungi

Margarito Ortíz Caton, Raquel Alatorre Rosas, Fernando Tamayo Mejia

Institute of Fitosanidad, Colegio de Postgraduados. Km. 36.5 Carretera México- Texcoco. CP 56230. Montecillo Edo of Mexico.
alatoros|@colpos.colpos.mx

The effect of relative humidity (RH) and degree days (GDD) on the rate of development of *Bemisia tabaci, B. argentifolii* and *Trialeurodes vaporariorum*, and five isolates of the fungus *Paecilomyces fumosoroseus*, 2 isolates of *Verticillium lecanii* and 2 isolates of *Beauveria bassiana* were determined under controlled conditions, on tomato and chilli. Temperature, RH and host plant can influence the life cycle duration and survival of white flies. The development of both the insects and the fungi are inversely proportional to the temperature. The thermal requirements of the different life stages of whiteflies on chilli and tomato were variable; *T. vaporariorum* and *B. argentifolii* required from 350 to 400 GDD and *B. tabaci* 300 GDD to complete their lifecycle. It is important to highlight that the developmental rate (Td) of the nymphal stages (N4) showed differences in the speed of growth among species and in relation to the host plant on which they were developing. The largest Td took place at 31.7ºC in *T. vaporariorum* (0.225) and *B. tabaci* (0.215) both on chili. The pathogenicity of the fungus varied against different developmental stages of the whiteflies. The greatest percent infection was obtained when whiteflies were maintained at 75% RH for 14 hours after inoculation. The GDD varied from 25.4 (*Verticillium*) up to 68.15 (*Paecilomyces*); the lower Td for the different entomopathogenic fungi corresponded to the largest/ fastest? development rate for the N4 of the different whiteflies species. Under favourable temperature and humidity conditions we would recommend the application of the fungus with the largest Td against pupae (N4) and young instars, with the assumption that N4 might escape the infection. Under field conditions (50% of RH) it was possible to confirm that the thermal requirements calculated for the fungus varied according to the cultivation cycle; in the December-March cycle in Tenextepango, Morelos the DGG were reached in 6 to 7 days, however mortality was recorded at day 4 after application. Sporulation was not evident suggesting that the entomopathogenic fungus could require less GDD to kill the insect.

POSTER BP5 - Tuesday (Bacteria)

Characterisation of the Cry1Ac-binding carbohydrate epitopes on *Manduca sexta* 120 KDa Aminopeptidase N

Catherine E. Chambers, Joseph Carroll and David J. Ellar

Department of Biochemistry, University of Cambridge
80 Tennis Court Road, Cambridge, U.K., CB2 1GA

Cry1 δ-endotoxins are a class of crystal proteins that specifically target lepidopteran larvae. Each type of Cry1 toxin has a unique spectrum of activity, targeting only a small range of species. This specificity is determined in part by recognition of receptors in the larval midgut. The 120 kDa aminopeptidase N (APN) from *M. sexta* is a glycoprotein located in the brush border membrane that has been identified as a possible receptor for Cry1Ac. Binding to *M. sexta* APN is specifically inhibited by GalNAc, suggesting that the toxin interacts with a carbohydrate epitope on the protein. Recently the GalNAc binding region of Cry1Ac has been localised to a site on and adjacent to β-16 in domain III (Burton *et al.* (1999) J. Mol. Biol. **287**:1011-1022). In this study the carbohydrate epitopes of *M. sexta* APN that are involved in Cry1Ac binding were localised on the glycoprotein and characterised by lectin mapping. The results confirm the presence of GalNAc on these peptides and identify several other monosaccharides. Further characterisation of the glycoprotein using glycosidases and mass spectrometry will be presented.

POSTER BP2 - Tuesday (Bacteria)

Regulation of the accumulation of the Cry1D toxin in *Bacillus thuringiensis* subsp *aizawai*

Lily Chang[1], Yu Zinui[1] and Arthur Aronson[2]

[1]Laboratory of Bacillus Molecular Biology, Huazhong Agricultural University, Wuhan 430070, PRC; [2]Department of Biological Sciences, Purdue University, W. Lafayette, IN, 47907

Bacillus thuringiensis subsp. *aizawai* HD133 produces inclusions comprised of the Cry1Ab9, Cry1Ca and Cry1Da protoxins. There are substantial differences in the relative amounts of these protoxins in inclusions with a ratio of 20:12:1, respectively, based on the quantitation of signature tryptic peptides (Masson et al, Appl. Environ. Microbiol. **64**:4782). Factors which could account for this great disparity in protoxin content were considered. (1) The *cry1Ab9* gene is on a plasmid of 45kDa and the *cry1Ca* and *cry1Da* genes are in close proximity on a ca 110mDa plasmid. There may be a greater copy number of the *cry1Ab9* gene relative to the other two but there are transcriptional differences among the three genes even relative to gene copy number. (2) These three genes contain very similar overlapping BtI (σ^E) and BtII (σ^K) promoters. There are differences in the sequences of the ribosome binding sites and changing the *cry1Da* region to the consensus sequence enhanced expression 2-3 fold, hardly sufficient to account for the substantial difference in relative protoxin content. (3) Expression of transcriptional fusions of the *cry1A* and *cry1Da* promoters to *lacZ* differed by about a factor of two and these differences were subspecies dependent. Northern hybridization results with *cry1Ab* and *cry1D*-specific oligonucleotides were consistent with the *lacZ* fusion data. (4) The stabilities of these two mRNA's were comparable with halve lives of 12-15 min. Overall, neither differences in translational initiation nor in gene transcription (4-5fold overall) appear to be adequate to account for the 20 fold greater amount of the Cry1Ab9 relative to the Cry1D protoxin in inclusions. It is likely therefore that some other aspect of translational efficiency, protoxin stability or packaging into inclusions is involved. Cry1Da polyclonal antibody was affinity purified and used to compare the relative content and stability of Cry1Da toxin in sporulating cell extracts as compared to the amount in inclusions. Similar comparisons were made for the Cry1Ab9 and Cry1Ca protoxins in *B. thuringienesis* subsp. *aizawai* HD133. The contribution of each of these factors to protoxin regulation and accumulation will be presented.

STUDENT PAPER - Monday, 12:15 (Bacteria I)

Genetic typing of the genus *Bacillus* and presence of two genes coding for the delta-endotoxin of *B. thuringiensis israelensis*

Sophie Chappuis[1,2], Mauro Tonolla[2], Raffaele Peduzzi[2], Nicola Patocchi[1], and Peter Lüthy[3]

Fondazione Bolle di Magadino, Casa comunale, CH-6573 Magadino, Switzerland[1]
Istituto Cantonale Batteriologico, via Ospedale 6, CH- 6904 Lugano, Switzerland[2]
Institute for Microbiology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland[3]

The use of *Bacillus thuringiensis israelensis* (Bti) against mosquito larvae represents an efficient method with minimum direct environmental impact. Our studies emphasise a natural reserve of high ecological value, located in the southern part of Switzerland, called Bolle di Magadino. In order to protect the population from the nuisance caused by large population of *Aedes vexans*, the Bti product Vectobac-G® has been used successfully over the last decade. Two to four treatments are required each year. Thus this natural reserve has become an ideal site to monitor the long term compatibility of Bti with the ecosystem. This requires reliable methods for genetic differentiation of the various subspecies of *B. thuringiensis*.

The aim of the present work was to differentiate between strains of Bti and other *B. thuringiensis* subspecies or other *Bacillus* species. Strains of the genus *Bacillus* were compared by partial sequencing of the 16S rDNA gene (n=32) as well as by ribotyping (n=44). The partial sequences were compared with sequences retrieved from the EMBL database (n=30). Analysis of alignments showed that the combination of 3 nucleotides located at the positions 77, 90 and 92 of the 16S rDNA gene (relative position on *E. coli* rDNA) yielded different profiles which allowed to distinguish Bti (G^{77}-C^{90}-A^{92} profile) from the *B. cereus* strains, as well as from most of the other *B. thuringiensis* subspecies (A^{77}-T^{90}-T^{92} profile). The digestion of the DNA with *PvuII*, followed by ribotyping revealed a unique pattern of the Bti strains including the strain present in the commercial product Vectobac-G®.

The delta-endotoxin genes of Bti (*cry4Aa, cry4Ba, cry10Aa*, *cry11Aa* and *cyt1Aa*) coding for the mosquitocidal proteins are located on a plasmid of approximately 125kDa, present in a few copies per cell. A PCR-based method allowed the detection of both the two genes *cry4Aa* and *cry4Ba*, selectively in strains belonging to the *israelensis* subspecies of *B. thuringiensis*.

This PCR result, combined with the differentiation of *B. thuringiensis israelensis* from other *Bacillus* species, should support the investigation of a possible horizontal transfer of the plasmids containing the genes coding for the delta-endotoxin of Bti. The final goal is the application of these methods under field conditions to monitor the long term use of Bti in samples of soil and water within the ecosystem of the natural reserve Bolle di Magadino.

WORKSHOP I - Thursday, 9:35 (Bacteria)

Status of food safety evaluation of Cry protein-containing crops

Bruce M. Chassy

Department of Food Science and Human Nutrition
University of Illinois-Urbana Champaign, Urbana, Illinois 61801

Genes encoding Cry proteins (Cry9C, CryIA9(b), CryIA(c), or CryIIIA) have been incorporated into crop plants such as soybean, corn, and potatoes. The principal food safety concerns for modified crops are: 1) potential toxicity of the newly introduced protein(s), 2) potential changes in allergenicity, 3) changes in nutrient composition, 4) unintended effects giving rise to allergenicty or toxicity, and 5) the safety of any antibiotic resistance marker-encoded proteins included in the transgene. Cry proteins are not new to the human diet, since they have a history of use as biopesticides. In *cry*-containing transgenics, the concentration of Cry proteins is usually well below 0.1% of the plant's total protein content. Additionally, none of the Cry proteins has been demonstrated to be toxic to humans. Cry proteins have also not previously been implicated as allergens, nor do they contain sequences resembling relevant allergen epitopes. For the most part, *cry*-containing trangenes are not consumed whole by consumers, but are fractionated into oil, starch, and/or denatured protein factions that would not contain active Cry proteins. It is likely that the majority of Cry proteins would be thermally denatured prior to consumption. Cry proteins are thought to be digestible. These factors point to a reasonable likelihood of safety, and would allow the claim that such crops are as safe as, or safer than crops produced by traditional methods. Indeed, after extensive safety testing and some five years of experience with such crops in the marketplace, there is not a single report that would lead one to question the safety of such transgenics. It is nonetheless, impossible to provide consumers assurance of absolute zero-risk, largely due to inadequacy of methods to screen for novel and previously unreported toxicity or allergenicity. The near zero-risk standard that is being applied to this new technology far exceeds the standard used for novel crops produced by conventional methods. Risk-benefit analysis suggests that investment in research on the leading food safety priorities such as food borne pathogens, natural toxicants, chance additives, and emerging safety issues associated with certain organic foods and dietary supplements would be a more effect use of resources.

CONTRIBUTED PAPER - Monday, 11:00 (Viruses I)

The *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus genome sequence

Xinwen Chen[1,2], Sander Peters[3], Wilfred F. J. IJkel[2], Hans Sandbrink[3], Hualin Wang[1], Xiulian Sun[1], Renato Tochini[3], René Klein Lankhorst[3], Douwe Zuidema[2], Zhihong Hu[1] and Just M. Vlak[2]

[1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, PR China, [2]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, and [3]Greenomics, Wageningen University and Research Center, P.O. Box 16, 6700 AA Wageningen, The Netherlands.

Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus (HaSNPV) has been extensively used to control the boll worm in cotton and in vegetable crops in China. In 1999 about 100,000 hectares of cotton have been treated with a commercial virus preparation based on HaSNPV. Complete nucleotide sequences have been obtained from MNPV and granulovirus (GV) genomes, but not from SNPVs. Here we present the complete sequence of the G2 strain of HaSNPV. This strain was obtained by an *in vivo* cloning procedure. The circular genome contains 131,403 base pairs and has a G+C content of 39.1%, the lowest value among baculoviruses to date. The HaSNPV genome contains five homologous regions (*hr*) with a unique pattern of repeats. Computer-assisted analysis revealed 135 non-overlapping ORFs larger than 50 amino acids. Hundred-and-fifteen ORFs have homologues in other baculoviruses, whereas twenty ORFs are unique to HaSNPV and without homologues in GenBank to date. Among the seven sequenced baculoviruses to date, AcMNPV, BmNPV, OpMNPV, SeMNPV, LdMNPV, HaSNPV and XcGV, sixty-five ORFs are conserved and hence considered as core baculovirus genes. The NPVs only have 80 ORFs in common. Three copies of 'baculovirus repeat ORFs' (*bro*) and two copies of 'inhibitor of apoptosis' (*iap*) genes were found. The HaSNPV genome lacks a homologue of the AcMNPV budded virus (BV) major glycoprotein gene *gp64*. Instead, a homologue of SeMNPV ORF8, encoding the major envelope protein in this virus has also been identified in HaSNPV. The mean overall amino acid identity of putative HaSNPV ORFs was the highest with SeMNPV and LdMNPV homologues. GeneParityPlot analysis of baculovirus genomes also suggests that the group II baculoviruses, HaSNPV, SeMNPV and LdMNPV, have a common ancestor and that they are more distantly related to the group I NPVs including AcMNPV and particularly to the granuloviruses such as XcGV on the basis of their genome organization.

POSTER FP17 - Thursday (Fungi)

Ultrastructural observations on the transovarial transmission of a yeast-like symbiote in the brown planthopper, *Nilaparvata lugens*

Dor-Jih Cheng and Roger F. Hou

Department of Entomology, National Chung Hsing University, Taichung, Taiwan 402, ROC

Transovarial transmission of a yeast-like symbiote (YLS) in the brown planthopper, *Nilaparvata lugens*, was observed with optical and electron microscopy. Optical micrographs showed that there was no YLS in testes and spermathecae of the mated females, indicating that sperm is not involved in the transovarial transmission of the symbiote. Adipose in fat body cells reduced as increased the female age. A great amount of proteins were seen in most part of cytoplasm of the fat body cells. YLS became free in hemocoel and ovaries. Electron micrographs revealed that abundance of endoplasmic reticula and granules were visible in cytoplasm of fat body cells and mycetocytes in reproducing females. Ultrastructural observations on transovarial transmission of YLS revealed that nucleus of the mycetocyte lost its boundary in the beginning; the YLS inside being enclosed by a membranous structure formed of fat body cells and discharged into hemocoel by exocytosis or extrusion through cell membrane, and that the free YLSs in hemocoel entered the oocyte from epithelial plug of ovariole by endocytosis through follicle cells, forming a symbiote ball away from ooplasm.

CONTRIBUTED PAPER - Tuesday, 8:15 (Viruses II)

The p34.8 (gp37, spindlin) gene is not essential for baculovirus replication

XiaoWen Cheng[1], Peter Krell[2], Qili Feng[1], Arthur Retnakaran[1] and Basil Arif[1]

Great Lakes Forestry Centre[1], Sault Ste. Marie, Ontario and Department of Microbiology[2], University of Guelph, Ontario, Canada.

The entomopoxvirus fusolin and the baculovirus spindlin (P34.8, GP37) are homologues that have highly conserved amino acid domains. Fusolin appears to enhance the infectivity of baculoviruses by several folds while the activity of spindlin is still a matter of inference to its entomopoxvirus homologue. Previous reports have indicated that *p34.8* may be essential for the replication of AcMNPV because no viral plaques with inactivated *p34.8* were successfully isolated. We were interested to see if fusolin could substitute P34.8 in AcMNPV but the baculoviral requirement for *p34.8* first had to be ascertained. We attempted to inactivate *p34.8* by inserting into its open reading frame (ORF) the gene encoding the green fluorescent protein (GFP) or by deleting all the conserved domains from the ORF. The gene encoding GFP was successfully inserted into the *Not*I site of the *p34.8* ORF of AcMNPV and a viral plaque containing the insertion was propagated in SF-21 cell. Similarly, a transfer vector was designed to eliminate 531 bp (*Not*I-*Xba*I containing all the conserved domains) from the ORF. SF-21 cells were co-transfected with the transfer vector and AcMNPV DNA. Ten progeny viral plaques were isolated, one of which lacking all the conserved domains was propagated. All viral isolates were authenticated by PCR amplification, restriction enzymes digestion, DNA sequencing and by Southern and Northern blots hybridization. The two independent methods clearly show that *p34.8* is not essential for baculovirus replication and that this locus could, therefore, provide another site for the engineering of baculoviruses.

SYMPOSIUM - Thursday, 9:00 (Microbial Control)

Microbial insecticides in Africa, current use and future prospects

Andy J Cherry[1], Nguya K. Maniania[2], George Oduor[3]

[1]International Institute of Tropical Agriculture, 08 BP 0932, Cotonou, Benin; [2]The International Centre of Insect Physiology and Ecology, PO Box 30772, Nairobi, Kenya; [3]CAB International, Africa Regional Centre, PO Box 633, Village Market, Nairobi, Kenya.

The development and use of microbial insecticides in Africa has a long history. Several of the continent's key insect pests have been the target of biopesticide development projects but, despite growing acceptance of the important role biopesticides have to play in IPM programes, few microbial insecticides have ever made it to the market place, and even fewer have established a sustained presence. Why is this?

Much of the work on biopesticide development in Africa has been conducted within the public sector, in international agricultural research centres and national research institutes within which the full range of skills needed to take a microbial control agent all the way from initial discovery to full commercial implementation often do not co-exist. While these centres may possess excellent research facilities for the initial stages of biopesticide development, few have the skills or expertise needed to take the product beyond the field trial stage to the market. Such constraints indicate the need for a degree of multidisciplinarity which only comes from successful partnerships with the private sector and regulatory authorities. Examples of such developments exist on the continent and critical examination of their success enables appraisal of the pitfalls in the process.

However, the commercial model of biopesticide implementation, particularly in Africa, is not the only route to achieve adoption and uptake. For those potential biopesticides based on pathogens which do not readily conform to the exacting standards of a chemical pesticide model, careful examination of alternative routes to implemetation should be undertaken early in their developmental stages.

CONTRIBUTED PAPER - Friday, 10:45 (Microsporidia II)

Thelohania solenopsae (Microsporida: Thelohaniidae) impact on imported fire ant populations at two locations in Texas

Tamara J. Cook[1], Jerry L. Cook[1], and S. Bradleigh Vinson[2]

Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341[1] and Department of Entomology, Texas A&M University, College Station, TX 77843[2]

Microsporidia are good biological control candidates for imported fire ants (*Solenopsis invicta*) because they possess a stable transmission stage and have an efficient means of transmission. Furthermore, in South America reduced densities of *Solenopsis richteri* are correlated with the presence of a microsporidian, *Thelohania solenopsae.* As part of an overall objective to utilize the microsporidian *Thelohania solenopsae* as a control agent of *Solenopsis invicta* in the United States, we investigated the impact of this pathogen on mound density and mound volume in natural unmanaged systems at Camp Swift, a Texas Army National Guard training post. We established two one-acre circular plots: the first plot was located in an area previously determined to have *T. solenopsae* infected fire ant colonies, and the second plot was in an area previously determined to be free from *T. solenopsae* infection. All colonies in both plots were flagged; width, length, and height of mounds were measured and samples from each mound were taken to the laboratory to determine inter- and intracolonial prevalence. Samples were collected bimonthly for eight months. At the positive site, intercolonial prevalence ranged from 17% to 50% and intracolonial prevalence ranged from 26% to 93%. Mound volume of infected colonies was significantly less than mound volume of uninfected colonies, and mound density was greater at the negative site. We also examined natural population dynamics in a heavily grazed pasture at the Texas A&M Beef Cattle Center. We collected samples from all fire ant colonies in a ½-acre study plot in October 1998 and April 1999. Intercolonial prevalence was 53% in October and 35% in April, and intercolonial prevalence ranged from 10% to 97%. Interstadial prevalence, which was recorded for those infected colonies from which we collected brood, was consistently higher in larvae and pupae than in workers.

SYMPOSIUM II - Monday, 17:50 (Viruses)

Sublethal infections – are they important in host-virus dynamics?

Jenny S. Cory

Ecology and Biocontrol Section, NERC Centrefor Ecology and Hydrology – Oxford, Mansfield Road, Oxford, OX1 3SR,UK

Despite many years of research and their widespread use as pest controlagents, we still know remarkably little about how natural baculovirusinfections persist in the wild. The main route of persistence hashistorically been assumed to be via occlusion bodies in environmental reservoirs. However, this does not explain theirpersistence during periods of low population density, in situations wherethe hosts are highly mobile, or where environmental reservoirs areunstable. In such situations it would seem highly likely that the viruses have evolved alternative meansof survival which rely on vertical transmission via the adult moths. Low levels of vertical transmission of baculoviruses have been frequently recorded, although little is known about its contribution to the persistence ofviruses in natural populations. Sub-lethal effects such as reductions infecundity and alterations in development time are also commonly reported, but we still donot know whether they are a cost of fighting off infection, a consequenceof low levels of sub-lethal infection or both. Perhaps, more importantly, we have no idea how frequentsub-lethal infections are in field populations, if they have a significantimpact on the host or whether these infections can be triggered into overtdisease. Sub-lethal infection has proved hard to study, in part, because techniquesfor identifying sub-lethal quantities of virus were lacking.This problem can now be overcome. Recently, with the development of highly sophisticatedmolecular tools such using RT-PCR to detect viral RNA, more informationabout sub-lethal baculovirus infections has emerged. Increasing evidence indicates that sub-lethal infection may bemore common than had previously been thought.It is also likely that so-called latent infections, often reported in the literature,are persistent low level infections, rather than being trulylatent.

POSTER FP14 - Thursday (Fungi)

Comparison of the Scotch tape method to washing or imprinting leaves for quantifying fungal spore deposition on leaf surfaces

Scott Costa, Vladimir Gouli and Bruce Parker

Entomology Research Laboratory, Department of Plant and Soil Science, University of Vermont, Burlington, Vermont 05405

The ability to reliably quantify the deposition microbes applied to a leaf surface is a critical step in the development of microbial control strategies. For example, in our work on entomopathogenic fungi we needed to determine how spray technology affects the quantity and distribution of spores on a plant. We compared a scotch tape method, which we have improved and standardized, with two other common methods, leaf washing and leaf imprinting, for quantifying deposition of *Metarhizium anisopliae* (GA-157, UVM-ERL). With the scotch tape method the spores are transferred from the leaf surface by a series of steps and then fixed for microscopic examination. With the other methods the spores are transferred volumetrically to selective media (solid) either by washing or direct contact (pressing) and the colonies that grow are counted. We conducted two separate studies: 1) the use of tape on poinsettia (Po), chrysanthemum (Ch), broccoli (Br), and bean (Be), 2) the use of the three spore count methods on the four plants listed above. For each study there were three experimental runs and data were collected from the lower and upper leaf surface of three plants per plant type. Data were analyzed in SAS using the general linear model of analysis of variance and P-diff ($\alpha = 0.05$). In the first study, there were no significant differences among plants in the total number of spores recovered using tape (Po 157.1 ± 11.5, Ch 141.3 ± 9.4, Br 125.7 ± 11.4, Be 138.8 ± 7.9) – direct counts on microscope slides used as a control found 250 (± 5.6) spores. In the second study, the total number of spores recovered with tape (140.2 ± 5.3) was more than 10 fold higher than with either the wash (12.6 ± 1.2) or print (7.1 ± 1.1) methods, and plant species again had no significant influence. The leaf surface (upper or lower) examined was not significant factor. These finding indicate that the scotch tape method is superior for enumerating the number of fungal spores deposited on a leaf surface. Our research has also found it to be effective with *Beauveria bassiana*, viral occlusion of NPV (Baculoviridae, subgroup A) and microsporidian spores of *Nosema* species.

CONTRIBUTED PAPER - Thursday, 11:45 (Viruses IV)

Isolation of a *Spodoptera exigua* baculovirus recombinant with improved biological activity against the beet army worm

Xiaojiang Dai[1,2], Monique M. van Oers[1], Nina N. Joosten[1], Wilfred F.J. IJkel[1], József P. Hajós[1], Douwe Zuidema[1], Yi Pang[2] and Just M. Vlak[1]

Laboratory of Virology[1], Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, and State Key Laboratory for Biocontrol[2] and Institute of Entomology, Zhongshan University, Guangzhou 510275, P.R. China

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) is a successful commercial pesticide to control the beet army worm in a variety of crops. However, attempts are made to improve the insecticidal characteristics of this virus either by in vivo selection of superior strains / genotypes or by genetic engineering. Selection so far has not yielded improved strains of SeMNPV although the potential has not yet been fully exploited. Until now engineering of the virus was hampered by the occurrence of defective viruses, when SeMNPV was grown in cell culture, a common procedure in the engineering process. Moreover, these defective viruses were unable to infect insects anymore. Here, we report the successful engineering of SeMNPV by alternate successive cloning of recombinants in vivo and in vitro. Recombinants were generated in insects after lipofection of the viral DNA and a transfer vector containing the green fluorescent protein (GFP) as a marker into the hemocoel. After a plaque assay in insects cells (Se301) the plaques containing both GFP and polyhedra were directly fed to insect larvae to screen and select for biological activity. The hemolymph of those larvae was also retrieved and the alternate cloning procedure repeated until genetic homogeneity was achieved.

Following this procedure a SeMNPV recombinant (SeXD1) was obtained which could replicate well in cell culture and which retained its biological activity in insects. Surprisingly, this recombinant showed an improved speed of kill (ST_{50}) by about 25% as compared to wild type SeMNPV. The biological activity (LD_{50}) of both types of virus was similar, but insects infected with the recombinant SeXD1 did not liquify. The recombinant lacked about 10.5 kbp of contiguous sequences. The deleted information, located between 13.7 and 21.6 map units of the SeMNPV genome, included the *ecdysteroid UDP glucosyl transferase* (*egt*), *gp37*, *chitinase* and *cathepsin* genes, as well as several other genes unique to SeMNPV. The result indicated, however, that these genes are dispensable for virus replication both *in vitro* and *in vivo*. Moreover, a mutant with a similar deletion as present in SeXD1 was identified by PCR in the parental wild type SeMNPV isolate suggesting that genotypes with differential biological activities exist in field isolates of baculoviruses.

POSTER VP24 - Thursday (Viruses)

Spatial distribution of pathogen explains non-linear LdNPV transmission

Vince D'Amico[1], Joseph Elkinton[2], John Podgwaite[1]

[1]USDA Forest Service, Hamden, CT 06514
[2]University of Massachusetts, Amherst MA 01003

Gypsy moth (*Lymantria dispar* L) larvae become infected with a nucleopolyhedrosis virus by feeding on foliage contaminated with cadavers of viral-killed larvae. Previous work has shown that transmission rates of the virus are non-linear functions of cadaver density. Here we show that the non-linearity is more pronounced when the cadavers are clumped than when they are placed at random on the foliage. Spatial clumping of viral inoculum may account for much of the departure of transmission in this system from the mass action assumption.

POSTER BP6 - Tuesday (Bacteria)

The α-glucosidase in *Culex pipiens* midgut which serves as receptor to *Bacillus sphaericus* binary toxin : cloning and expression

Isabelle Darboux[1], Jean-François Charles[2] and David Pauron[1]

[1]Institut National de la Recherche Agronomique, Unité Santé Végétale et Environnement,
123 Boulevard Francis Meilland, 06606 Antibes Cedex, France
[2]Institut Pasteur, Laboratoire des Bactéries et Champignons Entomopathogènes, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France

Mosquito larval midgut is the primary target of the binary toxin (Bin) present in crystalline inclusions produced by highly-toxic strains of *Bacillus sphaericus* (Nielsen-LeRoux & Charles, 1992; Silva-Filha et al., 1997).

Cpm1, a 60-kDa protein purified from brush border membranes from *Culex pipiens*, has been previously proposed as the receptor of the Bin toxin in the midgut epithelial cells of *C. pipiens* larvae (Silva-Filha et al., 1999).

We have cloned and characterized the corresponding cDNA from midgut of *Culex pipiens* larvae. The open reading frame predicted a 580 amino-acid protein with a putative signal peptide at the N-terminus and a putative GPI-anchoring signal at the C-terminus. The amino acid sequence of the cloned Cpm1 exhibited 39-43% identities with other insect maltases (α-glucosidases and α-amylases). Recombinant Cpm1 expressed in *E. coli* specifically bound to the Bin toxin and had a significant α-glucosidase activity but no α-amylase activity.

These results support the view that Cpm1 is an α-glucosidase expressed in *Culex* midgut where it constitutes the receptor for the Bin toxin. To date, this is the first component involved in the mosquitocidal activity of the *Bacillus sphaericus* Bin toxin to be characterized. Its identification constitutes a key step to elucidate the mode of action of the Bin toxin and the mechanisms of resistance developed against it by some mosquito strains.

Nielsen-LeRoux C & Charles J-F (1992). *Eur. J. Biochem.* 210:585-590.

Silva-Filha MH, Nielsen-LeRoux C & Charles J-F (1997). *Eur. J. Biochem.* 247:754-761.

Silva-Filha MH, Nielsen-LeRoux C & Charles J-F (1999). *Insect Biochem. Molec. Biol.* 29:711-721.

CROSS-DIVISION SYMPOSIUM - Friday, 8:50

Host specificity and virulence in the nematode hyperparasite *Pasteuria penetans*: the role of fibronectin

Keith G. Davies and Sharad Mohan

IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ

Pasteuria penetrans is a spore forming bacterium that binds to the cuticle of plant parasaitic nematodes and has potential as a biological control agent. Studies with different *Pasteuria* spore isolates have shown that those isolated from root-knot nematodes have a preference to attach to juveniles from root-knot nematodes while those spores isolated from cyst nematodes have a preference to attach to cyst nematodes. However, attachment studies using a single population of spores against over 200 root-knot populations showed there was no relationship in the levels of attachment of these spores to root-knot nematode phylogeny. Immunological studies of juvenile cuticles and *Pasteuria* spores have shown them to be highly variable and this variation appears linked to adhesion. Fibronectin is a heterodimer with Mr 440 kDa that mediates the adhesion of Gram-positive bacteria to the extracellular cell matrix. Experiments showed fibronectin to bind to *Pasteuria* spores hydrophobically and to inhibit spore binding. Western blot analysis of cuticle extract from J2s of root-knot nematodes has showed that anti-fibronectin polyclonal and monoclonal antibodies recognise a double band with Mr's 220 kDa and a series of polypeptides with Mr's between 30 – 55 kDa. Proteolytic fragments of fibronectin, one with Mr 30 kDa binds heparin and another with Mr 45 kDa binds gelatin bound to the spores of *Pasteuria* hydrophobically. The gelatin-binding fragment was the most effective at inhibiting spore attachment to *M. javanica* and *M. arenaria* , however, it was not as effective at inhibiting spore attachment to *M. acrita*. It is interesting that the smaller molecules, both gelatin-binding and heparin-binding molecules, were more effective at inhibiting spore attachment than the larger fibronectin molecule.

SYMPOSIUM - Thursday, 9:30 (Microbial Control)

Microbial pesticides - making them count

David Dent

CABI Bioscience, Silwood Park, Buckhurst Road, Ascot SL5 7TA
Email; D.Dent@CABI.ORG

The development, manufacture and use of microbial pesticides in developing countries is characterised by public sector led R&D, low capital based biopesticide companies, difficulties in meeting international standards for product quality, shelf life and safety and lack of farmer education and know-how regarding microbial products. In order to address these issues there is a need to target support in the most effective ways. This could range from large publicly funded product development projects or injections of R&D at appropriate times to an approach based on promoting demand for the products through public funded farmer training or commercial company marketing and farmer education. In many developing countries the opportunities for microbial pesticides are now growing. In order for this growth to be sustained and to meet the needs of developing, manufacturing and selling high performance, safe and environmentally friendly microbial pesticides the options for support of public sector R&D, the industry and the farmers need to be addressed.

POSTER BP7 - Tuesday (Bacteria)

Response of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to the selection with *Bacillus thuringiensis*

Ovidio Diaz-Gomez,[1] J. C. Rodriguez-M.,[2] A. M. Shelton,[3] A. Lagunes-T.,[2] N. M. Barcenas-O.,[2] and R. Alatorre R.[2]

Facultad de Agronomia, U.A.S.L.P. Alvaro Obregon 64, San Luis Potosi, S.L.P. 78000, MEXICO[1]. Inst. de Fitosanidad, C.P. Km 35.5 Carr. Mexico-Texcoco. Montecillo 56230, Edo. de Mexico[2]. Entomology, Cornell Univ. NYSAES, Geneva, NY 14456 U.S.A.[3]

In Mexico, the diamondback moth *Plutella xylostella* (L.) is the major pest of crucifers plants. Its control depends largely on the use of *Bacillus thuringiensis* and organosynthetic insecticide. Therefore, it is important to take measures to prevent the further buildup of resistance. In this research, five populations of *P. xylostella* , collected in three states of Mexico, were selected with *B. thuringiensis* subspecies *kurstaki* (Dipel 2X), *B. thuringiensis* subspecies *aizawai* (Xentari), delta endotoxins Cry1C of *B. thuringiensis* subspecies *aizawai* (MC), and Cry1A(c) of *B. thuringiensis* subspecies *kurstaki* (MVP), and finally, with the mixture of toxins contained in *B. thuringiensis* subspecies *kurstaki* and *aizawai* (Agree). Their responses were evaluated by leaf-dip bioassay. When it was compared a susceptible population with the response of those selected, in four out of five populations were obtained statistically significant differences. The most remarkable cases in resistance were detected to the delta endotoxins Cry1A(c) and Cry1C, and in smaller degree to the complex of toxins contained in the subspecies *kurstaki*. With the subspecies *aizawai* and the mixture of subspecies *kurstaki* and *aizawai* the developed resistance levels were low. This research shows the existence of genes for resistance to *B. thuringiensis* in populations of *P. xylostella* collected in Mexico. This implies a strong risk to the dependence of *B. thuringiensis* for the control of *P. xylostella* , and also, to the introduction of transgenic crucifers if they express a single delta endotoxin, mainly if appropriate strategies of management of the resistance are not implemented. Taking account these results and in order to ensure the continued performance of *B. thuringiensis* in field, we suggest that a resistance monitoring program be implemented to detect any changes in susceptibility to *B. thuringiensis* products and specific toxins; that their use be restricted to one generation per crop and that they be rotated with other groups of insecticides and incorporated in a integrated pest management program of cole crops.

CONTRIBUTED PAPER - Monday, 12:30 (Viruses I)

Expression analysis and promoter characterization of a cluster of genes from CfMNPV transcribed late in viral infection.

Clifford N. Dominy [1], Basif M. Arif [2] and Peter J. Krell [1]

[1]Department of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
[2]Great Lakes Forestry Research Centre, Sault Ste Marie, Ontario, P6A 5M7Canada.

Transcription analysis was conducted on a cluster of genes from *Choristoneura fumiferana* nucleopolyhedrovirus (CfMNPV) which were predicted to be expressed late in viral infection. The cluster was highly conserved among Group I nucleopolyhedroviruses, and consisted of the p24, gp16, calyx/pep, an unassigned ORF (ORF4) and the alk-exo genes. The CfMNPV locus and the encoded proteins from its genes were most closely related to the same locus in the genome of OpMNPV. DNA slot blot hybridization analysis showed that initiation of DNA replication occurred between 18 and 24 hpi in a Cf203 cell line infected at an moi of 10. Northern analysis of the alk-exo gene revealed that the gene cluster may be transcribed as two independent 3' coterminal nested sets unlike the corresponding genes in AcMNPV and OpMNPV. RT-PCR analysis revealed that four of the five genes were expressed after the initiation of viral DNA replication at the same time point as expression of gp64, a previously characterized "late" gene. The only exception was the ORF4 gene which was expressed at early (12 hpi), late (24 hpi) and very late (48 hpi) times post infection. Primer extension experiments confirmed these results and identified the 5' termini of the transcribed mRNA. Genes within the first nested set (p24, gp16 and calyx) were initiated from within the canonical PuTAAG late promoter consensus sequence, whilst genes from the second nested set were initiated from sites upstream of the canonical early and late transcription initiation signals. ORFs flanking the cluster were also examined for transcriptional activity. Messenger RNA transcripts for two small ORFs upstream of the cluster were not detected under the conditions used and might be spurious. Genes immediately downstream of the cluster (Cf-R1 and p26) were expressed early in infection, from sites +11 and -2 nucleotides from the respective early initiation signals. Cf-R1 encoding a 202 amino acid protein and is specific to two *Choristoneura fumiferana*-infecting nucleopolyhedroviruses (CfMNPV and CfDEF) suggesting a potential involvement in virus-host interactions.

POSTER BP8 - Tuesday (Bacteria)

Gene deletion indicates that Vip3A is required for the *Bacillus thuringiensis* spore effect against *Spodoptera exigua*

William P. Donovan, Judith C. Donovan and James T. Engleman

Ecogen Inc. Langhorne, PA 19047

The *B. thuringiensis* spore effect refers to the phenomenon whereby the addition of *B. thuringiensis* spores to purified crystal proteins results in increased insecticidal activity compared with purified proteins alone. To investigate this spore effect we used gene engineering to delete the vip3A gene encoding a lepidopteran-toxic protein that is secreted by *B. thuringiensis* during vegetative growth (Estruch et al. Proc. Natl. Acad. Sci 1996 93:5389). PCR was used to delete 1.6 kb of DNA from the interior of the 2.4 kb vip3A gene and the deleted gene was transferred into *B. thuringiensis* kurstaki strain HD1 by homologous recombination resulting in strain HD1Δvip3A. Strains HD1 and HD1Δvip3A produced similar levels of Cry1 and Cry2 proteins, and similar levels of heat-resistant spores. The supernatants of vegetative cultures of strains HD1 and HD1Δvip3A contained identical arrays of soluble proteins with the exception that HD1Δvip3A did not produce a 90 kDa Vip3A-size protein that was produced by HD1. Compared with HD1 strain HD1Δvip3A was slightly less toxic to *Trichoplusia ni* and *Heliothis virescens*, 1/4th as toxic to *Agrotis ipsilon*, and 1/20th as toxic to *S. exigua*. When streptomycin was included in the insect bioassay to inhibit growth of *B. thuringiensis* the toxicity of HD1Δvip3A was approximately half that of HD1 against *S. exigua*. These results suggest that the spore effect requires the synthesis of Vip3A by *B. thuringiensis* cells after ingestion of spores and crystal proteins by *S. exigua* larvae.

CONTRIBUTED PAPER - Monday, 12:30 (Fungi I)

Horizontal Transmission of *Beauveria bassiana* in the Colorado potato beetle

Francis A. Drummond[1], Eleanor Groden[1], and David Long[2]

Department of Biological Sciences[1], University of Maine, Orono, Maine, USA 04469 & 231 Salem Street[2], Lynnfield, MA 01940-2327

Factors influencing horizontal transmission of the entomopathogen, *Beauveria bassiana*, in the Colorado potato beetle were examined through a series of laboratory studies. Cadaver density, cadaver life stage, ambient temperature and conidial density were the factors manipulated. Mortality and sporulation of burrowing CPB prepupae both increased significantly with increased sporulating second instar cadaver density on the soil surface. Mortality and sporulation decreased significantly as temperature increased from 15°C to 30°C. An 86.1% decrease in conidial density per cadaver also had no significant effect on mortality or sporulation of prepupae indicating that this level of environmental degradation of cadavers may not significantly reduce the probability of horizontal transmission. We modeled horizontal transmission of *Beauveria bassiana* in Colorado potato beetle (CPB) between larval cadavers and soil inhabiting prepupae. The rate of disease transmission, based upon the probability of a prepupa contacting sporulating cadavers on the soil surface, is a nonlinear function of cadaver density and also dependent upon temperature. Observational field studies determined Johnson distributions to model the spatial pattern of cadavers and prepupae in the field. Potential for horizontal transmission is higher in simulations using weather data from the warmer year of 1995, than in simulations of the cooler growing season of 1993. Simulations of CPB populations under northern Maine climatic conditions recorded in 1993 and 1995 suggest that horizontal transmission can range from 3 - 24% depending upon the timing of primary infection of larvae in the field. Two simulated sequential applications of *B. bassiana* targeted at peak first instars resulted in maximum horizontal infection in both years. Sensitivity analysis suggests that horizontal transmission is most sensitive to changes in the proportion of cadavers that sporulate and least sensitive to changes in the time between larval death and the onset of cadaver sporulation. Field validation of the model indicates good prediction of one measure of horizontal infection, the proportion of prepupae which eventually sporulated after being released in controlled field experiments.

SYMPOSIUM II - Monday, 16:50 (Viruses)

Using Mathematical Models To Understand Disease Epizootics in Insects

Greg Dwyer

Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago IL 60637.

An important feature of disease epizootics is that a small number of infected hosts in a dense host population can lead to a high infection rate quite rapidly. Understanding this kind of nonlinear disease dynamic is difficult without mathematical models. Traditionally, however, mathematical disease models have been used only to provide qualitative explanations rather than to analyze data. My research in contrast has focussed on the practical uses of mathematical models in understanding epidemic data. In particular, I have shown that simple epidemic models can be combined with data from field transmission experiments to predict the timing and intensity of NPV epizootics in populations of the gypsy moth, Lymantria dispar. Similar models have likewise been useful in understanding epidemics of the gypsy moth fungal pathogen Entomophaga maimaiga, and in assessing the effects of a genetically engineered NPV in populations of Trichoplusia ni.

POSTER PP5 - Tuesday (Protozoa)

Exposure to trypanosomatids slows development in *Drosophila* hosts

Mercedes A. Ebbert, Jennifer J. Burkholder and Jennifer L. Marlowe

Department of Zoology, Miami University, Oxford, OH, 45056, USA.

Monoxenous trypanosomatids are widespread gut endosymbionts in Diptera and are often thought to have little, if any, detrimental effects on their hosts. Because trypanosomatid infections are common in our collections of local *Drosophila* , we investigated whether host fitness is affected by this interaction. We found that larvae allowed to feed on infected adults were significantly slower to pupate, eclose and reproduce when compared to larvae fed on uninfected adults. We concluded that the consequences of infection to these short lived hosts are negative, and continue to explore how this interaction phenotype varies with changing laboratory and field conditions.

SYMPOSIUM III - Tuesday, 17:10 (Bacteria)

Membrane pore architecture of a cytolytic toxin from *Bacillus thuringiensis*

David J. Ellar, and Boonhiang Promdonkoy

Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, CB2 1GA, UK

Bacillus thuringiensis subspecies *kyushuensis* synthesises a cytolytic mosquitocidal protein toxin, Cyt2Aa1.To obtain information about residues important in the pore-forming processes, second-site revertants of an inactive mutant toxin (I150A) were generated by random mutagenesis using error-prone PCR. Analysis of 28 revertants revealed that residues on αA, αB, αC, αD and loops between αA and αB, αD and on β5, β6 and β7 play an important role in the pore forming processes. In contrast, substitution of residues shown by X-ray crystallography to be buried inside the molecule had little or no effect on toxin activity.

To study the pore architecture, 14 residues from selected regions of Cyt2Aa1 were changed to cysteine to create modifiable sites in the molecule. The 5 modifiable mutants were labelled with polarity-sensitive acryloyl-dimethylaminonaphthalene (acrylodan). Spectrofluorimetry of the acrylodan-labelled mutants showed that all of them caused a green-blue shift in the emission spectrum on transfer from hydrophilic to hydrophobic media. Incubation of the membrane-bound labelled toxins with proteinase K did not cause significant decrease in the emission intensity from any mutant. These results suggested that β5, β6 and β7 insert into the hydrophobic zone of the membrane and therefore that the pore may be formed as a β-barrel.

Haemolysis assays of the wild type toxin showed a steep and sigmoidal dose response curve indicating that aggregation of toxin molecules is required before effective pores are formed. The presence of a lag time for haemolysis when using high toxin concentrations suggests that oligomerisation is required before a pore can be formed. Studies of the effect of temperature on pore formation revealed that the toxin can bind to the membrane at low temperature but oligomerisation is inhibited. The membrane-bound toxin cannot diffuse laterally so that the pore is formed only when a sufficient number of toxin molecules bind close together. Fluorimetric studies of a low activity mutant L189C revealed that the toxin can insert into the membrane even when used at very low concentrations. This result suggests that residue L189 plays an important role in stabilising the pore structure. Analysis of the interaction between the wild type and the inactive mutant I150A suggested that the pore comprises 6 or a multiple of 6 molecules. From the results in this study it is estimated that 18 toxin molecules may be required to form a pore.

POSTER FP15 - Thursday (Fungi)

Epizootiology of the mite-pathogenic fungus *Neozygites floridana* in the cassava green mite in north-eastern Brazil.

Sam L. Elliot[1], Gilberto J. de Moraes[2] and John D. Mumford[3]

[1]Section Population Biology, Univ. Amsterdam, Postbus 94084, 1090 GB Amsterdam. [2]Deptº Zoologia, Escola Superior de Agricultura, ESALQ/USP, 13418-900 Piracicaba, SP, Brazil. [3] T H Huxley School of Environment, Earth Sciences and Engineering, Imperial College, London SW7 1NA.

Monitoring of a natural epizootic of *Neozygites floridana* in a Brazilian population of cassava green mite (CGM), *Mononychellus tanajoa*, yielded some information of import to the use of natural enemies for biocontrol. In conjunction with predatory mites (Acari: Phytoseidae) in one field, the fungal pathogen appeared to keep the CGM population in check in July and August (1996) but as conditions warmed and the epizootic waned, the pest population began to increase. In a neighbouring field in which the pathogen occurred at very low levels, predatory mites, despite being very abundant, were unable to control the CGM population, leading to total leaf loss and a population crash.

From these data, a simple regression model was developed estimating host mortality on the basis of inoculum (dead, mummified CGM) and saturation deficit minima, to assess the epizootic potential under different conditions. This model was used to estimate the potential for the use of artificial introductions of the pathogen in the second of the fields. Three narrow windows of opportunity were identified in which an artificial epizootic might have been established, two coinciding with rising CGM levels. Concurrent field tests of transmission, however, showed only one period when transmission between mites might have occurred, and this was after leaf loss.

Further observed features were the near-absence of resting spores in mummified mites (ca. 0.1% of cadavers) and the low pick-up of capilliconidia of the fungus by juvenile stages of the mite, relative to adults.

We conclude that the fungus may be a useful adjunct to biological control with predatory mites. We advise caution, however, with introductions, as the windows of opportunity are narrow so repeat applications will probably be necessary (especially as juveniles appear behaviourally invulnerable), and resting spore formation may not occur, compromising establishment of the fungus.

CONTRIBUTED PAPER - Friday, 8:30 (Microbial Control II)

Can plants use entomopathogens as bodyguards?

Sam L. Elliot[1]*, Maurice W. Sabelis[1], Arne Janssen[1], Leo P.S. van der Geest[1], Ellen A.M. Beerling[1,2] and Joanne Fransen[2]

[1]Section Population Biology, Univ. of Amsterdam, Postbus 94084, 1090 GB Amsterdam, The Netherlands. E-mail elliot@bio.uva.nl. [2]Research Station for Floriculture and Glasshouse Vegetables, Linnaeuslaan 2a, 1431 JV Aalsmeer, The Netherlands.

For nearly thirty years, ecologists have been gathering evidence in support of the hypothesis that plants can use insect natural enemies such as predators and parasitoids as bodyguards to protect themselves from herbivory, but entomopathogens have escaped this consideration. We extend the bodyguard hypothesis to ask whether plants can use entomopathogens as bodyguards. We first discuss the evolutionary context of such tritrophic interactions and then categorise possible mechanisms as: (1) maintaining a population of bodyguards on the plant surface, (2) increasing contact rates between insect host and pathogen and (3) increasing the susceptibility of the host. We explore these mechanisms further, examining published studies for evidence for the hypothesis. We then discuss potential costs to the plant of promoting pathogens as bodyguards which may include a reduction in the efficiency of other "bodyguard" species, the incidental promotion of plant pathogens and the risk of entomopathogens developing phytopathogenicity. Aside from our intention to stimulate the testing of the bodyguard hypothesis with entompathogens and to provide a conceptual framework for this, we hope to bring evolutionary ecology and insect pathology closer together.

POSTER VP25 - Thursday (Viruses)

Prolongation of the UV-persistence of nucleopolyhedroviruses by the Lignin Derivative product

El-Salamouny S., El-Sheikh M.A.K., Elnagar S. and *Huber J.

Department of Economic Entomology and Pesticides,Faculty of Agriculture, Cairo University, Cairo, Egypt and *Institute for Biological Control (BBA), Heinrichstr.243 64287 Darmstadt, Germany.

It is generally accepted that protection of viral pesticides against UV radiation of the sun is most essential. Four materials were tested as UV protectants to increase the field persistence of polyhedrosis viruses: Fluorescent Brightener 28 (SIGMA) (=Calcofluor white M2R), Congo red, molasses and the lignin derivative product **(**Zschiegner, Leipzig, Germany**) (** Joint Patent).

Dry deposits of *Autographa californica* MNPV (AcMNPV), *Spodoptera littoralis* MNPV (SlMNPV)**,** and *S. exigua* MNPV (SeMNPV) were irradiated with artificial UV light from four Ultra-Vitalux lamps (OSRAM). The viruses were tested, in bioassays, against neonate larvae of their homologous hosts (except for AcMNPV, where neonate larvae of *A. gamma* were used). The results showed no significant differences between virus without protection and that with 15% molasses, 1% Congo red or 0.1% fluorescent brightener added. The Congo red material, at a concentration of 5%, seemed to even enhance the inactivation process. Contrary to these results, the lignin derivative showed a high protecting effect when used as an additive. The computed half-life values for SlMNPV were : 74.5, 10.3 and 2306 minutes with untreated virus, Congo red and the legnin 1%, respectively. Using the SeMNPV, the half-life values were 130.8, 153.9 and 1385 minutes for the same treatments, , respectively.

The present study suggests the importance of the lignin derivative as an UV protectant to increase the field persistence of viral insecticides. Further study is initiated to confirm the promising results, testing other lignin derivatives as well as undertaking field tests.

CONTRIBUTED PAPER - Monday, 11:30 (Bacteria I)

Identification of a *Bacillus thuringiensis* ECF (extracytoplasmic function) sigma factor gene involved in ß-exotoxin production and/or secretion

Sylvain Espinasse[1], Michel Gohar[1,2], Didier Lereclus[1,3] and Vincent Sanchis[1,3]

Unité de Lutte Biologique[1], INRA La Minière, 78285 Guyancourt Cedex, France ; Aventis Crop Science[2], Jozef Plateaustraat 22–B 9000 Gent, Belgium & Unité de Biochimie Microbienne[3], Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

In addition to the well known insecticidal -endotoxins (Cry proteins), several *Bacillus thuringiensis* (*Bt)* strains also produce a non proteinaceous thermostable exotoxin, also referred to as ß-exotoxin, that is excreted into the culture medium. This compound is an adenine nucleotide analogue highly toxic for a wide range of insect species ; its toxicity is due to inhibition of DNA-dependent RNA polymerase by competition with ATP. Due to its non-specific activity, and because mammalian RNA polymerases are quite sensitive to ß-exotoxin,

insecticidal preparations containing this material are generally not authorized. ß-exotoxin production can vary dramatically between strains and its detection and quantification is not straightforward. Very little is known about ß-exotoxin biosynthesis and the genes that control its expression and secretion are so far unreported. We found that a mutant *Bt* strain, initially isolated by O. Arantes, was able to secrete large amounts of ß-exotoxin (>100µg/ml) in the same time as it was producing a dark brown soluble pigment (melanin). Designated 407-Pig+, with respect to the pigment, this mutant is a derivative of *Bt* strain 407- (H1 serotype), a strain that neither produces ß-exotoxin (Etx-) or pigment (Pig-) in standard laboratory growth conditions. In the present study, a library of random mutants of strain 407-Pig+ was constructed, using a mini-Tn*10* transposon delivery system, and screened for variants affected in the production of both the pigment (white colonies) and ß-exotoxin (non toxic culture filtrates). One clone exhibiting a Pig-Etx- phenotype was further characterized. Sequencing of the region in which the mini-Tn*10* was inserted revealed the existence, downstream of the insertion locus, of a putative 3 genes operon. The first open reading frame shared similarity (approximately 45 to 50% similarity and 30% identity) with sigma factors of the ECF family ; this similarity was most pronounced with ECF sigma factors SigX and SigW of *Bacillus subtilis*. ECF sigma factors are believed to be activated to transcribe their regulons in response to a change in environmental conditions. Disruption of this putative ECF sigma factor gene in strain 407-Pig+ resulted in the absence of ß-exotoxin secretion but did not affect pigment production, indicating that the synthesis of ß-exotoxin or its secretion was positively controlled by the product of this gene. Expression of its promoter region fused to a *lacZ* reporter gene indicated that the expression of this gene was positively autoregulated as is the case for *sigX* and *sigW* in *B. subtilis*. Experiments are currently underway to identify the transcriptional start point and size of the transcript of this putative operon and to investigate the role and importance of the downstream ORFs in the regulation of its expression.

SYMPOSIUM I - Monday, 14:00 (Bacteria)

Role of Cyt1A in Managing Resistance to Mosquitocidal Proteins of *Bacillus thuringiensis* and *Bacillus sphaericus*

B. A. Federici[1,2,3], M. C. Wirth[1], D. K. Bideshi[1,2], H. W. Park[1], and W. E. Walton[1]

[1]Department of Entomology and Interdepartmental Graduate Programs in [2]Genetics & [3]Microbiology, University of California, Riverside Riverside, California 92521

Studies of resistance management for chemical insecticides have shown that resistance can be overcome or delayed by using combinations of different chemicals, especially if each chemical has a different mode of action. In mosquitocidal bacteria such as *B. thuringiensis* subsp. *israelensis* (Bti), the PG-14 isolate of *B. thuringiensis* subsp. *morrisoni* (Btm) and *B. thuringiensis* subsp. *jegathesan* (Btj), similar strategies have evolved in that these consist of multiple Cry toxins in combination with one or more Cyt proteins. Moreover, Cyt1A synergizes the toxicity of mosquitocidal Cry proteins. Commercial formulations of Bti have been in use for more than 20 years, yet no resistance has been reported in field populations of mosquito or blackfly larvae. In laboratory studies of recombinant Bt strains that produce mosquitocidal Cry proteins with or without Cyt1A, we have shown that this protein can delay resistance to Cry proteins in *Culex quinquefasciatus* as well as overcome resistance to these should it develop. In addition, in similar studies we have shown that Cyt1A can synergize the toxicity of the Bs 2362 binary toxin, and can be used to suppress very high levels of resistance to this toxin in populations of *Cx. quinquefasciatus*. Unlike Bti, resistance has already been reported to formulations of Bs under field conditions, in essence because this protein combination acts as a single toxin. The Bs 2362 binary toxin has a different mode of action than mosquitocidal Cry proteins. Thus by combining these two types of toxins in different ratios with Cyt1A, we have been able to produce Bt/Bs recombinants that have improved toxicity in combination with the potential for delaying resistance to both Cry toxins and the Bs binary toxin. In this paper, the development of these new mosquitocidal bacteria and their properties will be reviewed along with their potential use in operational vector control programs.

SYMPOSIUM IV - Friday, 11:45 (Bacteria)

Markedly improved bacterial insecticides for vectors by combining Bti and Bs proteins

B. A. Federici[1,2,3], D. K. Bideshi[1,2], H. W. Park[1], M. C. Wirth[1], W. E. Walton[1], and J. J. Johnson[1]

[1]Deparatment of Entomology and Interdepartmental Graduate Programs in [2]Genetics & [3]Microbiology, University of California, Riverside, CA 92521 USA

Insecticidal bacteria are being used increasingly as alternatives t chemical insecticides for controlling the mosquito vectors of malaria, filariasis, dengue, and the viral encaphalitides. Two bacteria, *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Bacillus sphaericus* (Bs), are used commonly in temperate and subtropical regions, but high costs limit their use in the tropics. Moreover, resistance to Bs has been reported in field populations of *Culex* mosquitoes in tropical regions of India and Brazil where it is used. Alternatively, Bti has been used extensively in some vector control programs without the development of resistance. The insecticidal properties of Bti and Bs are due to protein endotoxins; Bti produces a complex of Cyt and Cry endotoxins, whereas Bs only produces a single binary toxin. The lack of resistance to Bti has been attributed to its multiplicity of Cry proteins in combination with the Cyt protein. Previous attempts to improve insecticidal efficacy by combining Bti and Bs endotoxins have met with moderate success, achieving increases in efficacy of 2 or 3-fold. By using a novel recombinant expression, we have amplified the synthesis of the Bs binary toxin in Bti by approximately 8-fold. The recombinant bacterium, which produced the normal complement of Bti endotoxins in combination with the Bs binary toxin, was 15-fold more toxic to *Culex* mosquitoes than either parental bacterium. Moreover, it overcame high levels of resistance to Bs in mosquitoes. This recombinant bacterium combines remarkably high insecticidal activity with the capacity to substantially reduce the propensity for resistance. These properties should result in its rapid adoption worldwide for the control of vector and nuisance mosquitoes.

STUDENT POSTER FP12 - Tuesday (Fungi)

Evaluation of isolates of the fungus *Paecilomyces fumosoroseus* with relation to their physiology and effectiveness in the control of *Frankliniella occidentalis*

Figueroa[1], L.M., A. Varela[1] & D. Corredor[2].

[1]Depto. de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana. [2]Facultad de Agronomía, Universidad Nacional de Colombia. Santafé de Bogotá, D.C., Colombia.

F. occidentalis (Trips) causes large economic losses for flowers farmers in Colombia. The control of this pest solely involves the use of chemicals. An alternative less harmful for the environment could be the use of the fungus ***P. fumosoroseus***. In the present study, two isolates of this fungus were evaluated with respect to some physiological characteristics (growth rate, half time of germination (TG_{50}), sporulation rate and its compatibility with some chemical pesticides) and its efficacy to control ***F. occidentalis*** in lab and field tests. The imported and the native isolates were cultured in Sabouraud Dextrose Agar + yeast extract (SDAY) and incubated at 25±1ºC during 3-10 days. These cultures were used to evaluate the physiological characteristics. The isolates were also cultured in a natural standardized culture media for its massive production, to perform the lab and field tests. It was established a breeding of ***F. occidentalis*** for the bioassays, in chambers with Chrysanthemum flowers as a food source. The bioassays was performed in flasks with different concentrations of fungal conidia for establishing the half lethal concentration (LC_{50}). For the field test, an inoculum of 1 x 10^6 conidia/ml of each one of the isolates was applied in three different weeks, in beds of a Chrysanthemum crop. In the crop, mature and immature alive insects were monitored from the flowers and from blue traps, every week during a month, before harvest. The isolates were significantly different ($p<0,01$) for all the physiological characteristics, but in general terms, the imported isolate was superior to the native, except for the growth rate. The growth of both isolates were less inhibited by insecticides than by fungicides and no growth was detected in the presence of four fungicides. The CL_{50} of imported isolate was $1x10^7$ conidia/ml against $8x10^8$ for the native one, which implies differences

with lab control: 60% and 50% of insect mortality from the imported and native isolates, respectively. In the field test, there were no significant differences ($p>0,05$) between isolates in the percentage of control of ***F. occidentalis***; however, there were differences ($p<0,05$) with the control, which suggests a little level of control in the field. It is proposed to examine other native isolates and to improve them for a better level of control in the field.

STUDENT PAPER - Monday, 11:30 (Fungi I)

Abiotic factors influencing resting spores of the forest tent caterpillar pathogen *Furia crustosa* (Zygomycetes: Entomophthorales)

Melanie J. Filotas and Ann E. Hajek

Department of Entomology
Cornell University, Ithaca, NY 14853

The activity of resting spores produced by many species of entomophthoralean fungi is critical to their ability to initiate epizootics after periods of host scarcity. Understanding how abiotic environmental conditions influence this activity can help in predicting when epizootics will occur. *Furia crustosa* is an important but little known entomophthoralean pathogen of the forest tent caterpillar (FTC), a pest of North American hardwood forests. Maximizing resting spore activity is particularly important to the initiation of *F. crustosa* epizootics, as FTC larvae predominantly remain in their natal tree and do not encounter soil-dwelling resting spores until they disperse during the fourth instar. In the present study, we investigated the influence of temperature and soil moisture on infection of forest tent caterpillars by germ conidia produced from *F. crustosa* resting spores and on subsequent fungal reproduction within hosts. FTC larvae were exposed to soil containing *F. crustosa* resting spores under nine moisture-by-temperature treatments (all combinations of 70%, 95% and 130% soil moisture and 15°, 18° and 23°C), selected to approximate the range of ambient abiotic conditions present during the early spring period when host and pathogen are active. Temperature was significantly associated with both host mortality and spore production while moisture was never important. Host mortality remained the same at 15° and 18°C, but deaths due to *F. crustosa* were almost halved at 23°C ($p<0.03$). The percentage of cadavers producing conidia was negatively associated with temperature ($p<0.0002$), declining from 70% at 15°C to 27% at 23°C. In contrast, fewer cadavers at 15°C produced resting spores than at either of the higher temperatures ($p<0.002$). *F. crustosa*, therefore, maximizes infection and dispersal to new hosts at the cooler temperatures occurring when its host, an early spring insect, is active and shifts to producing long-lived resting spores when temperatures increase in late spring and hosts pupate. We were surprised to find that host infections initiated by *F. crustosa* germ conidia could result in the production of resting spores in cadavers. Previous studies of other entomophthoralean fungi have shown that infections initiated by germ conidia yield only conidia.

POSTER VP33 - Thursday (Viruses)

***Cydia pomonella* granulovirus: Lethal time of neonate codling moth varies with temperature**

Eva Fritsch[1], Karin Undorf-Spahn[1], Susanne B. Steineke[2], Jürg Huber[1] and Johannes A. Jehle[2]

[1] Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Pest Control, Heinrichstr. 243, 64287 Darmstadt, Germany
[2] State Education and Research Centre Neustadt/Weinstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany

Lethal time is an important measure in the efficacy of a baculovirus as insect control agent. We determined the lethal times at different temperatures to describe the activity of *Cydia pomonella* granulovirus (CpGV) more accurately. *Cydia pomonella* neonates were placed on artificial medium containing 5000 occlusion bodies per ml (this is the equivalent of a LC_{80}). Larvae were incubated at each of the following five temperatures: 19°C, 22°C, 26°C, 28°C and 32°C. Independent observations warranted a probit analysis to determine the LT_{50}. The lethal time followed an optimum curve. At 19°C the LT_{50} took 19 days and 7 days at 28°C. Even though the insects developed very rapidly at 32°C, only 45% died of virus by the end of the experiment. It appeared that the virus replication was inhibited at this temperature. To better understand the correlation between lethal time and temperature, we also noted the length of the larval stages at different temperatures. There may be practical implications of these findings. In field studies the decreased insecticidal effect of CpGV is mostly attributed to physical inactivation caused by the UV radiation. Our experiments suggest that higher temperatures which are also correlated to higher intensity sunshine, may place an additional constraint on the efficacy of the virus. With our data it would be interesting to find out what component of this loss of effect is caused by the lowered physiological activity of CpGV.

POSTER VP3 - Tuesday (Viruses)

Midgut cell cultures from *Pseudaletia unipuncta* and *Trichoplusia ni* larvae for baculovirus studies.

J. Garcia, J. Zhong, G. Li, P. Wang and R.R. Granados

Boyce Thompson Institute for Plant Research, Cornell University Ithaca, NY 14853

Midgut epithelial cells were isolated from mid-fourth and fifth instar *Trichoplusia ni* and *Pseudaletia unipuncta* larvae, respectively, by collagenase treatment of midgut tissues, and cultured in TNMFH medium. The primary cultured cells remained viable for several days and were suitable for binding and fusion studies with the *Autographa californica* nucleopolyhedrovirus (AcMNPV). Long term continuous culture and maintenance of midgut cells was achieved with *P. unipuncta* armyworm intestinal cells. Several cell lines were obtained from these armyworm primary cultures and have been subcultured and maintained for over 18 months. The three major cell types are present in cultures, including stem cells (regenerative cells), columnar, and goblet cells. Morphogenesis of columnar and goblet cells from stem cells was followed in vitro. There appears to be a cycle of apoptosis of goblet and columnar cells and their replacement by stem cells. After approximately six passages, the cell density in T-flasks appeared to be somewhat constant, reaching 10^3 and 10^4 cells per ml of medium. The columnar cells are round to rectangular in shape and possess a brush border while the goblet cells have a classic flask-like shape with a central cavity. Peritrophic membrane-like secretions were observed in the culture flasks. Infection of these cells with AcMNPV was confirmed and we conclude that these midgut cells can be used as an *in vitro* model system to study early events in baculovirus infection.

STUDENT PAPER - Monday, 12:15 (Fungi I)

Toxicological assessment of *Beauveria bassiana* against Mexican bean beetle

García-Gutiérrez[1], H. Medrano-Roldán[2] and V. Hernández-Velázquez[3].

[1]CIIDIR-IPN Unidad Durango, Sigma s/n Fracc. 20 de Nov. II 34220 Durango, Mex. Tel y Fax: 01 (18) 14-20-91
[2] Instituto Tecnológico de Durango, Felipe Pescador 1830 Ote. Tel . y Fax: 01 (18) 18-69-36
[3]Centro Nacional de Referencia de Control Biológico. Tecomán Colima, 28120. Tel y fax (332) 4-07-45

In Mexico, the Mexican bean beetle MBB, *Epilachna varivestis* (Mulsant) Coleoptera: Coccinellidae, is a pest that defoliates soybean plants. Natural control of MBB by the parasitc wasps *Pediobius foveolatus* (Crawford), *Aplomyiopsis epilachnae*(Aldrich) and *Perilus bioculatus* (Fabricius) is sporadic in Mexico. Control of this pest using entomopathogen fungi such as *Beauveria bassiana* (Bals) has not been studied in depth. In Mexico, reports have been published about isolates with pathogenicity for insects of economical importance such as *G. senilis*., *Phyllophaga* sp., *Diabrotica* sp., *D. balteata* ., and *H. hampei.* For *E. varivestis*, basic information regarding toxicity of *B. bassiana* is not available. Consecuently, this study was conducted to evaluate the toxicity of ten isolates of *B. bassiana* against *E. varivestis* and to detect the sporulation time of the fungi. Spore toxicity of ten *Beauveria bassiana* strains, grown on SDA medium at 27 ± 2^0C, and 80% RH, were evaluated

against 3-day old larvae of *Epilachna varivestis* (Mulsant). Bioassays, using a dipped bean leaf assay, at ten doses between 1.23 and $1.27x10^7$ spores/ml were performed to evaluate toxicological activity. Treatments were incubated at 25± 2^0C, 60% RH, and 12:12 (L:D) per 72 h. All strains of *B. bassiana* showed toxicity to *E. varivestis*. A strain isolated from *Diabrotica* sp. showed the highest toxicity with 96.6% mortality, while isolates from *Geraeus senilis, Diabrotica balteata , Hippodamia convergens*, and *Hypothenemus hampei* resulted in mortalities of 93, 90, 90, and 86.6%, respectively; strains isolated from Curculionidae showed mortalities of 86.5 and 80%. The lowest mortality was observed for strains isolated from *Sphenarium* sp. at 76.6%, *Oebalus mexicanus* at 70%, and *Cydia pomonella* at 66%. The isolates from coleopterous insects showed higher toxicity than isolates from other orders. The time required for emergence of fungus from infected insects was 24 hours. *Diabrotica* sp., *G. senilis*, and *D. balteata* strains sporulated from over 90% of infected insects, while the strain from *C. pomonella* sporulated from only 67% of those infected.

POSTER FP21 - Thursday (Fungi)

Beauveria bassiana and _Metarhizium anisoplae_ Isolations Virulence Against _Phyllophaga crinita_ (Coleoptera: Melolonthidae) Larvae in Laboratory

Garcia-Martinez, M.[1], M. Najera-Rincon[2], Robert L. Crocker[1], V. Hernandez-Velazquez[3], and L. A. Rodriguez del Bosque[4]

[1]Texas Agricultural Experimental Station, Texas A&M University Research & Extension Center, 17360 Coit Road, Dallas, Texas, U.S.A.
[2]National Research Center for Sustainable Production,
[3]National Reference Center for Biological Control,
[4]North East Regional Research Center.

Three *Metarhizium anisopliae* (Metschnikof) (Sorokin), and one *Beauveria bassiana* (Balsamo) isolations from western Mexico were evaluated as potential biological control agents against *Phyllophaga crinita* (Burm) (Coleoptera: Melolonthidae) third stage larvae. That insect is an economically important pest in Southeastern United States, and Northeastern Mexico. Entomopathological fungi were provided by the National Reference Center for Biological Control Collection (CNRCB-DGSV-SAGAR-Mexico) through an international cooperative project coordinated by the National Research Center for Sustainable Production of the National Institute of Forestry, Agriculture, and Animal Production Research, Mexico (CENAPROS-INIFAP). The M498 isloation of *M. anisopliae* showed the highest virulence level (80 % mortality) 30 days after inoculation. M492, and M 493 were the least virulent showing a behavior similar to hat of Bb50 of *B. bassiana.* All isolation were evaluated at $2X10^8$ conidia per gram of culture medium concentration. Those isolations showed statitiscally significant differences in comparison to control, and were capable to grow, to produce spores, to provoque mycosis, and therefore they showed potential to cause an epizooty. Based on results, the isolation with highest *P. crinita* third stage larvae control potential was M498 from Jalisco, Mexico, isolated from *Phyllophaga* genus larvae.

POSTER BP56 - Thusday (Bacteria)

Specific binding and pore formation activity of Cry3A toxin in membranes isolated from _Leptinotarsa decemlineata_ and _Tenebrio molitor_.

Inmaculada Garcia-Robles[2], Jorge Sanchez[1], Carolina Rausell[2], Amparo C. Martinez-Ramirez[2], Ruud de Maagd[3], M. Dolores Real[2] and Alejandra Bravo[1]

[1]Instituto de Biotecnología, Universidad Nacional Autonoma de Mexico, Ap. Postal 510-3 Cuernavaca 62250 Morelos, Mexico. [2]Dep. Genética, Universidad de Valencia, Dr. Moliner 50, 46100-Burjassot, Valencia, Espana. [3]Plant Research International, Wageningen, Netherlands.

Bacillus thuringiensis Cry3A toxin is highly toxic to a limited number of coleopteran insects, including *Leptinotarsa decemlineata* (colorado potato beetle) and *Tenebrio molitor* larvae. The aim of the present work was to investigate the mechanism of action of Cry3A toxin in membranes from these two coleopteran insects. The specific binding of Cry3A to BBMVs prepared from larvae midguts and the pore formation activity of this toxin were analyzed. Binding assays with biotin-labeled Cry3A trypsin activated toxin were performed with BBMVs from *L. decemlineata* and *T. molitor*. In both cases binding of the toxin to midgut BBMVs proteins was found. Heterologous competition experiments using Cry3B and Cry3C toxins showed no competition of these two toxins for Cry3A binding. The analysis of the pore formation activity was carried out using a fluorescent positively charged dye sensitive to membrane potential changes. Toxins that alter the permeability of the membrane modify the transmembrane electric potential and therefore resulting in the movement of the dye across the vesicles with the consequent changes in fluorescence. Hyperpolarization causes dye internalization into the BBMV and fluorescence decrease whereas depolarisation causes the opposite effect. Using this technique we have found that Cry3A affect membrane permeability causing a fast hyperpolarization and increase in the transport of K+ ions in both coleopteran systems. The data indicates that pH and activation of the toxin are critical on pore formation activity of Cry3A.

POSTER BP52 - Thursday (Bacteria)

Cloning and sequencing of BTR-CAD, a gypsy moth midgut protein related to a putative receptor for the insecticidal toxins of _Bacillus thuringiensis_

Karen J. Garner and Algimantas P. Valaitis

USDA Forest Service, Northeastern Research Station
Delaware, OH 43015

Bacillus thuringiensis (Bt) is the most widely used biopesticide for control of the gypsy moth (*Lymantria dispar*). Bt receptors present in the membranes of insect midgut epithelial cells have an important role in the insecticidal activity of Bt toxins. Exploitation of the selective toxin-binding properties of the Bt receptors will be valuable in developing technology to improve Bt-based products for gypsy moth management programs.

The Cry1Aa and Cry1Ab Bt toxins, which are highly toxic to *L. dispar* larvae, bind to a large molecular weight molecule present in brush border membrane vesicles from larval midguts. Similar in size to the putative Bt receptor in gypsy moth, two Cry1A toxin-binding glycoproteins from *Manduca sexta* (Bt-R_1) and *Bombyx mori* (BtR-175) have been cloned and found to share sequence similarity with the cadherin family of proteins. Reverse transcriptase PCR using primers designed from *M. sexta* BT-R_1 sequence information was used to isolate a related cDNA fragment from the gypsy moth. Subsequently, a complete cDNA called Ld BTR-CAD was cloned and sequenced. The cDNA is 5484 bp in length and encodes a polypeptide with a predicted size of 192.3 kDa. The cDNA sequence is 62% and 66% identical to Bt Cry1A toxin receptor clones from *M. sexta* and *B. mori*, respectively. The *L. dispar* Bt Cry1A receptor is predicted to have seven potential N-linked glycosylation sites, a leucine zipper sequence, and a large extracellular domain containing cadherin-related motifs. Current studies are aimed at expression of the clone and determination of its role in the insecticidal activity of Bt.

POSTER BP26 - Tuesday (Bacteria)

Cloning and characterization of four distinct gypsy moth midgut aminopeptidase-N enzymes related to the _Bacillus thuringiensis_ Cry1Ac receptor.

Karen J. Garner and Algimantas P. Valaitis

USDA Forest Service, Northeastern Research Station
Delaware, OH 43015

The host range for *Bacillus thuringiensis* ä-endotoxins is largely determined by the specific interaction of the toxins with their insect midgut receptors. Several Cry1A toxin-binding proteins have been identified in various insects as putative *B. thuringiensis* toxin receptors that include members of the aminopeptidase-N (APN) gene family. Previously we have reported isolation, cloning and characterization of two distinct APNs from gypsy moth. Ligand blots and binding studies using a BIAcore optical biosensor revealed that APN-1 purified from

Lymantria dispar binds Cry1Ac toxin with a 1:1 stoichiometry and does not interact with Cry1Aa or Cry1Ab. However, several distinct but related APNs purified from other lepidopteran insects have been shown to interact with Cry1Aa, Cry1Ab and Cry1C toxins suggesting that structurally different APN isoforms with differences in toxin binding activities may be present in *L. dispar*.

Four APN cDNAs from a gypsy moth midgut cDNA library have been isolated to date. The two new APNs, ëAPN3 and ëAPN4, encode proteins that are related to but are distinct from two previously cloned gypsy moth aminopeptidases, ëAPN1 and ëAPN2. The ëAPN3 and ëAPN4 cDNAs are 3065 basepairs and 3320 basepairs in length, respectively. The ëAPN3 encodes a predicted protein of 947 amino acids and nine potential glycosylation sites. The ëAPN4 cDNA is predicted to encode a 995 amino acid protein with one glycosylation site. Each of the two polypeptides has a predicted N-terminal signal peptide and a C-terminal glycosylphosphatidylinositol (GPI) anchor addition site. All four gypsy moth APNs contain conserved sequences characteristic of the APN family including the zinc-binding sequence HEXXHXW. The phylogenetic analysis derived from eighteen complete APN sequences has five deep branches. Although four distinct gypsy moth APNs have been characterized, phylogenetic analysis of the published lepidopteran APN sequences suggests that at least one additional APN form is present in *L. dispar*. Experimental evidence for toxin binding activity has been reported for APNs in three of the five branches of the sequence similarity tree. The characterization of any toxin binding activity of these new gypsy moth APN isoforms remains to be determined.

POSTER PP6 - Thursday (Protozoa)

Nosema* disease of the encyrtid parasitoid *Tachinaephagus zealandicus

Christopher J. Geden[1], Maria Ferreira de Almeida[2], James J. Becnel[1] and Carl K. Boohene[1]

USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA 32604[1]; and Departmento de Parasitiologia, University of Campinas, CP 6109, Campinas, Brazil, CEP 13083-97[2]

Tachinaephagous zealandicus is an encyrtid parasitoid of house flies and other muscoid Diptera inhabiting livestock and poultry manure, and is widely distributed in the southern hemisphere. The parasitoids attack mature fly larvae as they seek pupation sites, depositing multiple eggs in the hemocoel of the larva. The host pupates and continues developing slowly while the parasitoid larvae develop within the body cavity. The host is killed near the end of the parasitoids' development, and the *T. zealandicus* immatures pupate within the mummified host remains. Development is completed in 22-25 days at 25°C. A colony of *T. zealandicus* from Brazil recently was found to be 100% infected with a microsporidian pathogen resembling *Nosema muscidifuracis*, a pathogen of the pteromalid parasitoid *Muscidifurax raptor.* Transmission testing indicates that the pathogen is transmitted transovarially. Horizontal transmission occurs when larvae of uninfected and infected parasitoids both occur within superparasitized hosts. Infected females have reduced longevity and produce about half as many progeny as healthy females. Infection has no substantial effect on sex ratios or development time of the parasitoids. Heat shock was not effective for managing the disease because of the sensitivity of the host parasitoid to elevated temperatures. *Per os* administration of a 1.5% solution of rifampicin to adult *T. zealanidus* resulted in reduced rates of maternal transmission. This reduction was sufficient to allow the isolation of clean females and the establishment of an uninfected colony of the parasitoid.

STUDENT PAPER - Monday, 11:15 (Bacteria I)

***Bt* Resistance and Dominance Levels**

Anne Génissel[1,2] Michel Raymond[3] and Denis Bourguet[1]

Unité de Recherches de Lutte Biologique[1], INRA La Minière, 78 285 Guyancourt, France
Station de Zoologie forestière[2], INRA Centre de recherche d'Orléans, 45 160 Ardon, France
Institut des Sciences de l'Evolution[3], CC 065 Université Montpellier II, 34 090 Montpellier, France

Dominance has been assessed in different ways in pesticide resistance studies, based on three phenotypic traits: the pesticide concentration required to give a particular mortality (referred to as D_{LC}), mortality at a particular pesticide dose (D_{ML}) and fitness in treated areas (D_{WT}). We propose a general formula for estimating dominance on a scale of 0 (complete recessivity) to 1 (complete dominance). D_{LC}, D_{ML} and D_{WT} are not directly related and their values depend on genetic background and environmental conditions. We also show that pest management strategies can have the consequence to increase D_{WT} via the selection of dominance modifiers. Studies on resistance to *Bacillus thuringiensis* toxins provide the ultimate example of the complexity of the definition of the concept of dominance. Almost all studies have focused on calculation of D_{LC} which provide little information about the efficiency of pest management programs. For instance, one assumption of the high dose / refuge strategy is that *Bacillus thuringiensis* resistance must be effectively recessive (i.e. D_{ML} must be close to zero). However, D_{WT}, rather than D_{ML}, is relevant to the resistance management strategy. Therefore, we strongly suggest that the time has come to focus on fitness dominance levels in the presence and absence of pesticide in natural populations.

CONTRIBUTED PAPER - Monday, 15:15 (Fungi II)

Determination of the rate of production and size of conidia of the aphid-pathogenic fungus *Erynia neoaphidis* using image analysis.

Simon Gray[1], Tony Bonner[1] and Judith Pell[2].

[1]Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., LU1 3JU, U.K., simon.gray@luton.ac.uk.
[2]Entomology and Nematology Dept., IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, U.K.

The zygomycete fungus *Erynia neoaphidis* frequently causes epizootics in aphid populations and is consequently regarded as a potential biological control agent for these insects. The results of trials using *E. neoaphidis* against aphids in the field have been inconsistent. Problems which have been identified include lack of standardisation of the physiological status of the inoculum and lack of virulence of inoculum produced on artificial media against aphids.

The strategy currently favoured for introduction of *E. neoaphidis* as a biological control agent is to apply formulated fungal biomass which sporulates in the field, releasing conidia, the infective propagules of this fungus. Monitoring the rate of release of conidia is necessary in order to optimise the efficacy and consistency of the biological control agent. A sporulation monitor, which allows quantification of the number of conidia produced by biomass of entomophthoralean fungi, has been described previously. We have refined this method by using image analysis to quantify the numbers of conidia produced. This greatly increases the rate at which data can be processed compared to manual counting of conidia, and also allows automated measurement of the length and width of each conidium counted. The number of experimental treatments which can be compared and the statistical power of the results are therefore increased, and inferences can be made about the physiological status of the conidia being released based on their dimensions.

The method was used to evaluate production of conidia by: mycelial plugs cut from agar plates; mycelial pellets produced in liquid batch culture; and mycosed cadavers of the pea aphid *Acyrthosiphon pisum*; over a 168 h period at 18°C and 100% R.H. In each case, conidia were produced for 65 to 75 h, with the greatest rate of release of conidia at 10 h. Fewer, larger conidia were produced by the fungus grown on solid and in liquid culture (714 conidia h^{-1} with a mean volume of 13,753 μm^3 and 750 conidia h^{-1} with a mean volume of 13,992 μm^3 respectively) than by mycosed cadavers (2,332 conidia h^{-1} with a mean volume of 6,856 μm^3). Mycosed aphid cadavers produced the greatest mass of conidia per unit fungal biomass (2,785 g.d.w. g.d.w. biomass^{-1} h^{-1}), followed by pellets from liquid culture (595 g.d.w. g.d.w. biomass^{-1} h^{-1}) and plugs from plate culture (138 g.d.w. g.d.w. biomass^{-1} h^{-1}). The ratio of length to width of the conidia remained constant throughout discharge, suggesting that the ratio of primary to secondary conidia did not change with time.

SYMPOSIUM - Tuesday, 10:55 (Nematodes)

Nematode environmental tolerance

Itamar Glazer

Department of Nematology, Volcani Center, Bet Dagan 50250

Entomopathogenic nematodes (EPN), in association with symbiotic bacteria, live in the soil where they locate suitable insect hosts, penetrate into their haemocel and release the bacterial symbionts. The bacteria multiply and kill the insect within two days. The wide host range, lack of pathogenicity to humans and livestock, and the availability of procedures for mass culture, make EPN attractive environmentally friendly bio-insecticides. However, their sensitivity to the extremes of the environment prevents them from reaching their full biocontrol potential. Selection, screening for new natural isolates, and mutagenesis have been attempted in an effort to enhance EPN tolerance to environmental harshes. Manipulation of genes involved in heat and desiccation tolerance offers a powerful attractive alternative. Recently, the technology for generating transgenic nematodes has been successfully adapted from *C. elegans* to EPN, opening the way for generating transgenic EPN with enhanced tolerance to environmental extremes. Indeed, transgenic EPN carrying a heat shock gene from *C. elegans* were shown to have increased heat tolerance. Yet, in view of the growing concern regarding the use of genetically modified organisms, we reasoned that field release of transgenic EPN will meet less opposition if the engineered genes were their own rather than from a foreign species. As a first step towards this goal we have isolated and characterized two heat shock genes from the EPN *H. bacteriophora*, and a glycogen synthase gene, involved in the biosynthesis of trehalose, which confers desiccation tolerance, from the EPN *S. feltiae*. Our progress along these lines will be described.

CONTRIBUTED PAPER - Tuesday, 16:45 (Protozoa I)

Influences of a new microsporidian isolate on development of gypsy moth (*Lymantria dispar* L.) - a comparison with *Nosema "Germany"*

Dörte Goertz, Andreas Linde, Judith Golldack

Fachhochschule Eberswalde, Dept. of Forestry, Applied Ecology, Alfred-Möller-Str.1, 16225 Eberswalde

Four natural populations of gypsy moth (*Lymantria dispar* L.) were screened for presence of microsporidian infections in Bavaria in 1999. A new microsporidian isolate was found near by Schweinfurt (Bavaria, Germany).

Larvae of *Lymantria dispar* L. were individually infected with the new isolate and either kept in small populations of 10 larvae per diet cup or **as** individual larva. The influence of the infection on development, stage specific mortality, pupation and eclosion of the adults was investigated. Adults were mated and the effects of microsporidiosis on the reproductive success of females and the hatch rate of the progeny were examined.

The two isolates caused different stage specific motality rates. No mortality occured during larval stage and 3,6% to 5,6% of infected individuals died as pupae, when larvae were infected with the new isolate. Up to 8,4% and 34,7% of all individuals died as larva resp. pupa, when they were infected with Nosema "Germany". Infection with the new isolate caused a significantly retarded development of male larvae and a longer pupal stage of both sexes.

The results of the first experiments will be presented. The impacts of the new microsporidian isolate and *Nosema "Germany"* on gypsy moth will be compared.

CONTRIBUTED PAPER - Monday, 12:00 (Fungi I)

Lack of effects of sublethal doses of the chemical pesticides imidacloprid, diflurobenzuron and buprofezin on the susceptibility of thermoregulating grasshoppers to the fungus *Beauveria bassiana*

Mark S. Goettel[1], Grant M. Duke[1] and G. Douglas Inglis[2]

[1]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1

[2] Department of Entomology, Mississippi State University, Mississippi State, MS 39762

We have recently demonstrated that behavioral thermoregulation in grasshoppers has a profound influence on the ability of the fungus, *Beauveria bassiana*, to overcome its host. Grasshoppers basking for as little as one hour per day are able to halt disease progression and escape death. We tested the hypothesis that the negative effects of host thermoregulation can be overcome through the use of sublethal doses of chemical insecticides that inhibit the basking behavior of grasshoppers. We tested three insecticides, imidacloprid (Admire), diflurobenzuron (Dimilin), and buprofezin (Applaud) on the susceptibility of third instar nymphs of the migratory grasshopper (*Melanoplus sanguinipes*) to *B. bassiana*. The effects of the chemicals and fungus alone or in combination were evaluated under thermoregulating and non_thermoregulating conditions. Treatments included nymphs treated with: 1) carrier alone; 2) conidia alone; 3) chemical alone; and 4) chemical and conidia. Individual nymphs were treated orally using an oil-bait method. Following treatment, groups of 20 individuals from each treatment were combined in cages maintained at constant 25°C and a photoperiod of 16/8 (L/D). Grasshoppers in half the cages were allowed to bask for 4 h/day (*i.e.*, adjacent to a 25W incandescent light bulb), and mortality was assessed daily for 2 weeks. The doses administered per nymph were as follows: 5×10^4 conidia of *B. bassiana*; 2.5×10^{-3} mg buprofezin; 2.5×10^{-4} mg diflurobenzuron; and 2.5×10^{-5} mg imidacloprid. Assays demonstrated that these concentrations of the chemicals approached a lethal level when administered to grasshopper nymphs and were not detrimental to *B. bassiana* vegetative growth *in vitro*. Within minutes of ingestion of imidacloprid, nymphs became immobilized for 6 to 12 hours. There were no apparent effects after ingestion of diflurobenzuron or buprofezin. Mortalities never exceeded 28% for all non-*B. bassiana* treatments within 14 days of treatment. Under non-thermoregulating conditions, mean mortalities at the end of the 2 week experimental period exceeded 90% for all *B. bassiana* treatments regardless of the chemical co-treatment. When grasshoppers were allowed to thermoregulate, mortality due to *B. bassiana* was substantially reduced (20 - 50%). However, there were no significant differences in mortality between the *B. bassiana*-alone and *B. bassiana*-insecticide combination treatments under either thermoregulating or non-thermoregulating conditions. We conclude that a sublethal dose of the insecticides, imidacloprid, diflurobenzuron, and buprofezin do not sufficiently affect the thermoregulatory behavior of third instar *M. sanguinipes* nymphs to increase their susceptibility to *B. bassiana*.

SYMPOSIUM - Thursday, 17:25 (Insect Immunity)

The role of opsonins in cellular immune reactions of insects

Peter Goetz[1], Alexandra Graebert[1], Marc Niere[1], Chris Weise[2] and Ute Wernig-Pohl[1]

[1]Institute for Zoology, Free University of Berlin, D-14195 Berlin, Germany

[2]Institute for Biochemistry, Free University of Berlin, D-14195 Berlin, Germany

Various factors influencing phagocytic activity in insects have already been detected.

In larvae of the Greater Wax Moth *Galleria mellonella* Wiesner et al. (1997) discovered the stimulating effect of apolipophorin III (ApoLpIII) on phagocytosis and identified <3 kDa factors in the hemolymph with opsonic activity (Wiesner et al. 1996). In contrast, an inhibitory effect upon phagocytosis is mediated by toxins liberated by entomopathogenic fungi (Vilcinskas et al. 1997) but proteases released from such fungi stimulated the immune response of insect hosts.

Stimulation of phagocytic activity might be caused by increased cellular activity of the phagocytes as well as by alterations on the surface of the foreign objects which favor phagocytosis. Such opsonization is widespread among vertebrates and invertebrates but only in very few cases the factors, so called opsonins, have been really isolated and characterized.

Incubation of yeast cells in cellfree haemolymph of *Galleria mellonella* larvae causes an up to tenfold increase in phagocytosis as shown with a quantitative phagocytosis assay using FITC labelled yeast cells and monolayers of isolated plasmatocytes (Rohloff et al.1994). This effect is tranferable: after treatment of opsonized yeast cells with glycin

buffer the supernatant can successfully be used for opsonization of so far untreated yeast cells. SDS-PAGE of the supernatant exhibited several protein bands, the most conspicuous (ca 70 kDa) of them was transferred to terminal Edman sequence analysis. Homologies were detected with a Gram negative bacteria binding protein from *Bombyx mori* (Lee et al.1996) and the CCF-1 cytolytic factor from *Eisenia foetida* (Beschin et al. 1998) which binds LPS as well as ß-1,3-glucans. Marc Niere (PhD thesis, in preparation) was able to gain the complete c-DNA sequence of this yeast cell attaching protein from G. mellonella and to show, that its expression is up-regulated upon immunization. Further biological characterization of this immune factor will be performed as soon as the cleaning procedure from E. coli cultures has been optimized.

The implications of these results with *G. mellonella* are discussed in comparison with other known mechanisms regulating phagocytosis in insects.

POSTER BP34 - Thursday (Bacteria)

Improvement of the HPLC analysis of beta-exotoxin : down to 0.3 µg/ml in culture supernatants

Michel Gohar[1] and Stéphane Perchat [2]

INRA, station de recherche de lutte biologique, La Minière, 78285 Guyancourt Cedex, France[1,2]
Aventis Crop Science[1], Jozef Plateaustraat 22, B-9000 GENT, Belgium

Beta-exotoxin (thuringiensin) is a heat-stable, water soluble toxin produced by some strains of *Bacillus thuringiensis* (Bt). The toxin is a ATP analogue, which inhibits the DNA-dependent RNA polymerase. It is secreted in the fermentation medium during the stationary phase and is active against several species of insects and mites. For commercial or research needs, it may be necessary to determine the amount of beta-exotoxin present in Bt cultures.

Several methods are used for the quantification of beta-exotoxin, including bioassays, UV absorption after solvent extraction, HPLC, capillary electrophoresis or enzyme-linked immunosorbent assay (ELISA). The most sensitive of these methods is ELISA, but this method is not easily transferable as it is strongly dependent on the quality of the available antibodies. Furthermore, some proteins can interfere with the assay. HPLC using reversed-phase columns is rapid, with a limit of detection around 5µg/ml, due to contaminants co-migrating with beta-exotoxin.

We have defined two new HPLC methods, differing by the sample preparation, for the analysis of beta-exotoxin in fermentation broth. For both methods, the chromatography step is performed on a C18 column, with a gradient of methanol in potassium phosphate buffer from 5 to 15%. The first method is rapid and inexpensive as it relies, for sample preparation, on solvent precipitation. Its detection limit is 2µg/ml. The second method is based on the use of two successive solid-phase extractions, and has a sensitivity of 0.3µg/ml.

STUDENT POSTER BP35 - Thursday (Bacteria)

Characterization of scFv antibodies that interfere Cry1Ab receptor interaction

Isabel Gómez, Jorge Sánchez, Alejandra Bravo and Mario Soberón

Instituto de Biotecnología
Universidad Nacional Autónoma de México, Apartado Postal 510-3
Cuernavaca 62250, Morelos, México

Bacillus thuringiensis, a Gram-positive bacterium, produces various types of crystal inclusions during sporulation. These crystal inclusions are composed of an insecticidal protein, δ-endotoxins. δ-endotoxins have a high insecticidal activity. The crystal inclusions are solubilized in the alkaline environment of the insect midgut and processed proteolytically to yield smaller active toxins. The toxin bind to specific receptor molecules on the midgut epithelial cells of host insect and disrupt the permeability of the midgut cell membrane, resulting in a net influx of ions and an accompanying influx of water, so that the cell swells and lyses.

Two types of putative Cry1 toxin receptor proteins were identified in the lepidopteran insects. One is a cadherin-like glycoprotein receptor found in *Bombix mori* and *Manduca sexta* and the other is aminopeptidase N, which is found in *B. mori, M sexta, Heliothis virescens, Lymantria dispar* and *Plutela xylostella*.

Recently, filamentous bacteriophages have been used to display peptides and protein fused to the minor g3p or g8p coat proteins. After rounds of selection, each of which involves the binding of phages to appropriate ligand, elution and amplification variants of the original protein can be derived of the basis of modified binding activity: phage display technology.

In the present study, we analyzed the binding of Cry1Ab protein previously incubated with scFv antibodies, obtained by phage display, to brush border membrane vesicles (BBMV) of the tobacco harnworn, *M. sexta*. First we obtain scFv antibodies that bind to Cry1Ab toxin using phage display technology, we analyzed which scFv molecules are capable of interfering the binding to the toxin receptor. Two antibodies were analyzed showing that one was capable to interfere with Cry1Ab receptor interaction. The CDR3 region of this antibody has 71% homology with cadherin receptor region (Bt-R1). A 10 aa peptide representing the CDR3 region of this antibody was capable of interfering also with Cry1Ab receptor interaction.

Our results suggests that we have identified the region of the Bt-R1 receptor that binds Cry1Ab toxin.

POSTER BP16 - Tuesday (Bacteria)

Oligopeptide permease is required for expression of the *Bacillus thuringiensis plcR* regulon and for virulence

Myriam Gominet [1], Nathalie Gilois[2] and Didier Lereclus[1, 2]

Unité de Biochimie Microbienne, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris[1], Station de Lutte Biologique, INRA, La Minière, 78285 Guyancourt cedex, France[2]

PlcR is a pleiotropic regulator of virulence factors in the insect pathogen *Bacillus thuringiensis* and in the opportunistic human pathogen *Bacillus cereus*. It activates the transcription of at least 15 genes encoding extracellular proteins, including phospholipases C, proteases and enterotoxins. It was shown that the *plcR* gene expression is autoregulated and activated at the onset of stationary phase. We used mini-Tn*10* transposition to generate a library of *B. thuringiensis* mutants, with the goal of characterizing genes involved in the expression of the *plcR* gene. Three mutant strains, carrying distinct mini-Tn*10* insertions, were identified. The mutations negatively affect *plcR* expression, and result in a deficient hemolytic phenotype, similar to the phenotype of a *B. thuringiensis* strain in which the *plcR* gene has been disrupted. The insertion site of the mini-Tn*10* transposons mapped in a five-gene operon encoding polypeptides homologous to the components of the oligopeptide permease (Opp) system of *B. subtilis*, and showing a similar structural organization. By analogy, the five *B. thuringiensis* genes were designated *oppA, B, C, D* and *F*. *In vitro* disruption of the *B. thuringiensis oppB* gene reproduced the effect of the mini-Tn*10* insertions (*i.e.* the loss of hemolytic activity) and reduced the virulence of the strain against insects. These phenotypes are similar to those of a *plcR* mutant. Since Opp is required for the import of small peptides into the cell, the results indicate that *plcR* expression may be activated at the onset of stationary phase by the uptake of a signaling peptide acting as a quorum-sensing effector.

POSTER BP10 - Tuesday (Bacteria)

Reliability evaluation of K_Lap as a parameter for *Bacillus thuringiensis* scale-up fermentations

Andrés González, Alexandre Restrepo, and Sergio Orduz

Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas, Apartado Aéreo 7378, Medellín, Colombia

Bacillus thuringiensis commercialization has been focused on a few subspecies. All Diptera control products use *B. thuringiensis* subsp. *israelensis*, and this represents a risk of development of insect resistance in the future. *Bacillus thuringiensis* subsp. *medellin*, isolated in Colombia, is a new dipteran-active subspecies (Orduz *et al.* 1992) that could be used as an alternative to *B. thuringiensis* subsp. *israelensis*.

In *Bacillus thuringiensis* scale-up fermentation studies, several parameters have been used, one of which is the oxygen transfer volumetric coefficient corrected by pressure (K_Lap). Flores *et al.* (1997)

found a correlation between spore productivity and $K_L$ap for *B. thuringiensis* subsp. *kurstaki*, but unfortunately they did not report effects of $K_L$ap on toxicity. In scale-up fermentations similar hydrodynamic behavior is assumed in all scales but, with equal $K_L$ap, it is not possible to assure equal liquid velocity profiles inside the fermentor and bacteria physiological response could be affected. In this study, we investigated the reliability of $K_L$ap as a parameter for *B. thuringiensis* scale-up fermentations. Fermentation runs were carried out in 20 l and 200 l bioreactors with 11 l and 110 l cultures, respectively. Stirrer speed and air flow were fixed, based on the $K_L$ap, between 73.58 and 152.16 atm/h. Biomass and spore concentration were not affected by $K_L$ap in 11 and 110 l fermentations, with average values of 6.39 g/l and 5.3 $x10^8$ spores/ml, respectively. Toxicity towards third instar *Aedes aegypti* larvae and total fermentation time were different at different hydrodynamic conditions, but equal $K_L$ap. A half lethal concentration (LC_{50}) of 17.91 ng/l and total fermentation time of 22.5 h was obtained at stirrer speed 300 rpm and air flow 22 lpm, whereas a LC_{50} of 110.94 ng/l and total time fermentation of 41 h was obtained at 400 rpm and 11 lpm, both at $K_L$ap 86.43 atm/h.

SYMPOSIUM IV - Friday, 10:30 (Bacteria)

Strategies for mass production of mosquitocidal strains of *Bacillus thuringiensis* in pilot plant

González, A. Restrepo, E. Habeych, and S. Orduz

Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas (CIB)
A. A. 7378, Medellín, Colombia

Several approaches have been considered to increase *Bacillus thuringiensis* biomass and ä-endotoxin production, such as, culture media development, alternate culture methodologies to batch fermentation, and process optimization in laboratory and pilot plant operations. We evaluated *B. thuringiensis* subsp. *medellin* intermittent fed-batch to increase biomass production and the scale-up of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *meddellin* in 20 and 200 l fermentors.

In fed-batch experiments, maximum values obtained were a biomass of 38.6 g/l, 8.9 x 10^8 spores/ml, and half lethal concentration (LC_{50}) of 153 ng/ml. However, maximum values of biomass obtained with the addition of 8 times the culture medium concentration produced a decrease of 60 times the potency of the final whole culture. Addition of 4 fold the concentration of the original medium produced the best results in biomass and spore concentration while potency of the product was maintained within acceptable limits.

The scale-up results with *B. thuringiensis* subsp. *israelensis* showed an increased linear correlation between specific growth velocity and oxygen transfer volumetric coefficient corrected by pressure $K_L$ap, obtaining a maximum value of 0.77/hr at 132.8 atm/h. Additionally, a linear decrease of growth time and total fermentation time was found when increasing $K_L$ap of 20.41 atm/h. It is necessary to take into consideration the hydrodynamic behavior of the liquid inside the fermentor when scaling-up *B. thuringiensis* subsp. *medellin* with $K_L$ap. Biomass concentraion was increased up to a Klap of 125 atm/h, where the maximum value of 5 g/l was reached. The LC_{50} presented logarithmic decay below the KLap of 20.31 atm/h and therefore the optimum $K_L$ap for industrial production found was 125 atm/h. It is necessary to take into consideration the hydrodynamic behavior of the liquid inside the fermentor when scaling-up *B. thuringiensis* subsp. *medellin* with $K_L$ac. The LC_{50} was different in fermentations at stirrer speed of 300 rpm and air flow of 22 l/pm, and 400 rpm and 11 l/pm, both at $K_L$ap of 86.4 atm/h (17.9 ng/ml and 110 ng/ml, respectively).

CONTRIBUTED PAPER - Monday, 11:00 (Bacteria I)

Evidence for different Cry1Ab resistance genes in a colony of *Plutella xylostella.*

Joel González-Cabrera, Salvador Herrero and Juan Ferré

Department of Genetics, University of Valencia, 46100-Burjassot, Valencia, Spain

To date, the only insect species that has developed high levels of resistance to *Bacillus thuringiensis* (Bt) in the field is the diamondback moth, *Plutella xylostella.* Episodes of resistance have been reported in many places of the globe, especially in those with tropical climate where this insect has many generations per year. We have been working, since 1993, with a sample from a diamondback moth population collected in the Philippines from a Bt treated area. At the time the laboratory colony was established, the insects showed high resistance to Cry1Ab, but not to other toxins or Dipel, and lack of binding of this toxin to the insect midgut was demonstrated. After years of laboratory rearing, including selection with different Bt toxins, we observed that the insects became resistant to Cry1Aa, Cry1Ab, and Cry1Ac. This change in resistance spectrum strongly indicated the occurrence of more than one mechanism of resistance to Bt toxins.

At the start of the present work, the resistance of the colony from the Philippines had reverted to very low levels of resistance, with resistance ratios (RR) from 1 to 4.3. A sample of the colony was selected with a spore/crystal mixture of a strain only producing Cry1Aa. After 11 generations of selection the RR had increased from 1 to 6. A different sample of the same original colony was selected with trypsin activated Cry1Ab. After 3 generations of selection the RR varied from 3.5 to 238. Surprisingly, toxicity tests with the 3 Cry1A toxins showed that both selection lines had developed a practically identical resistance phenotype, both with very low resistance to the spores/Cry1Aa crystal mixture, no resistance to activated Cry1Aa, and similar high levels of resistance to Cry1Ab and Cry1Ac. However, the Cry1Ab-selected line had become cross-resistant to Cry1F, but not the spore/crystal-selected line. Genetic analyses using single-pair crosses showed another difference between the two selection lines. Resistance to Cry1Ab was dominant in the spore/crystal-selected line whereas recessive in the Cry1Ab-selected line.

Our results show that certain insect populations have a high potential to evolve resistance to Bt toxins and that on the type of toxin used as selecting agent (or the physical state in which it is administered) may be a determining factor in the type of resistance finally generated.

CONTRIBUTED PAPER - Thursday, 17:15 (Bacteria V)

Variability in tolerance to *Bacillus thuringiensis* insecticidal crystal proteins between standard susceptible laboratory strains of diamondback moth

J. González-Cabrera[1], S. Herrero[1], B. Escriche[1], A.H. Sayyed[2], D.J. Wright[2], S. Meyer[3], Y.B. Liu[3], B.E. Tabashnik[3], and J. Ferré[1]

[1] Dep. Genética, Fac. CC. Biológicas, Universitat de Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia) SPAIN. [2] Dep. Biology, Imperial College of Science, Silwood Park, Ascot, Berkshire SL5 7PY, U.K. [3] Dep. Entomology, University of Arizona, Tucson, AZ 85721, U.S.A.

The diamondback moth, *Plutella xylostella* (L.), is an important worldwide pest of crucifers that has developed resistance to practically all classes of insecticides, including formulations based on insecticidal crystal proteins (ICPs) from *Bacillus thuringiensis* (Bt). Variation in the susceptibility of standard laboratory strains used to calculate resistance levels in field-derived strains has been attributed largely to technical differences in bioassay protocols and it has been assumed that the ICP source has little effect. In addition, the reported data from populations with different geographical origins show little variability in this tolerance to Bt formulations and ICPs.

The present work examined the toxicity of ICPs against three strains of diamondback moth (LAB-V, ROTH, and LAB-PS) that have been used as control susceptible strains in studies with resistant colonies in different laboratories. These strains were tested in side-by-side experiments with five ICPs, from two different sources, using three different bioassay protocols. The ROTH strain was found to be the most susceptible to all ICPs (from 17- to 170-fold more susceptible). LAB-V and LAB-PS also showed some differences in susceptibility (e.g., Cry1D was 13-fold more toxic against LAB-PS than against LAB-V) and ICPs from different sources showed up to 20-fold differences in toxicity. In contrast, no significant variation in susceptibility to ICPs was observed when a single strain was tested with different bioassay protocols.

Our results show the existence of different biotypes in diamondback moth with respect to ICP susceptibility. Resistance levels of a given population can change drastically depending of the "reference" susceptible population used for comparison.

STUDENT POSTER GP4 - Thursday (General)

Evaluation of the clotting response of the hemolymph of cochineal (*Dactylopius sp.*) and its predator (*Laetilia coccidívora*).

González López L.[1], Alba Murillo M.[1], García-Gil M.F.[1-2], Lanz Mendoza H[4], Rojas Martínez A.[1], del Río Dueñas I[2], Hernández-Hernández F.C.[3].

Universidad Simón Bolivar[1], CICATA-IPN[2], CINVESTAV-IPN[3], CISEI-INSP[4]

Insects possess, for their defense against parasites and predators, many mechanisms that include melanization, phagocytosis, encapsulation, nodule formation, agglutination, and bactericin production (Sugumaran, *et al* , 1991, *Biochem. Biophys. Res. Commun*. 176:1371-1376). Cochineal scales (*Dactylopius sp: homóptera*) are parasites of the prickly pear cactus (*Opuntia sp*.), they have in their hemolymph the pigment carminic acid that has been stated as a potential deterrent to predators and invading organisms (Eisner T. *et al*, 1980, *Science*, 208:1039-1041). Recently it has been proposed that carminic acid participates during clotting reactions of the insect (García-gil, *et al*, 1999, *Imaggen*, 48:16-22). In spite of the defensive mechanisms of the cochineals, the pyralid moth (*Laetilia coccidívora*) predates cochineals and has the remarkable habit of keeping the ingested carminic acid and maybe using it for its own defense. The objectives of this work were to evaluate the activity of *Dactylopius sp.* hemolymph in the clotting and hemoagglutination responses and observe if these are maintained in the hemolymph stored by *L. coccidívora* . To study *in vitro* the clotting system of *Dactylopius sp* . and *L. Coccidívora* , hemolymph was obtained by injecting a saline solution (NaCl at 0.9%) and bleeding them afterwards. With this hemolymph, clotting kinetics were made using the following inducers: Galactosamine (25mcg/ml), Laminarin (25mcg/ml), Zymosan (25mcg/ml), and Lipopolysaccharid LPS (10mcg/ml). The samples were incubated and read with the help of the spectrophotometer at 495 nm. It was observed that in the hemolymph of both insects inducers, except LPS, triggered clotting reactions as well as carminic acid abduction. Dexametasone (20mcg/ml), an inhibitor of prostaglandin synthetase, blocked the hemolymph clotting of both species. Proteins present in hemolymph after clotting reactions were analyzed through PAGE-SDS, showing the protein loss in the activated hemolymph and differences in the abducted protein for each one of the species.

CONTRIBUTED PAPER - Tuesday, 9:30 (Fungi III)

On the beginnings of entomophthorous epizootics in insect populations

Svetlana Gouli and Vladimir Gouli

Entomology Research Laboratory, University of Vermont,
P.O. Box 53400, Burlington, VT 05405-3400

Questions concerning the beginning, large-scale outbreak and subsequent decline of mycoses caused by entomophthorous fungi among insect populations are of continual interest for researchers. These questions concern not only theoretical aspects of the problem, but are also of applied importance because knowledge of mechanisms moving the epizootic process is the key for effective utilization of microbial pathogens to regulate pests. Most epizootic diseases of insects involve semisaprophytic deuteromycetes, which explains the widely spread fungal propagules in place of insect inhabitation. However, there are difficulties in explaining the mechanisms of the explosive entomophthorous epizootics. Not infrequently, these epizootics develop rapidly and are widely spread. For epizootics among insects living in colonies, it is possible to gain insight form the biological characteristics of the conidial stage, but for all other insects those characteristics do not offer a convincing explanation. In particular, panzootics among gypsy moth populations on the vast spaces of the North-American continent are very difficult to explain based only on the activity of resting spores and conidia. In the last several years explosive epizootics caused by the fungus *Neozygites fresenii* were observed in populations of the aphid, *Dactynotus nigrotuberculatus*. The aphids exist as local isolating groups, but epizootics were registered simultaneously across large areas of New England (U.S.A.). The mechanisms of such phenomena are not understood.

During our work on the isolation and cultivation of entomophthorous fungi in different geographic zones we paid attention to the periodical appearance of specific crescent-shaped (CS) fungal propagules in cultures. These propagules grow well on various artificial media. These unusual fungal structures are observed only when we worked with insects that had typical signs of entomophthorous infections. The research was conducted with the following species: *Entomophaga maimaiga, Pandora neoaphidis, Neozygites fresenii, Furia sp.* and others. Often the CS propagules could be isolated from nutrient medium when isolation of the entomophthorous fungus was not successful. Sometimes slow growth of typical entomophthorous propagules was accompanied by appearance of CS formations, which grow very quickly. In the case when CS structures grow together with common entomophthorous propagules, the CS structures grow on the surface of medium, whereas the typical hyphal bodies and conidia are found in the upper layer of agar medium.

On the basis of these observations, we hypothesize that these fungi can develop into a form with a specific CS morphological structure and a saprophytic type of nutrition. If this is confirmed, then the beginnings of entomophthorous epizootics in insect populations can find explanation.

CONTRIBUTED PAPER - Friday, 8:15 (Microbial Control II)

Simple and rapid method for estimating microbial propagules on plants after a application of microbial formulations

Vladimir Gouli, Bruce Parker and Svetlana Gouli

Entomology Research Laboratory, University of Vermont
P.O.Box 53400, Burlington, VT 05405-3400

The total volume of microbial formulations used to pest control has been increasing from year to year. Most formulations contain different microbial propagules as active ingredients. All these propagules have a size within the limits of the light microscopic range. Estimating the distribution of active ingredients in different zone of the plant canopy is critical in developing and evaluating spray technologies. There are currently several methods for estimating distribution of microbial ingredient after application of pesticides based on different types of microorganisms. One method uses the wash procedure to remove the propagules from the plants or other substrata. A second technique involves making a leaf print on a solid nutrient medium. Microbial propagules can also be counted directly on the plant using specialized microscopes. Finally, there is possibility to receive replicas based of liquid silicon or silicon spray. Unfortunately all these methods are a labor-intensive and not very accurate.

We propose a new quick method for counting microbial propagules on the leaf surfaces by transferring the propagules to adhesive cellophane tapes. One tape has strong adhesive characteristics (375 0 Tape/Cinta/Ruban, 3M St. Paul, MN 55144-1000) and second tape has soft adhesive characteristics (Removable Magic Tape) for guaranteeing of taking down necessary side of leaf. The method includes several steps. Three 16x120 mm strips of tape are cut from standard tape (48 mm). One of them is put on a glass plate sticky side up. Forth 3x16 mm removable strips are placed sticky side down across the large strip at 20 mm intervals, beginning at one end. Four leaf disks (15 mm) are now applied to the strip such that the edge of each disk overlaps half the middle of narrow tapes. Than each leaf disk is closed the removable tapes from opposite sides. As a result, each leaf disk is located on the adhesive scotch tape and between two removable tape bands. All the leaf disks are covered with the second piece of the adhesive tape. The leaf disks are pressed hard to the scotch tape. It needs to do in different directions. The upper piece of the scotch tape is separated from the leaf disks. It need to do from side when is located the nearest removable band in upper position. This piece of scotch tape has the leaf imprints with all microscopic particles. Third piece of the adhesive scotch tape is used for elimination of leaf disks from first piece of scotch tape. Third piece is separated from opposite side when is nearest removable band. After these operations we have the first and second adhesive scotch tape piece with the reprints from both side of leaf. The reprints can place on the glass slide with any stain prepared on non-aggressive solvents. The study these slides are possible with any objectives.

CONTRIBUTED PAPER - Monday, 15:15 (Fungi II)

Determination of the rate of production and size of conidia of the aphid-pathogenic fungus *Erynia neoaphidis* using image analysis.

Simon Gray[1], Tony Bonner[1] and Judith Pell[2].

[1]Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., LU1 3JU, U.K., simon.gray@luton.ac.uk.
[2]Entomology and Nematology Dept., IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, U.K.

The zygomycete fungus *Erynia neoaphidis* frequently causes epizootics in aphid populations and is consequently regarded as a potential biological control agent for these insects. The results of trials using *E. neoaphidis* against aphids in the field have been inconsistent. Problems which have been identified include lack of standardisation of the physiological status of the inoculum and lack of virulence of inoculum produced on artificial media against aphids.

The strategy currently favoured for introduction of *E. neoaphidis* as a biological control agent is to apply formulated fungal biomass which sporulates in the field, releasing conidia, the infective propagules of this fungus. Monitoring the rate of release of conidia is necessary in order to optimise the efficacy and consistency of the biological control agent. A sporulation monitor, which allows quantification of the number of conidia produced by biomass of entomophthoralean fungi, has been described previously. We have refined this method by using image analysis to quantify the numbers of conidia produced. This greatly increases the rate at which data can be processed compared to manual counting of conidia, and also allows automated measurement of the length and width of each conidium counted. The number of experimental treatments which can be compared and the statistical power of the results are therefore increased, and inferences can be made about the physiological status of the conidia being released based on their dimensions.

The method was used to evaluate production of conidia by: mycelial plugs cut from agar plates; mycelial pellets produced in liquid batch culture; and mycosed cadavers of the pea aphid *Acyrthosiphon pisum*; over a 168 h period at 18°C and 100% R.H. In each case, conidia were produced for 65 to 75 h, with the greatest rate of release of conidia at 10 h. Fewer, larger conidia were produced by the fungus grown on solid and in liquid culture (714 conidia h^{-1} with a mean volume of 13,753 μm^3 and 750 conidia h^{-1} with a mean volume of 13,992 μm^3 respectively) than by mycosed cadavers (2,332 conidia h^{-1} with a mean volume of 6,856 μm^3). Mycosed aphid cadavers produced the greatest mass of conidia per unit fungal biomass (2,785 g.d.w. g.d.w. biomass^{-1} h^{-1}), followed by pellets from liquid culture (595 g.d.w. g.d.w. biomass^{-1} h^{-1}) and plugs from plate culture (138 g.d.w. g.d.w. biomass^{-1} h^{-1}). The ratio of length to width of the conidia remained constant throughout discharge, suggesting that the ratio of primary to secondary conidia did not change with time.

SYMPOSIUM IV - Friday, 9:30 (Bacteria)

Standardizing black fly bioassay techniques in the laboratory and field

W. W. Gray[1], R. A. Fusco[2], and Ray Noblet[1]

[1]Department of Entomology, University of Georgia, Athens, Georgia 30602
[2]Valent BioSciences, Mifflintown, Pennsylvania 17059 USA

The discovery of *Bacillus thuringiensis* ssp. *israelensis* (Bti) in the late 1970's and its efficacy against black flies ushered in a new era of black fly research. As a result of studies on black fly control conducted in different laboratories around the world, a variety of techniques, protocols and standards were used and accepted. Today we have time proven, standardized techniques for both the field and laboratory evaluation of Bti products and formulations. Field trials are conducted by measuring the flow of a given waterway, treating at the designated dosage, collecting larval samples after exposure and returning them to a laboratory for a 24 hour, post-exposure determination of mortality. Untreated, control larvae are handled and counted in an identical manner. This protocol enables scientists or program technical staff to make accurate comparisons of products and formulations in a repeatable, scientific manner at both research (low) and label rates. Laboratory techniques have also been standardized with the development and refinement of the orbital shaker bioassay. This technique was originally developed at Clemson University with field collected larvae and has been refined with laboratory reared black flies and modifications proposed by the WHO to make the technique more applicable to working conditions in West Africa. The orbital shaker technique involves placing black fly larvae in flat-bottomed extraction flasks with a set volume of test water. Larvae are acclimated to the shaker movement and dosed in unison via syringe. After a given period, the shaker is turned off and the larvae are held until mortality evaluations are conducted. These field and laboratory protocols are used simultaneously during research and development to facilitate the production of highly efficacious, uniform and dependable formulations of Bti for black fly control. The development of high quality formulations of Bti has contributed to the increased number of effective black fly control programs throughout the world.

CROSS-DIVISION SYMPOSIUM - Friday, 9:30

Nematicidal effects of entomopathogenic nematodes and their symbiotic bacteria on plant-parasitic nematodes

Parwinder Grewal

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

Innundative application of entomopathogenic nematodes *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae and Heterorhabditidae) suppress populations of plant-parasitic nematodes in several ecosystems including citrus, turfgrass, woody ornamental landscapes, and potatoes. Recent studies in turfgrass has shown that both native and non-native entomopathogenic nematodes produce similar effects on plant-parasitic nematode communities. Interestingly, the entomopathogenic nematodes have no adverse effects on the bacterivorous and fungivorous nematodes in the soil. Both entomopathogenic nematodes and their symbiotic bacteria *Xenorhabdus* (for *Steinernema*) and *Photorhabdus* (for *Heterorhabditis*) appear to play a role in this desirable non-target effect. Three mechanisms have been proposed: 1. Entomopathogenic nematodes crowded along the roots of plants force plant-parasitic nematodes away, 2. Massive doses of entomopathogenic nematodes led to a build-up of nematode antagonists, resulting in nematode-suppressive soils, and 3. Allelochemicals produced by the entomopathogenic nematodes or their symbiotic bacteria either repel or intoxicate plant-parasitic nematodes. Studies provide support to the allelopathy hypothesis because (i) applications of dead entomopathogenic nematodes decreased root penetration of the root-knot nematode *Meloidogyne incognita;* (ii) *M. incognita* infective juveniles were strongly repelled by the presence of *Xenorhabdus* spp. bacteria, represented either by an infected insect cadaver or as a cell-free exudate; (iii) *M. incognita* IJs were killed when exposed directly to cell-free *Xenorhabdus* exudates, and (iv) *M. incognita* egg hatch was reduced by *Xenorhabdus* exudates. Further studies supported that the application of entomopathogenic nematodes also interferes with the root-knot nematode *Meloidogyne incognita* life cycle at two points; reproduction is decreased in females exposed as juveniles to soil treated with entomopathogenic nematodes and egg hatch and subsequent plant penetration are decreased by 5 or 6 week old applications of entomopathogenic nematodes. At least two compounds produced by *Xenorhabdus/Photorhabdus* bacteria, ammonium and stilbene derivatives have been shown to produce some these allelopathic interactions.

SYMPOSIUM - Tuesday, 11:45 (Nematodes)

Molluscicidal nematodes and their potential for slug control in North America

Parwinder Grewal

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

Phasmarhabditis hermaphrodita (Rhabditida: Rhabditidae), originally isolated from England is a lethal parasite of slugs in the families Arionidae, Limacidae, and Milacidae (Gatropoda: Stylommatophora). This nematode has been successfully mass-produced and has been available commercially for slug control in Europe for several years. This

nematode has been fairly successful in providing slug control in Europe where soil temperatures typically are close to 10°C when the slugs are active. The potential of this nematode to control pests slugs in the USA and other warmer climates is unknown. Also its occurrence in North America has not been documented In several tests conducted in Ohio, the UK strain of *P. hermaphrodita* provided 80-100% control of the gray garden slug, *Deroceras reticulatum* in Hosta at soil temperatures ranging from 14-19°C. The nematodes reduced leaf are consumed by slugs to 10-24% as compared to over 75% in the controls. The nematodes showed remarkable recycling potential and provided up to 100% control of the slugs introduced in the plots previously treated. This species was not recovered from North America in a survey conducted during 1999-2000. Over 10,000 slugs and 600 snails were collected from 157 localities in USA (Oregon, Colorado, Michigan, Wisconsin, Indiana, Ohio, Pennsylvania, Maryland, and New Jersey) and Canada (Ontario and British Colombia). Nine species of slugs, *Arion ater, A. fasciatus, A. intermedius, A. hortensis, A. subfuscus, Deroceras lavae, D. reticultum, Lehmannia valentiana*, and *Limax maxmus* were found. Nematodes were recovered from slugs collected from 44 localities and they included: *Caenorhabditis elegans*, *C. formusana*, *C. remanei*, *Caenorhabditis* spp., *Curviditis* sp., *Cuticularia oxycera*, *Diplogaster ltheriteri*, *Diplogaster* spp., *Dolichorhabditis dolichura*, *Panagrolaimus* spp., *Rhabditella axei*, *Rhabditis* spp., *Rhabditophanes schneideri*, *Rhabditophanes* sp., *Saprorhabditis adentifera*, *Steinernema* sp., and *Xylorhabditis* sp. Only *C. remanei* was recovered from snails in all the three localities. Studies to determine the nature of the association between these nematodes and slugs are continuing.

CONTRIBUTED PAPER - Tuesday, 9:15 (Fungi III)

Multiple exposure and the importance of foliar persistence of *B.bassiana* for control of *Leptinotarsa decemlineata*

E. Groden, F.A. Drummond, H.B.H. Jorgensen, and S. Fernandez

Department of Biological Sciences
University of Maine, Orono, Maine 04469

Successive applications of *Beauveria bassiana* conidia have been suggested for management of foliar feeding insects, as successive applications would minimize the likelihood of loss of conidia when single applications coincide with host molting. Natural infections may also be acquired over time with repeated exposure to an inoculum source. A laboratory study was conducted to determine the effect of successive exposure of second instar *Leptinotarsa decemlineata* to topical applications of *B. bassiana* conidia (strain GHA, Mycotech Inc., Butte, MT). Larvae were treated with a conidial suspension of *B. bassiana* (ca. LD_{10}) or a control solution of tween-20 on day 0 and day 0 (second dose), 1, 2 or 3. Treated individuals were maintained individually at 25°C with fresh potato foliage, and mortality was assessed over 14 days. Mortality of individuals receiving two applications at 1, 2, or 3 day intervals was significantly higher than that experienced by individuals receiving two applications, one after the other, on day 0. The time to death was shorter for those individuals treated at the one day interval than those treated at the two day interval , and those treated at the two day interval died more quickly than those at the three day interval. However, there was no difference between these intervals in total mortality experienced after 14 days.

Florescent microscopy studies demonstrated that larvae do pick up lethal doses of *B. bassiana* while feeding on inoculated foliage. The persistence of conidia on potato following foliar applications was monitored throughout a field season in Central Maine. Persistence was found to be negatively correlated with cumulative exposure of foliage to solar radiation, with a half-life of 357,000 W per m^2 (< 24 hours). The consequences of this persistence for cumulative infection of *Leptinotarsa decemlineata* larvae was explored with computer simulation, and the implication of these results for field application strategies will be discussed.

STUDENT POSTER BP11 - Tuesday (Bacteria)

Specificity of *Bacillus thuringiensis* Cry1 proteins Against Colombian *Spodoptera frugiperda* population

Grosso V, Martinez W, Uribe D and J Cerón

Instituto de Biotecnología, Universidad Nacional de Colombia,
A.A. 14-490, Santafe de Bogotá, Colombia

Spodoptera frugiperda in a very important insect pest for the Colombian agriculture in crops like cotton, sorghum, corn and rice. It produce economic losses and the increment in the use of chemical insecticides. It is worth to mention that *S frugiperda* is a world-wide pest insect.

Some proteins of *B thuringiensis* like Cry1D, Cry1F and Cry1C have been described as highly active against *S frugiperda* first instar larvae. On the other hand other proteins like Cry1Ab and Cry1E have been shown different results depending on the country where the evaluation have been made. Such kind of results may be explained as a result of differences of geographic location of each studied population.

In this work it is shown the specific biopesticide activity of the Cry1 proteins against a Colombian population of *S frugiperda*. The evaluation were made by using first instar larvae on artificial diet. The cristal dilutions obtained from *E coli* recombinant strains which express each Cry 1 gene were applied onto the diet surface.

The Cry proteins were evaluated individually and in mix of two toxins in order to determine the synergistic or antagonistic effect between the proteins. The results were compared with similar international reports.

The main aim of this work was to known the real susceptibility of the Colombian *S frugiperda* population in order to make a good design of the control strategy of this pest. With that information it is possible to make a good management program which permit us to avoid any possibility of resistance of *S frugiperda* to the Bt toxins in the agricultural fields of our country.

POSTER BP12 - Tuesday (Bacteria)

Effects of *Bacillus thuringiensis* Insecticidal Crystal Proteins on Adult *Heliothis virescens* (F.) and *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae).

Mike Grove [1], Wendy Little [1,2], and William J. McCarthy[1]

Pesticide Research Laboratory and Graduate Study Center, Department of Entomology, The Pennsylvania State University, University Park, PA, 16802.
Present address: American Type Culture Collection 10801 University Blvd. Manassas, VA, 20110

Bacillus thuringiensis (Bt) insecticidal crystal proteins, or ICPs are usually thought to act only on the actively feeding larvae of susceptible species by a mechanism which involves consumption and proteolytic processing of the protein followed by binding to, and lysis of, midgut epithelial cells. However, few authors have reported *Bt* toxicity to adult insects. In the following presentation, we expand on previous reports of toxicity to adult insects and present data which demonstrate that: 1) Proteolytically activated ICPs are toxic to adult *Heliothis virescens* and *Spodoptera exigua*, 2) Individual activated ICPs are differentially toxic to adult *H. virescens* and *S. exigua*, and 3.) Adult *S. exigua* are sensitive to Cry1C protoxin.

CONTRIBUTED PAPER - Tuesday, 11:45 (Viruses III)

Developmental resistance of *Lymantria dispar* to *Lymantria dispar* Nucleopolyhedrovirus

Mike Grove, Kit O'Connor, Andrea Dreger, Maria Geleskie, Bailey Klinestiver, Heather Emminger, Kelli Hoover

Department of Entomology, The Pennsylvania State University,
University Park, PA 16802

Developmental resistance to baculovirus infection as larval Lepidoptera age within the instar is a well known phenomenon. To date, this form of resistance is best characterized for several noctuid species in which a mechanism of increased midgut cell sloughing with increasing larval age has been proposed. We conducted similar studies in our laboratory to investigate developmental resistance of the gypsy moth, *Lymantria dispar*, to its nucleopolyhedrovirus. *L. dispar* exhibits a similar pattern of developmental resistance within the fourth instar to oral inoculation with LdMNPV as that described for several noctuids. An LD_{80} dose of polyhedra for newly molted fourth instar larvae produced

42, 39, and 26% mortality in 24, 48, and 72 hour-old fourth instar larvae, respectively. To test whether this phenomenon was midgut-based, we conducted intrahemocoelic injections of developmentally staged cohorts anticipating that at a given dose of budded virus, 4th instars, regardless of age post-molt, would exhibit equivalent mortality levels. This was not the case. At a dose that produced 80% mortality in newly molted fourth instar larvae, mortality in 24, 48, and 72 hour old fourth instar larvae was 54, 24, and 56%, respectively, and these results were highly reproducible. We are currently extending our time points for both oral and intrahemocoelic inoculations to include 96 and 120 (premolt) hour-old larvae to determine whether these trends continue of increasing oral but decreasing intrahemocoelic resistance beyond 72 hours post-molt. These results suggest that developmental resistance in *L. dispar* may not be exclusively midgut-based. We are currently investigating the hypothesis that age-related resistance in *L. dispar* to baculoviral disease occurs as a result of a hormonally regulated age-dependent immune response.

POSTER BP36 - Thursday (Bacteria)

Partial characterization of midgut proteases of non-target Lepidoptera relevant to *Bacillus thuringiensis* sensitivity

Mike Grove[1], Brenda Oppert[2], Kris Hartzer[2], and Heidi Appel[1]

Department of Entomology, The Pennsylvania State University, University Park, PA 16802.
2. USDA-ARS Grain Marketing and Production Research Center 1515 College Ave., Manhattan, KS 66502.

We partially characterized the midgut proteases of thirteen species of nontarget Lepidoptera found in the Northeastern United States to determine whether specific characteristics of luminal proteases correlated with sensitivity to *Bacillus thuringiensis* insecticidal crystal proteins (ICP). By transferring electrophoretically separated midgut proteins to ICP or casein containing indicator gels, we identified multiple proteases capable of hydrolyzing ICP and/or casein. Individual species varied dramatically in protease profiles, with species having one to five major proteolytic bands with molecular masses of approximately twenty five to thirty seven kDa. Further characterization of luminal protease activity using trypsin or chymotrypsin specific chromogenic substrates identified individual bands as trypsin-like or chymotrypsin-like. Multiwell plate assays revealed that ICP sensitive species had greater ratios of chymotrypsin to trypsin activities (5:1 to 10:1) than ICP insensitive species (1:1 to 2:1). Treatment of midgut proteases with EDTA enhanced total proteolytic activity in eight ICP-insensitive species, but either did not affect, or reduced, the activity observed from three of four ICP-sensitive species. Ca++ reduced protease activity in all species from 50-100%. Mg++ reduced protease activity by 40-50% in five species, but somewhat enhanced the activity in four other species. However, there was no delineation between sensitive and insensitive species.

SYMPOSIUM - Thursday, 10:30 (Microbial Control)

Promotion and development of viral biopesticides in India and Thailand: lessons for the promotion of microbial control products in developing countries

David Grzywacz[1], Uthai Ketunuti,[2] and R Jebamoni Rabindra[3]

[1:] Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK. [2:] Department of Agriculture, Chatuchak, Bangkok 10900, Thailand [3:] Department of Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

India and Thailand are two developing countries where there has been significant progress in promoting the local production and use of biopesticides. A study of these two cases can help to identify valuable lessons on how to better promote microbial pesticides. The main driving force to the adoption of biopesticides has undoubtedly been the appearance of chemical pesticide resistance by key pests such as *Spodoptera* and *Helicoverpa armigera*. Another factor has been the need to meet strict chemical residue targets for export vegetables and fruit. In both countries, while safety is an issue, in practice this probably not a significant in increasing the adoption of biopesticides.

There are interesting differences in the models of development and registration in these two countries. India the state sector intervened by creating a market for biopesticides but production is largely through the local commercial companies. In Thailand local production is currently by government (though efforts are underway to move it to the local private sector) but most is sourced through the import of products such as "Gemstar" and "Spod-X". Regulatory structures are also radically different. In Thailand strict regulation means only a few high quality products are available to farmers and NPV is used mainly in high value vegetable and horticultural crops. In India there is currently no regulation of NPV products as yet. There are therefore many low cost products from small producers that generally compete on cost alone. Farmers use low cost state subsidised products on field crops such as cotton and legumes. However the existence of low cost, poor quality products makes expanding the market for high quality products difficult.

In both countries though biopesticide suppliers have evolved certain similarities. They develop NPV products as part of a larger "green" product portfolio including fungal products, pheromones, predators and parasites. Producers are moving to selling not single products but packages of compatible IPM solutions and this may represent the best approach to commercialising biopesticides in developing countries.

CONTRIBUTED PAPER - Tuesday, 12:15 (Bacteria III)

Isolation of *Bacillus thuringiensis* from Faeces of Insectivorous Bats from Upper Rhine Valley, Germany

Thania V. Guaycurus[1], Andreas Arnold[2] and Norbert Becker[2]

1.Department of Genetic, Institut Oswaldo Cruz, FIOCRUZ, Brasil1
2.German Mosquito Control Association, KABS, Germany

B.thuringiensis is an endospore-forming gram-positive bacterium that in its growth cycle produces crystalline proteinaceous inclusions that are toxic mainly to lepidopteran, coleopteran and dipteran insects. The crystal production led to the development of bioinsecticide for biological control of numerous human disease vectors as well as agriculture pests. In our continuing search for a novel insecticide strains we conducted the studies for the detection of *B.thuringiensis* in the faeces of insectivorous bats from different localities of the Rhine River Valley. In Germany it is known around 22 bat species which have as food only arthropods. This report described the isolation of *B.thuringiensis* from faeces of 8 species which serve as very effective 'collectors of insects'. About 140 faece samples were collected and the selection of endospore *bacilli* was attempted by eliminating germinated cells and and anothers contaminants using heat treatment at 80^0C he isolation of *B.thuringiensis* from faeces of different insectivorous bat species. This work open a new source for screening and search of this entomopathogenic bacterium.

STUDENT POSTER PP7 - Thursday (Protozoa)

Spatial distribution and prevalence of trypanosomatids in natural populations of the stream-dwelling gerrid *Aquarius remigis*

Kata Gurski and Mercedes A. Ebbert.

Department of Zoology, Miami University, Oxford, OH 45056 USA

To document patterns in the presence and prevalence of trypanosomatid parasites in the stream-dwelling gerrid *Aquarius remigis*, we investigated infection rates in eight host populations from May to October, 1999. We asked whether gerrid age, gerrid sex, time of collection or spatial distribution (upstream vs. downstream) were important in explaining prevalence of the interaction in SW Ohio. We showed that (1) trypanosomatids were present in all four streams surveyed in the watershed, (2) prevalence increased with host age as adults were more likely to be infected than nymphs, (3) prevalence did not differ between males and females, (4) prevalence did vary between streams but not between upstream and downstream locations, and (5) prevalence decreased from spring to fall. We concluded that prevalence patterns were related to host behavior. Opportunities for transmission are highest in the spring when adult host contact rates are high because of mating behaviors. Gerrid contact rates decrease over the summer corresponding to the decrease in trypanosomatid prevalence.

POSTER BP45 - Thursday (Bacteria)

A Theoretical Model of the Tridimensional Structure of *Bacillus thuringiensis* subsp. *medellin* Cry 11Bb Toxin Deduced by Homology Modelling

Pablo Gutierrez[1*] Oscar Alzate[2] and Sergio Orduz[1]

[1] Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas, CIB. Medellin, Colombia, [*] Current address, Department of Biochemistry, McGill University, Montreal, Canada, [2] Biochemistry Department, The Ohio State University, 484 w. 12th. Ave., OH. 43210

Cry11Bb is an insecticidal crystal protein produced by *Bacillus thuringiensis* subsp. *medellin* during its stationary phase; this -endotoxin is active against dipteran insects and has great potential for mosquito borne disease control. Here, we report the first theoretical model of the tridimensional structure of a Cry11 toxin. The tridimensional structure of the Cry11Bb toxin was obtained by homology modelling on the structures of the Cry1Aa and Cry3Aa toxins. Structural comparison of the Cry1Aa, Cry3Aa toxins with the theretical model of the Cry11Bb protein indicates corresponds to the general model for a Cry protein, and the few differences found were located in the loops of domain II and III. The superimposed backbone traces of Cry1Aa and Cry3Aa displayed 0.66 and 0.83 Å RMS deviations for Cα. The Ramachandran plot indicate that most (95%) of residues have ϕ and ψ angles in the core and allowed regions, except for some proline and glycine residues and few residues located in the loop regions. Most bond lengths, bond angles, and torsion angles were in the range of values expected for a naturally folded protein. The structural model shown in figure 2 indicates that it contains all the general features of the Cry toxins (an $\alpha+\beta$ structure with three domains). In this work we give a brief description of the model and hypothesize the residues of the Cry11Bb toxin that could be important in receptor recognition and pore formation. This model will serve as a starting point for the design of mutagenesis experiments aimed to the improvement of toxicity, and to provide a new tool for the elucidation of the mechanism of action of these mosquitocidal proteins.

STUDENT POSTER BP37 - Thursday (Bacteria)

Identification of *Bacillus thuringiensis* virulence genes by signature-tagged mutagenesis (STM)

Daniel M. Guttmann and David J. Ellar

Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge CB2 1GA, UK

The study of virulence genes has been greatly assisted by the development of signature tagged mutagenesis (STM) [Hensel, M. *et al* (1995) *Science*, 269:400-403]. This technique has been used with a wide range of organisms, both gram-negative and gram-positive including *Staphylococcus aureus* [Mei, J.M. *et al* (1997) *Mol. Microbiol.*, 26:399-407] and *Streptococcus pneumoniae* [Polissi, A. *et al* (1998) *Inf. Imm.*, 66:5620-5629]. To our knowledge, all reports to date have used ^{32}P to label the probes for the hybridisation to blots. As an alternative we designed oligonucleotides that, for the first time, allow highly specific, zero background hybridisations using DIG-labelled probes. A large pool of uniquely tagged transposons in a transposon delivery vector was constructed and transformed into *Bacillus thuringiensis*. Over 2000 insertion mutants were generated and several were analysed to establish whether insertions were targeted into several different locations. Using the tobacco hornworm (*Manduca sexta*) as a model of infection 1152 mutants were screened and over 30 mutants were recovered. Analysis of the disrupted open reading frames reveals the involvement of genes as diverse as transcriptional regulators, transporters and enzymes involved in cell wall synthesis and metabolism, in the pathogenesis of Bt.

CONTRIBUTED PAPER - Friday, 11:30 (Microsporidia II)

Microsporidia and other pathogens in associated spruce bark beetles (Col., Scolytidae)

U. Händel *[1], R. Wegensteiner *[1], E. Führer *[1] and J. Weiser *[2]

*[1] Institute of Forest Entomology, Forest Pathology and Forest Protection, Univ.-BOKU-Vienna, Austria
*[2] Institute of Entomology, Academy of Sciences, Ceske Budejovice, Czech Republic

Bark beetles are known to be the most serious insect pest species in managed forests of northern and Central Europe and often cause considerable economic damage, especially in secondary stands of Norway spruce (*Picea abies*). In spite of the importance of bark beetles, there is a great lack of knowledge about natural enemy complexes, especially about pathogens, and even less is known about the potential for manipulation of pathogens as biological control agents. Several recent investigations focused on the pathogen complex of the most economically important spruce bark beetle, *Ips typographus*, but there are only few reports about pathogens in other bark beetle species that occur sympatrically with *I. typographus*. Therefore, the goal of this study was to obtain information about pathogens that are specific to each bark beetle species as well as about pathogens with broader host ranges that occur in more than one (alternate) host species. The topic of these studies was a survey of pathogens (evidence and prevalence) in associated spruce bark beetles (with particular focus on *I. typographus* and *Pityogenes chalcographus*) from selected spruce stands in Austria.

Investigations were conducted at six sampling sites (9 sampling plots in total). Bark beetle infested log sections were collected from four areas with secondary spruce stands and in two "control" localities, one natural forest type stand and one conservation forest. Log sections were incubated separately in insectary breeding chambers. All emerging bark beetles were removed daily and stored in an incubator until they could be examined microscopically.

More than 15000 living specimens of 10 bark beetle species were dissected and examined under light microscopy. Several pathogen species (Poxviruses, Rhizopoda species, Gregarinia species and Microsporidia species), some of them not previously reported, were isolated. Differences were found in the pathogen complex of each beetle species in the different collection sites. Pathogen complexes in bark beetles were found to be extremely heterogenous and pathogens in beetles from different sampling plots within an area, even from single logs, were different. Furthermore, date of sampling and mode of sampling resulted in differences recorded in pathogen species recovered. Some pathogen species seem to have a broader host range, which is an important aspect concerning transmission and persistence of pathogens in bark beetle communities.

STUDENT POSTER PP1 - Tuesday (Protozoa)

A multiple infection of entomopathogenic microsporidians in *Bombyx mori*

Mihoko Hashiguchi[1], Rie Teramoto[1], Chisa Yasunaga-Aoki[1], Takeshi Kawarabata[1] and Hidehiro Tomimaru[2]

[1] Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
[2] Fiacao De Seda Bratac S/A, Caixa Postal 39-Cep, 17690-000 Bastos-Est, Sao Paulo, Brazil

A microsporidian isolate TB-2 was originated from a diseased silkworm larva, *Bombyx mori*, found in a Brazilian silkworm farm. Spore suspension of the TB-2 isolate contained at least three morphologically different microsporidian spores. Partially purified spores were recovered from bands formed in a Percoll density gradient after centrifugation. The lower, middle and upper bands contained large, medium and small sized spores, respectively.

After priming with 0.1 N KOH solution, small sized spores were inoculated into an *Antheraea eucalypti* cell culture and maintained at 27_C. A life cycle of a microsporidian isolate producing small sized spores was similar to that of Pleistophora species. However, persistently infected cultures of a *Pleistophora* like microsporidian was not established in *A. eucalypti* cell cultures.

By a limiting dilution method, *Spodoptera frugiperda* SF21AEII cell cultures were inoculated with medium sized spores primed with 0.1 N KOH solution and a microsporidian TB-2M-H1 producing medium sized spores was cloned. A cloned microsporidian TB-2M-H1 was transmitted transovarially in the silkworm. The growth, development and spread of infections of a cloned microsporidian TB-2M-H1 were investigated and compared in A. eucalypti, *S. frugiperda* SF21AEII, and *B. mori* BmN-4

cell lines. Cells of TB-2M-H1 observed in vitro at 27ºC were similar to those of *Vairimorpha* species.

A cloned microsporidian TB-2L-H1 was established similarly in *B. mori* BmX cell cultures inoculated with large sized spores from TB-2 isolate. When the primed spores of TB-2L-H1 were mixed with physiological salt solutions for germination, higher germination rates were obtained in Rinaldini's solution. The growth, development, and spread of infections of a microsporidian TB-2L-H1 were also studied in *A. eucalypti, S. frugiperda* SF21AEII, *B. mori* BmN-4, and B. mori BmX cell lines. The life cycle of TB-2M-H1 in vitro at 27ºC was similar to that of disporous species such as *Nosema bombycis.*

It was strongly suggested that the diseased silkworm larvae occurred in a Brazilian silkworm farm died due to a multiple infection of microsporidians including TB-2M-H1, and TB-2L-H1, and *Pleistophora*-like species.

POSTER FP22 - Thursday (Fungi)

Prevalences of fungal pathogens of cereal aphids in wheat under dryland and irrigated conditions in South Africa

Justin L. Hatting[1], Tadeusz J. Poprawski[2], and Ray M. Miller[3]

[1]South African Agricultural Research Council, Small Grain Institute, Private Bag X29, Bethlehem 9701, South Africa; [2]USDA-ARS Beneficial Insects Research Unit and Texas A&M Agric. Exper. Station, 2413 East Hwy 83, Weslaco, TX 78596; [3]Univ. of Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa.

Surveys were conducted in the summer and winter rainfall wheat producing regions of South Africa in a first attempt to investigate the impact on, and identity of epizootic-causing fungi within the cereal aphid complex. Wheat produced under dryland and irrigated conditions was surveyed during the 1996 and 1997 seasons. Six cereal aphid species were recorded of which the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), was the most abundant under dryland conditions (summer rainfall region) as opposed to the oat aphid, *Rhopalosiphum padi* (L.) in the winter rainfall region. Rose grain aphid, *Metopolophium dirhodum* (Walker) was most prevalent under irrigated conditions in the summer rainfall region. Five species of entomopathogenic fungi were recorded including four Entomophthorales and the Hyphomycete, *Beauveria bassiana*. The Entomophthorales included *Pandora neoaphidis*, *Conidiobolus obscurus*, *C. thromboides*, and *Entomophthora planchoniana*. *Pandora neoaphidis* was the most important etiological agent recorded from *D. noxia*, with up to 50.0% mycosis recorded under dryland conditions (summer rainfall region). Similarly, *P. neoaphidis* was the most prevalent species (28.2% infection) within populations of *M.dirhodum* under irrigated conditions in the summer rainfall region. However, mycoses of *R. padi* remained below 5% despite favorable aphid numbers and apparently suitable environmental conditions, suggesting some level of non-susceptibility of *R. padi* to fungal infection. In contrast, epizootics in populations of *D. noxia* under dryland conditions in both the winter and summer rainfall regions indicate a high level of susceptibility to fungal infection in this aphid.

SYMPOSIUM - Friday, 11:00 (Fungi)

Propagule production in submerged culture of the entomopathogenic Hyphomycete, *Paecilomyces fumosoroseus*.

Zenguo He,* Héctor Cárdenas-Cota, Pablo Fernández-Sumano y Mayra de la Torre.

Departamento de Biotecnología y Bioingeniería. CINVESTAV-IPN. Apdo. Postal 14-740, 07000 México D.F. *Centro de Ciencias de Sinaloa. Ave. Apdo. Postal 1889,Culiacán,Sin.

The entomopathogenic Hyphomycete *Paecilomyces fumosoroseus* is a powerful biocontrol agent against several pest insects, including the white flies *Bemisia tabaci* and *B. Argentifolii* Bellows & Perring (Homoptera: Aleyrodidade). Aerial conidia of this fungus are currently produced in Mexico by solid state fermentation. Several groups have focused on submerged culture of fungal propagules in order to increase productivity and improve process control to reduce production costs. Some patents have been issued covering submerged production of blastospores and mycelium of *P. fumosoroseus*, which are less resistant to adverse environmental conditions than aerial conidia. Our group first reported production of conidia in submerged culture. *P. fumosoroseus* propagules production, morphology and growth in liquid medium is dependent on several operating conditions and media composition. By modifying them it is possible to produce mainly submerged conidia, blastospores, pellets or free mycelium, and to induce microcycle conidiation from conidia or blastospores. Submerged conidia were infectious for whitefly nymphs and were as effective as aerial conidia to control whitefly in field test. Propagules production in submerged culture could be optimized to reduce production costs without affecting resistance and virulence of the fungus.

SYMPOSIUM I - Monday, 15:00 (Bacteria)

Do *Heliothis virescens* and *Plutella xylostella* have the same mechanism of Cry1Ac resistance? Evidence from comparative mapping studies.

David G. Heckel[1], Linda J. Gahan[2], Bruce E. Tabashnik[3] and Fred Gould[4]

[1]Dept. Genetics, University of Melbourne, Parkville, Victoria 3052, Australia; [2]Dept. Biological Sciences, Clemson University, Clemson, South Carolina 29634, USA; [3]Dept. Entomology, University of Arizona, Tucson, Arizona 85721, USA; [4]Dept. Entomology, North Carolina State University, Raleigh, North Carolina 27695, USA.

The YHD2 strain of tobacco budworm *Heliothis virescens* is >10,000-fold resistant to Cry1Ac toxin of *Bacillus thuringiensis* (encapsulated MVP formulation) as measured by a diet-incorporation bioassay. Up to 80% of this resistance level is conferred by a single gene (or tightly linked cluster) on linkage group 9 of that species. The NO-QA strain of diamondback moth *Plutella xylostella* is >6,800 fold resistant to the same formulation of Cry1Ac in a leaf dip bioassay. Most if not all of this resistance is conferred by a single gene (or tightly linked cluster) on linkage group 7 of that species. However, the correspondence between linkage groups of these two species is still not known; making it difficult to evaluate whether resistance in both cases is conferred by the same genetic mechanism. Although both resistance genes are autosomal and recessive; and there are similarities in the spectrum of cross-resistance (to Cry1Aa, Cry1Ab, and Cry1F); the physiological resistance mechanisms may not be identical. Resistance in NO-QA is caused by loss of the ability of midgut epithelial brush-border membranes to bind Cry1Ac; whereas the first report of Cry1Ac binding in YHD2 found no reduction relative to a susceptible strain (although Cry1Aa binding was abolished). However, the existence of several Cry1Ac binding proteins in *H. virescens* complicates the issue. By mapping these proteins in *H. virescens*, and their homologues in *P. xylostella*, we can test their involvement in each resistant strain separately, as well as establishing the correspondence between linkage groups that is necessary to answer our question.

CONTRIBUTED PAPER - Friday, 9:45 (Microbial Control II)

DrCP1, a putative developmental cysteine protease in Crucifer root maggot (*Delia radicum*): Target for inactivation?

Dwayne Hegedus[1], Michael O'Grady[1], Mahmood Chamankhah[1], Doug Baldwin[1,2], Lorraine Braun[2], and Martin Erlandson[2]

Department of Molecular Genetics[1] & Ecological Crop Protection[2] Agriculture and Agri-Food Canada, Saskatoon, SK, Canada, S7N 0X2.

All higher organisms express a vast suite of proteases that are involved in a diverse array of functions ranging from digestion to cell signaling and development. In virtually all instances, proteolytic activity must be temporally and spatially coordinated in order to carry out the prescribed function without affecting the integrity of host tissues. The inactivation of insect digestive proteases as a pest control strategy has met with some success and, as such, has been the primary focus of insect protease research. Conversely, insect proteases involved in more complex developmental processes have received little attention despite playing an equally vital role in the insect's life cycle.

During an investigation of the proteolytic components of the *D. radicum* midgut we isolated an abundant cDNA encoding a cysteine

protease, subsequently termed DrCP1. Previously, other researchers had found DrCP1 homologues in *Sarcophaga* (SpCP1) and *Drosophila* (DmCP1), however its precise function has not been determined. Northern blot analysis revealed that DrCP1 is expressed in all developmental stages including eggs, larvae, pupae and adults. Interestingly, Western blot analysis confirmed this observation but also showed that DrCP1 exists almost exclusively as an inactive 37 kDa proenzyme precursor. Employing "in-gel" separation and analysis of protease isoforms we demonstrated that a single 26 kDa cysteine protease, corresponding to the activated enzymatic form, was present specifically in mid to late third instar larvae. No activity was observed in second instar larvae or pre-pupae. Starvation of mid third instar larvae was observed to simulate the third instar/pre-pupal boundary and resulted in the loss of the 26 kDa active form.

Based upon these and other studies we propose: 1) that the activity and not expression of DrCP1 is highly regulated throughout development and, 2) that DrCP1 may be involved in some aspect of morphogenesis during the larval to pupal transition.

WORKSHOP I - Thursday, 11:10 (Bacteria)

Consequences for Non-Target Insects: Monarch Butterfly Update

Richard L. Hellmich

USDA-ARS, Corn Insects and Crop Genetics Research Unit, and Department of Entomology, Iowa State University, Ames, Iowa.

Research was conducted summer 1999 to determine if *Bacillus thuringiensis* (Bt) corn pollen presents a toxic risk to monarch butterflies, *Danaus plexippus*. Preliminary data strongly indicate that pollen from Bt corn events MON810 and CBH351 do not influence monarch larval mortality or development at levels normally found near cornfields during pollen shed, although more analysis remains to be done and peer-reviewed. With Bt corn event 176, the results were problematic when the pollen was considered only from a toxic view rather than a toxic plus exposure possibility. EPA has made the assumption that Bt pollen would affect all moths and butterflies, but that the exposure levels would be low to nonexistent. Our pollen deposition results suggest that there are many mitigating factors: pollen is heavy and most is deposited within a 2-3 m of cornfield, only a fraction of pollen (our study ~ 30% on average) stays on milkweed leaves, and rain washes most of pollen (our study ~ 90%) off of milkweed leaves. Choice and feeding results suggest that monarch larvae are influenced by the presence of pollen. When presented with leaves with no pollen and very high amounts (~ 600 grains/cm^2) of pollen, more larvae were found on the leaves with no pollen. Weight and survival data indicate that larvae feeding on milkweed leaves with high (~150 grains/cm^2) and moderate (~ 60 grains/cm^2) amounts of pollen from the MON810 and CBH351 are not affected. At the very high levels of pollen (~ 600 grains/cm^2) these same larvae were smaller than larvae from the control treatments, but survival was not different from that of the controls. Larvae feeding on leaves with pollen from the 176 type of corn were affected at each of the pollen density levels. Bt 176 corn represents about 2.5% of total corn planted in the United States. A Monarch Workshop was convened February for planning research activities for the 2000 growing season. A summary these activities also will be presented.

SYMPOSIUM - Friday, 11:30 (Fungi)

Biochemical and Molecular characterization of native strains and the development of autonomous replicating vector of the entomopathogenic fungus *Metarhizium anisopliae*.

Angélica González Hernández, Eduardo Calderón Alanís, Leslie Román Reyes, Claudia Morales Hernández, Eduardo Salazar Solis*, Félix Gutiérrez Corona and Juan Carlos Torres Guzmán.

Instituto de Investigación en Biología Experimental de la Facultad de Química, *Instituto de Ciencias Agrícolas, Universidad de Guanajuato. AP187, Noria Alta s/n, 36050 Guanajuato, Gto. México.
gonzang@quijote.ugto.mx

The genus *Metarhizium* is one of the most ubiquitous entomopathogenic fungal genera (Tulloch 1976[1], Romback et al. 1987[2]). Since the best biopesticides are the native strains of the particular region, is important the identification and characterization of the native strains to be used in the pests control. In our laboratory, we characterized 14 native strains from the states of Colima and Guanajuato (Mexico). The analysis of the chitinase activity was perform, determining the induction of this activity and its behavior on native acrylamide gels according to the methodology described by St. Leger et al (1993[3]); the results indicated that some isolates share the same pattern of enzyme activity with one, two or three activity bands. The physiological test included the determination of the induction of lethality and the time necessary to produce the death in the infected insects (*S.podoptera frugiperda*); the results indicated that all of them are virulent, efficiently killing more than 90% of the insects in 3-5 days. Finally we obtained DNA fingerprinting patterns of the isolates by the amplified fragment length polymorphisms technique (AFLP) (Vos et al, 1995[4]). The results indicated that the isolates have a low variability. The above mentioned studies of characterization are used to monitoring the strain used in the massive production of biopesticide of *M anisopliae* named "BIOFUNG M" as quality control.

Other aspect of our interest is the construction of an autonomous replicating vector for the genetic transformation of *M. anisopliae* based in the use of a new dominant selectable marker. In this sense we constructed some vectors containing different heterologous ARS sequences (from *U. maydis, S. cerevisiae* and *M. circinelloides*) and the cbx^r gene from *U. maydis* (Keon et al, 1991[5]), which confers resistance to the fungicide carboxin (5,6-dihidro-2-methyl-1,4oxathiin-3-caroxanilide). After transformation of *M anisopliae* with the different plasmids we observed the recovery of transformants able to grow in the presence of toxic concentrations of carboxin at a frequency of 6000 transformants by 50 υg of plasmidic DNA; the ARS from *U. maydis* allowed the highest efficiency of transformation. Southern bloth experiments indicated the presence of the plasmid as autonomous element. These results indicated that the ARS element and the carboxin resistant gene (cbx^r) from *U. maydis* are functional in *M. anisopliae* making feasible the use of these elements for the development of new vectors for the transformation of this fungus.

Transactions of the British Mycological Society, **66**: 407-411.
Transactions of the British Mycological Society, **88**: 451-459.
J. Invertebrate Pathology, **61**: 81.84.
Nucleic Acids Research. **23**: 4407-4414
Current Genet., **19**: 475-481.

STUDENT POSTER BP60 - Thursday (Bacteria)

ANALYSIS OF ß-EXOTOXIN TYPE STRAINS OF *Bacillus Thuringiensis* AND IN SELECTED INSECTICIDAL STRAINS.

Carmen Sara Hernández[1], Isabelle Larget-Thiéry[2] and Juan Ferré[1].

[1] Departament de Genètica, Universitat de València. Spain.
[2] Laboratoire des Bactéries et Champignons Entomopathogènes, Institut Pasteur. France.

Some *Bacillus thuringiensis* strains produce, independently to the crystal δ-endotoxin(s), a non-specific heatstable ß-exotoxin that is secreted to the culture medium. This is an insecticidal adenine-nucleotide analog with a molecular weight of 701 Daltons. Toxicity of the ß-exotoxin is likely due to inhibition of DNA-directed RNA polymerases by competition with ATP, inhibiting the synthesis of RNA. ß-exotoxin has been used commercially as an insect control in some countries and it could be found as a contaminant in commercial formulations of Bt. Bioassay and high-performance liquid chromatography (HPLC) have been successfully used to detect β-exotoxin in fermentation supernatants. The current work to analyzed, by HPLC, the presence of type I β-exotoxin in a collection of type-strains of *B. thuringiensis* and in some strains selected for their insect toxicity. HPLC results have been compared with those obtained in bioassays with *Ephestia kuhniella* (Lepidoptera Pyralidae). Presence of type I ß-exotoxin was only detected in type-strains representing serotypes H1, H9, and H10a,10b. Supernatant of the H8a,8b type-strain gave positive in the *E. kuhniella* bioassay but not by HPLC, indicating the occurrence of another soluble toxin different than type I ß-exotoxin, possibly type II ß-exotoxin. In a selection of 18 insecticidal strains, ß-exotoxin activity was also found in some strains lacking type I ß-exotoxin. These results show the need to use both bioassay and HPLC to determine the presence of ß-exotoxin activity, however, HPLC is a fast and sensitive technique if only type I ß-exotoxin is to be determined.

CONTRIBUTED PAPER - Monday, 11:00 (Fungi I)

A *Paecilomyces fumosoroseus* mutant strain with enhanced virulence against *Bemisia tabaci* synthetizes additional chitinase enzyme.

Claudia Hernández, Magdalena Iracheta, Reyes Tamez, Carlos Hernández-Luna
Luis Galán-Wong and Benito Pereyra-Alférez

Departamento de Microbiología e Inmunología. Fac. Ciencias Biológicas/UANL. San Nicolás de los Garza, N. L. México.

Paecilomyces fumosoroseus is a fungi that has been used for the control of several agricultural pests, such as the whitefly *Bemisia tabaci*. Currently the insects are infected by the fungus, after that fungus begin tha synthesize of several cuticle hydrolysis enzymes as chitinases and proteases. These enzymes are involved in the infection process. In this work, we report the partial characterization of chitineases from parental and mutant *P. fumosoroseus* strains. Both strains were grown on YPD (% w/v, yeast extract 0.3; peptone ,1.0 and glucose, 2.0) for 4 days. Cultures were centrifuged, washed and used as inoculum for enzyme induction. Three Erlenmeyer flasks containing 100 ml of minimum media supplemented with colloidal chitin as sole carbon source (1.0 % w/v) were inoculated and incubated at 28 °C under orbital agitation. Chitinolytic activity was evaluated indirectly for N-acetylglucosamine production. It was checked every 24 h. After 7 days, supernatants were ontained and dialysed against bidistiled water. Activity and protein content were evaluated in both strains. Results showed that chitinolityc activit of mutant strain was around 5-fold than parental one. Supernatants were precipited with ammonium sulfate at 90% saturation, dialysed and resuspended in 20 ml of tetradistilled sterile water. Optimal temperature and pH activity were determined, and we found that in both parameters, mutant strain gave two activity pics at pH 4.0 and 6.0, but parental one showed maximum activity at pH 4.0. Temperature showed similar effect, the mutant show two activity pics at 35 and 45-50°C, while parental one had its maximal activity at 45°C. Samples were loaded on SDS-PAGE plus glycol chitin. Gels were stained with calcofluor white and visualized with UV. Mutant strain gave two activity bands while parental only one.

STUDENT POSTER BP13 - Tuesday (Bacteria)

Role of the tryptophans residues in toxicity of Cry1Ab toxin.

Georgina Hernández , Alejandra Bravo and Ma. Eugenia Nuñez

Instituto de Biotecnología Universidad Nacional Autonoma de México Ap. postal 510-3 Cuernavaca Morelos. e-mail yinna@ibt.unam.mx

Bacillus thuringiensis is a Gram-positive spore-forming bacteria that forms crystals during the stationary phase of growth. The crystals are composed of proteins denominated Cry. These proteins are toxic against insects as Lepidoptera, Diptera, Coleoptera and others.

The toxic fragment of the Cry proteins consist of three domanis. Domain I is involved in pore formation and Domain II and III are involved in toxin especificity.

The tryptophan (Trp) is a nonpolar and aromatic aminoacid, this residue has been conserved during evolution in many proteins. The Cry1Ab toxin contains nine Trp in its sequence eigth of them are highly conserved among the whole Cry family. The Trp 219 located in α-helix 7 is the only one not conserved. Seven of the typtophans of Cry1Ab toxin are located in Domain I.

In several membrane proteins as bacteriorhodopsin, porins and potassium channel the Trp residues are located at the membrane surface. It is generality assumed that Trp interact with bilayer in an amphipathic manner. That is, it bury itself in the hidrocarbon core with its imino group H-bounded to the phospholipid carbonyl and water. Therefore, it has been proposed that Trp residues serve as anchors on the periplasmic side of the membranes.

To evaluate the role of these amonoacids in the mecanism of action of Cry toxins each one of the Trp were substituted with conservative and non conservative changes (phenylalanine and alanine respectively) by site-directed mutagenesis.

Toxicity against *Manduca sexta* larvae and the diferent steps in the mechanism of action of the Cry proteins: 1)solubilization, 2) activation of the protoxin with trypsin and midgut juice, 3) binding of the activated toxin to brush border membrane vesicules and 4) ion channel activity were analysed.

In this work we will present the functional analysis of the conservative mutants. Those mutants that were not affected in toxicity will be useful for the studies of membrane insertion by using the fluorescencet properties of the tryptophans.

POSTER FP2 - Tuesday (Fungi)

Variability of *Beauveria bassiana* isolates from *Hypothenemus hampei*: Germination, sporulation and effect of water activity.

Francisco Hernandez-Rosas[1], Raquel Alatorre-Rosas[1] and Gerardo Saucedo Castañeda[2]

[1]Instituto de Fitosanidad, Colegio de Postgraduados, Km 36.5 Carr. México-Texcoco. C.P. 56230. Texcoco, Edo. de México. México.
[2]Departamento de Biotecnología. Universidad Autónoma Metropolitana. Unidad Iztapalapa. Apdo. Postal 55-535, México.

The germination rate, germ tube length (GTL) and sporulation of 94 isolates of entomopathogenic fungi (18 multisporic (parents) and 76 monosporic isolates) were compared. Thirteen of the multisporic, parent isolates were from the species *Beauveria bassiana* and originated from coffee berry borer, *Hypothenemus hampei*. The remaining isolates were from different fungal species and originated from different insect hosts. The effect of water activity (a_w) on sporulation of some of the *B. bassiana* isolates was determined on different substrates. Percent germination after 24 h allowed differentiation between *B. bassiana* isolates obtained from *H. hampei* and species isolated from other hosts; the LGT range among 1-40 um let to separate isolates of the genus *Beauveria* and *Verticillium lecanii*; and sporulation with 10^7 sp/ml was used as the suitable concentration for the isolates selection. The cluster analysis of germination rate, LGT and sporulation data showed a dendrogram with a tendency to separate isolates by species and hosts from each other (Hernández-Rosas, F. and Alatorre, R. R. Submited to Rev. Mex. Micol.). Five isolates (four *B. bassiana* and one *B. amorpha*) were selected in a preliminary experiment to determine the minimum water activity for growth on bacteriological agar supplemented with insect powder and ethylene glycol (EG), plus or minus anhydrous dextrose. The minimum a_w for growth was 0.97 and the best growth at this a_w occurred on agar without dextrose. In a second assay dead *H. hampei* were used as the substrate and conidia of all (1X105 sp/ml) isolates were suspended in EG and 0.1% Tween 80 to achieve even conidia distribution at a a_w of 0.97. Control suspensions for each isolate were made up without EG. Twenty insects were placed on a slide with the conidia suspension and maintained at 25 º C, 70% RH, 12:12 L:D in a Petri dish for five days. Thirty two isolates grew under these conditions but only 16 sporulated to produce more than 5×10^5 conidia/ml. One isolate did not sporulate (*B. bassiana* isolate reference number 17) and one *V. lecanii* isolate grew more at a a_w of 0.97 than in the control. Data from this experiment allowed us to select isolates that were likely to tolerate and develop at low water availability, a useful trait if they are to be developed for biological control.

STUDENT PAPER - Thursday, 11:00 (Viruses IV)

Comparative genomics and baculovirus phylogeny

Elisabeth Herniou[1&2], Teresa Luque [1], Ruth Finch [3], Doreen Winstanley [3], Jenny Cory [2], David O'Reilly [1]

[1] Department of Biology, Imperial College, London SW7 2AZ, UK.
[2] NERC Institute of Virology and Environmental Microbiology, Oxford, UK
[3] Horticultural Research International, Wellesbourne, Warwickshire, UK.

The recent publication of 6 baculovirus genome sequences has generated an unprecedented wealth of data, which is especially useful to molecular biologists. However they have so far not been utilised to the full for phylogenetic studies. In the light of the newly completed sequence of *Cydia pomonella* granulovirus (CpGV), we now compare 7 complete genomes reconstruct phylogenies based on this.

First the genomes are compared using gene parity plots which allow the visualisation of conserved gene arrangements between pairs of genomes like AcMNPV and BmNPV, and the outline of conserved gene

clusters between NPVs and GVs. However this approach doesn't provide any solid phylogenetic information for tree reconstructions.

We therefore tried a second approach based on classical cladistic methodology. A matrix recording the presence or absence of genes in each genome was generated and used for parsimony analysis. The resulting tree shows the evolution of the genomes with respect to gene acquisition and loss. However this method does not take into account gene arrangements. To investigate the phylogenetic signal present in the genome organisation, we used a method known as gene order breakpoint analysis.

Breakpoints in gene order provide a general measure of genomic distance, requiring no assumptions about the mechanisms of genomic evolution and have the additional advantage of being easy to calculate. Using relative breakpoint distances, we have reconstructed a baculovirus phylogenetic tree based on genome organisation

STUDENT POSTER BP38 - Thursday (Bacteria)

Biochemical mechanisms of resistance to Bt toxins in *Plodia interpunctella.*

Salvador Herrero[1], Brenda Oppert[2] and Juan Ferré[1]

[1]Departament de Genètica, Universitat de València, Dr Moliner 50, 46100 Burjassot (València), Spain. [2]USDA Agricultural Research Service, Grain Marketing and Production Research Center, 1515 College Ave., Manhattan, KS 66502.

Insect resistance to *Bacillus thuringiensis* (Bt) might be due to an alteration in any step involved in the Bt toxic process: solubilization of the crystals inclusions, trypsin-activation to the active form, binding of the toxin to the midgut receptor and pore formation. However, only changes in the midgut receptor or in the proteinase activities have been described in the resistant insects. These two mechanisms have been described independently in resistant colonies of the Indianmeal moth, *Plodia interpunctella* . The absence of a major gut proteinase implied in the toxin processing was detected in a colony (198r) selected with Bt-*entomocidus* HD198. We have observed that the 198r insects, in addition to show 250-fold resistance to Cry1Ab protoxin, also showed a 20-fold resistance to trypsin-activated Cry1Ab. These results suggest that impaired toxin activation is not the only resistance mechanism in this strain. Receptor binding studies with ^{125}I-Cry1Ab using brush border membrane vesicles of 198r larvae indicated slightly lower binding affinity and concentration of receptors. This binding alteration may account for the resistance of this colony to the activated Cry1Ab toxin. A different resistant colony (Dplr), for which proteinase alteration was not involved in resistance, showed a much lower ^{125}I-Cry1Ab binding affinity (58-fold compared with a susceptible colony) than the 198r colony. Experiments with ^{125}I-Cry1Ac did not reveal any difference in binding affinity or concentration of receptors among the three colonies.

CONTRIBUTED PAPER - Friday, 11:15 (Microsporidia II)

Physiological aspects of host-parasitoid-microsporidia interactions

Gernot Hoch[1], Michael W. Henn[2], and Axel Schopf[1]

[1] Institute of Forest Entomology, Forest Pathology and Forest Protection,
Universität für Bodenkultur, Hasenauerstr. 38, A-1190 Vienna, Austria;
[2] Institute of Applied Zoology, Technical University Munich, Am Hochanger 13,
D-85354 Freising, Germany

Both infection with the entomopathogenic microsporidium *Vairimorpha* sp. (Protista, Microsporidia) and parasitization by *Glyptapanteles liparidis* (Hym., Braconidae) influence growth and development of *Lymantria dispar* (Lep., Lymantriidae) larvae. In the first part of this study we investigated the extent to which parasitoid associated factors of *G. liparidis*, such as polydnavirus and venom, affect the development of microsporidia infected and uninfected hosts, and whether a parasitoid induced suppression of the host immune system can promote the microsporidiosis. To study the action of parasitoid associated factors, *G. liparidis* females were treated with γ-radiation resulting in oviposition of infertile eggs plus active polydnavirus and venom into the host. Both parasitism and polydnavirus/venom treatment alone caused a slight increase in growth rate in fourth instar larvae. *Vairimorpha* infection extremely reduced this parameter. Microsporidiosis caused a delay in host larval molts, while additional polydnavirus/venom treatment or parasitization induced significantly earlier molting. Polydnavirus/venom treatment of uninfected *L. dispar* resulted in prolonged larval development due to supernumerary molts and in higher pupal mortality. Infected larvae treated with polydnavirus/venom died earlier than infected larvae that were not treated and produced more *Vairimorpha* spores per unit fresh weight of the host. A *Vairimorpha* infection of *L. dispar* larvae negatively affects development of *G. liparidis* larvae without causing infection of the parasitoids. Due to this fact we began to study microsporidia induced changes in the composition of various nutrients in the host hemolymph. Preliminary data on such changes in the concentrations of free amino acids and trehalose, the main blood sugar, in the hemolymph as well as changes in the glycogen level of host tissues, caused by both parasitism and microsporidiosis are presented in this paper.

CONTRIBUTED PAPER - Monday, 11:45 (VIRUSES I)

Phenotypic variation between genotypic variants of a nucleopolyhedrosis virus attacking pine beauty moth, *Panolis flammea*

D.J. Hodgson[1], R.S. Hails[2] & J.S. Cory[3].

[1,2,3]CEH Oxford, Mansfield Rd., Oxford, OX1 3SR, United Kingdom.

Natural populations of the pine beauty moth, *Panolis flammea*, nucleopolyhedrovirus (*Pf*NPV) have been shown to contain high levels of genotypic variability, with individual host larvae containing at least 24 distinct viral genotypes. Four of these genotypic variants have been compared in bioassay experiments to ascertain whether this variation in genotype translates into differences in key phenotypic traits. Variants differed in their infectivity, speed of kill and yield upon death of the host, all factors which have the potential to profoundly influence host-virus interaction in the field. Such phenotypic variation ought to promote selection for the 'fittest' viral genotype: this begs the question why so much variation is observed in the field in this and other baculovirus systems. Coexistence of virus genotypes may be promoted by tradeoffs between phenotypic traits. Bioassay results were used to examine such tradeoffs, using mean phenotypic traits for each variant. There was a negative relationship between speed of kill and virus yield, but no correlation between these variables and infectivity. We discuss further ecological features of the *P. flammea* - virus interaction which may promote genotypic variation in the pathogen population.

SYMPOSIUM II - Monday, 17:30, Viruses

Inhibition of baculoviral disease mediated by phytochemicals

Kelli Hoover and Heidi Appel

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

Several studies demonstrate that both constitutive and inducible plant defenses can inhibit disease caused by baculoviruses. For example, viral-induced mortality of *Heliothis virescens* (tobacco budworm) larvae fed on cotton foliage can be up to 60% lower than insects fed on lettuce. Similarly, viral-induced mortality of *Lymantria dispar* (gypsy moth) larvae fed on red oak can be up to 60% lower than aspen-fed insects. These two tritrophic systems appear to share a common mechanism of inhibition -- redox cycling of foliar phenolics catalyzed by plant-derived oxidative enzymes. Where these systems differ is in the identity of the oxidative enzyme that is responsible for biological activity. In the cotton system, the biological activity against baculoviruses appears to be mediated by phenolic oxidation catalyzed by plant peroxidase, whereas in the oak system, inhibition appears to be mediated by phenolic oxidation catalyzed by polyphenol oxidase. This means that investigators interested in the tritrophic effects of plant phenolics should measure the activating enzymes in addition to the phenolics themselves.

From an ecological or agricultural perspective, both of these enzymes, and their substrates, are inducible in many systems, and thus may cause even greater inhibition of disease as feeding continues prior to death.

Thus, paradoxically, induction of "plant defenses" by insects can benefit the insect through attenuation of viral disease, despite the putative enhanced resistance of the induced state. For example, in the gypsy moth system, studies by Foster, Hunter, & Schultz demonstrated that the negative impacts of oak tannins on gypsy moth growth and fecundity are far outweighed by the positive impacts on survival in the presence of the virus. Although there have been no field studies of the agricultural relevance of plant-mediated inhibition of baculoviral disease on cotton, industry representatives have stated that this inhibition is a significant problem in their efforts to bring baculovirus biopesticides to the world market for control of noctuids on cotton. Increasing our understanding of the mechanisms of inhibition of baculoviruses by oxidative processes should lead to design of better formulations to reduce plant effects on the efficacy of biopesticides.

CONTRIBUTED PAPER - Thursday, 16:30, Bacteria V

Evidence of a new family of insecticidal proteins from similarity between *Serratia* and *Photorhabdus* gene products

Mark R.H. Hurst [1,2], Travis R. Glare [1] Trevor A. Jackson and Clive W. Ronson [2].

[1]Biocontrol and Biosecurity, AgResearch, PO Box 60, Lincoln, New Zealand
[2]Department of Microbiology, University of Otago, PO Box 56, Dunedin, New Zealand

Amber disease of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), can be caused by strains of either *Serratia entomophila* or *S. proteamaculans*. Previously, we have demonstrated that a large plasmid, designated pADAP, is the common factor shared by disease-causing strains and that expression of the plasmid in other Enterobacteriaceae confers disease-causing ability to the host cell. More recently, we have identified and sequenced a virulence-encoding region on pADAP. It consists of a ~17kb region with 9 open reading frames (ORFs). Three ORFS -*sepA*, *sepB* and *sepC*, show strong amino-acid similarity to recently described toxin proteins from the nematode-associated bacterium, *Photorhabdus luminescens* (Bowen *et al.* 1998), although phenotypically the effect on insects varies greatly between the two bacterial genera. One protein SepB, has amino-acid similarity to the *P. luminescens* TcaC toxin protein and the *Salmonella* virulence-enhancing factor SpvB. The translated products of the remaining ORFs in the *Serratia* virulence-encoding region have protein similarity to various *E. coli*, *S. typhimurium*, phage and transposon proteins, but appear to be unrelated to the disease function. We believe the similarity between proteins produced by *Serratia* and *Photorhabdus* points to a new family of insecticidal proteins, the action of which is yet to be completely determined.

Bowen, D., Rocheleau, T.A., Blackburn, M., Andreev, O., Golubeva, E., Bhartia, R., and Ffrench-Constant R.H. (1998) Insecticidal toxins from the bacterium *Photorhabdus luminescens*. *Science* **280:** 2129-2132.

SYMPOSIUM II - Tuesday, 11:10 (Bacteria)

Molecular characterization of *Bacillus thuringiensis* type strains.

Jorge E. Ibarra [1], Víctor Juárez-Pérez [2], Octavio Martínez [1], and Armelle Delecluse [2].

[1]CINVESTAV-IPN, 36500 Irapuato, Gto. México; [2]Bactéries et Champignons Entomopathogènes, Institut Pasteur, Paris, France.

The ever-increasing number of new isolates has proved the great diversity and wide distribution of *Bacillus thuringiensis* strains throughout the world. Although most of the isolates from nature show the typical bipyramidal crystal and a variable toxicity against lepidopteran larvae, many other strains show a great diversity of crystal morphology and insecticidal activity (if any). Many techniques have been developed to try to classify the different isolates. Among these, serotyping has been the most successful and standardized technique; however, it has limitations in establishing evolutionary relationships between strains. This report shows other alternative techniques that may help to understand possible evolutionary trends at the subspecific level. Three molecular approaches were used to determine relationships between the type strains from the Institut Pasteur *B. thuringiensis* Collection. Ribotyping was based on an RFLP-like genome analysis, using the rDNA operon as a probe, and digesting the *B. thuringiensis* genome with *Eco*RI. A dissimilarity matrix was constructed according to the "Simple Matching Coefficient", defined as the proportion of non-shared bands, and the method to construct dendrograms was the "Average Cluster" or UPGMA. A second approach was used, based on the variability of *Alu* I restriction profiles of amplicon SG-749, among *B. cereus* and *B. thuringiensis* strains. Digestion profiles were analyzed as described previously for the ribotyping. In a third approach, consensus oligonucleotides matching Repetitive Extragenic Palindromic (REP) elements were used in PCR trials, to produce amplification profiles. These profiles were also analyzed as described previously for the ribotyping. Although some variation was observed between the dendrograms obtained from the different techniques, in general, strains from the same serotype were grouped together. Also, some highly related strains but belonging to different serotypes, were grouped together (i.e. *israelensis* and *malaysiensis*). Likewise, some strains with highly different Cry proteins, but belonging to the same serotype were also grouped together (i.e. *morrisoni* type, *tenebrionis* and PG-14). Interestingly, three *B. cereus* stains and one *B. anthracis* were grouped among the *B. thuringiensis* stains. Other species, like *B. mycoides*, *B. megaterium* , *B. licheniformis* and *B. subtilis*, were separated from the rest of the strains, in that order.

POSTER FP19 - Thursday (Fungi)

Screening of stink bug infectious entomopathogenic fungi.

Fumio Ihara [1], Katsuhiko Yaginuma [2], Norio Kobayashi [3], Koji Mishiro [1], and Takeru Sato [1]

National Institute of Fruit Tree Science, 2-1 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan [1] & 92 Nabeyashiki, Shimo-kuriyagawa, Morioka, Iwate 020-0123, Japan [2]. Horticultural Institute, Ibaraki Agricultural Center, Sekijo, Makabe, Ibaraki 308-0125, Japan [3].

To develop a method for controlling stink bugs of fruit tree pest, entomopathogenic fungi with high pathogenicity against stink bug were selected. Fungi used were isolated from insect cadavers or from soil samples in various parts of Japan. Laboratory reared adult stink bug, *Plautia stali* SCOTT (Hemiptera: Pentatomodae), was used for bioassay. Ten stink bugs were put on a conidia forming plate and were transferred into a clean container. The pathogenicity was determined by counting dead insects for up to 10 days after contact with fungus at 25°C with conditions of high humidity and 16L/8D. Fungi that killed more than 90% of stink bugs within 4 days were decided as high virulence strain. From 718 isolates, 36 strains of *Beauveria* spp. and 20 strains of *Metarhizium* spp. were selected. From tested 82 strains of *Paecilomyces* spp., no high virulency was obtained. To determine LC_{50} (medium lethal concentration required to achieve 50% mortality) values of them, stink bugs were dipped into conidia suspensions for 5 min and reared under the same conditions as written above. After treatment for 7 days, *Metarhizium* spp. strain FRM515 and FRM569 showed the lowest LC_{50} values of $1 \times 10^{4.8}$ and $1 \times 10^{4.9}$ conidia/ml, respectively. *Metarhizium* strains were more virulent than *Beauveria* strains; the minimum LC_{50} value of *Beauveria* sp. was $1 \times 10^{5.7}$ conidia/ml of strain FRB205. When 1×10^{7} conidia/ml were used for the bioassay, the LT_{50} (medium lethal times required to achieve 50% mortality) values of FRM515 and FRM569 were 4.9 and 4.7 days, respectively. The pathogenicities against stink bug suggest that *Metarhizium* spp. would have potential to control stink bugs.

CONTRIBUTED PAPER - Monday, 17:00 (Bacteria II)

Expression of Cry1Aa receptor variants from silkworm in cultured mammalian cells

Satoshi. Ikawa, Yoko Tsuda, Takashi Fukada, Kenji Sugimoto and Michio Himeno

Department of Applied Biochemistry, College of Agriculture, Osaka Prefecture University,
1-1 Gakuen-cyo, Sakai , Osaka 599-8531, Japan

Crystalline protein bodies produced by *Bacillus thuringiensis* are highly toxicity to susceptible insects after ingestion by them. The protein

bodies are dissolved in their midgut and change to an active toxin (δ-endotoxin; Cry toxin). This Cry toxin binds to a protein in brush border membrane (BBM) of midgut epithelial cells, causing subsequently cell-swelling and finally specific insect mortality. Two proteins in the silkworm BBM, aminopeptidase N and a cadherin-like protein designated as BtR175 (Y. Nagamatsu *et al*., 1998, *Biosci. Biotechnol. Biochem*., 62: 718-726) are shown as a Cry1Aa-binding protein and a Cry1Aa - receptor protein, respectively. In order to determine a mode of action of the Bt-toxin, we report in this presentation cloning the receptor protein genes from silkworm, and expressions of the receptor genes in originally non-susceptible mammalian cells.

We independently isolated and purified a 180kDa Cry1Aa-binding protein in *Bombyx mori* BBM by affinity chromatography, which was found to be close related to BtR175 by analyzing its partial cDNA. Three variant genes of BtR175 cloned by PCR were named BtR175a, BtR175b,and BtR175c. Compared with BtR175, these variants, BtR175a, -b, and -c have 2, 29, and 26 base substitutions and 1, 6, and 5 amino acid substitutions, respectively. The amino acid substitutions were localized in the C-terminal half of extracellular region and Cry1Aa-binding region. These genetic heterogeneity of silkworm suggested that BtR175a, -b, and -c may be polymorphous alleles.

cDNAs of BtR175 a, -b, and -c protein were joining to an expression vector, pcDNA3.1(-)-Myc-His A (Invitrogen), adding the myc-epitope tag to the C-terminus of BtR175 genes. The reconstructed vectors carrying the BtR175 gene were transformed by electroporation into non-susceptible cultured mammalian cells; COS7, MDCK, and HEK293 cells. Expression of the BtR175 genes in the cells were observed with a fluorescent microscopy by Cy3- labeled anti-myc IgG antibody. BtR175b expressed in COS7 cells were detected by western blotting as a 180kDa protein. The expression of BtR175a and -b in COS7 cells were detected and BtR175b in MDCK and HEK 293 cells was also done, respectively. Moreover, BtR175b were expressed on the plasma membrane, ER membrane, and surface membrane of the COS7 cells. The BtR175b expressing on COS7 cell surface could bind to Cry1Aa toxin was detected by using the anti-Cry1Aa antibody.

CONTRIBUTED PAPER - Tuesday, 9:15 (Viruses II)

Functional analysis of the LdMNPV *hrf*-1 gene in recombinant AcMNPV-infected Ld652Y cells

Motoko Ikeda[1], Elizabeth A. Reimbold[2], and Suzanne M. Thiem[2,3]

Graduate School of Bioagricultural Sciences[1], Nagoya University, Chikusa, Nagoya 464-8601, JAPAN; Depts of Microbiology[2] and Entomology[3], Michigan State University, East Lansing, MI 48824

Ld652Y cells are non-permissive for *Autographa californica* M nucleopolyhedrovirus (AcMNPV). A gene, *hrf*-1, isolated from *Lymantria dispar* nucleopolyhedrovirus (LdMNPV) expands the AcMNPV host range to Ld652Y cells and *L. dispar* larvae. In cell culture *hrf*-1 precludes global protein synthesis arrest that is observed in AcMNPV-infected Ld652Y cells. The deduced amino acid sequence of *hrf*-1 provides no clues to suggest how it might function. In Ld652Y cells hrf-1 controlled by its own promoter was expressed beginning at 3 h pi and accumulated maximally by 12 h pi, but was then degraded. To analyze hrf-1 function we generated recombinant AcMNPV bearing mutated hrf-1, which were tested for their ability to rescue AcMNPV protein synthesis and virus production in Ld652Y cells. Mutations included amino- and carboxyl-terminal truncations, two-amino acid insertions, and specific amino acid substitutions. We also constructed and tested recombinant AcMNPV bearing a chimeric hrf-1 comprised of the carboxyl-terminus of LdMNPV hrf-1 fused with the OpMNPV hrf-1 homologue, which is truncated after 79 amino acid residues. Chimeric hrf-1 did not support AcMNPV replication indicating that the sequence of the amino-terminus of LdMNPV hrf-1 is important for its function. Most of the mutations in LdMNPV hrf-1 abolished the ability of hrf-1 to promote AcMNPV replication in Ld652Y cells. In addition most mutations dramatically reduced hrf-1 accumulation in Ld652Y cells suggesting that the conformation of hrf-1 is important for its stability. Changing aspartic acid residues to alanine residues within a highly acidic domain resulted in incomplete arrest of protein synthesis suggesting that the acidic domain may be important to hrf-1 function.

POSTER VP27 - Thursday (Viruses)

Localization of host range factor 1 (hrf-1) protein in Ld652Y cells infected with recombinant AcNPV bearing hrf-1 gene

Motoko Ikeda[1], Wade A. Williams[2] and Suzanne M. Thiem[2,3]

[1]Graduate School of Bioagricultural Sciences
Nagoya University, Chikusa, Nagoya 464-8601, Japan;
Department of Entomology [3] & Microbiology[2]
Michigan State University, East Lansing, Michigan 48824, USA

Autographa californica nucleopolyhedrovirus (AcNPV) infection induces protein synthesis shut down in Ld652Y cells, and this shut down is precluded by a gene, host range factor-1 (*hrf*-1) isolated from *Lymantria dispar* NPV (LdNPV). So far no similarity to genes and proteins with known functions has been indicated. To clarify the function of the hrf-1 protein, cellular localization was analyzed using HA-tagged hrf-1 recombinant AcNPV (vHAhrf-1). vHAhrf-1 was infected into Ld652Y cells. At 12hr post infection, cells were collected, fractionated and HAhrf-1 protein accumulation was analyzed by western blot by using an anti-HA antibody. HAhrf-1 protein was predominantly detected in the cytoplasm. Cellular localization of hrf-1 protein was further analyzed by stably transforming Ld652Y cell line expressing hrf-1 (Ld652Y-C9). This cell line allowed productive infection of wild type AcNPV (L1), indicating that cellular hrf-1 is able to preclude protein synthesis shut down induced by AcNPV infection. Western blot analysis by using an anti-hrf-1 antiserum showed that the hrf-1 protein in Ld652Y-C9 was predominantly localized in cytoplasm regardless of AcNPV infection. These results suggest that hrf-1 protein may function in cytoplasm by connecting with protein synthesis machinery. Over-expression of the hrf-1 protein by recombinant virus bearing the hrf-1 gene under p6.9 (cor) promoter (vCORHAhrf-1) showed abundant hrf-1 protein in infected cells from 0 hr postinfection. This suggests that hrf-1 protein is packaged into budded virus (BV) and transported to the infected cells. This result was further confirmed by co-purification of the hrf-1 protein with BV from Sf21 and Ld652Y cells infected not only with vCORHAhrf-1 but also with vHAhrf-1.

STUDENT POSTER VP28 - Thursday (Viruses)

Effects of host age and virus dosage on the yield of a nucleopolyhedrovirus in larvae of *Adoxophyes honmai* (Lepidoptera: Tortricidae)

Takayoshi Ishii, Madoka Nakai, and Yasuhisa Kunimi

Department of Applied Biological Science, Faculty of Agriculture,
Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan

The effects of host age and virus dosage on the yield of *Adoxophyes honmai* nucleopolyhedrovirus (AdhoNPV) were investigated in larvae of *A. honmai*. Neonate, newly molted 2nd-, 3rd-, 4th-, and 5th-instar larvae were infected by feeding them droplets of polyhedral suspension ($10^{5.5}$ to 10^{8} PIBs/ml) containing 10% sucrose and 5% red food coloring. Larvae that completely ingested a droplet of suspension were individually transferred into 1/2-ounce cups and observed daily for mortality. All virus-killed larvae were weighed and homogenized with a hand homogenizer. The yields of polyhedra were determined using a Thoma hemocytometer under phase-contrast microscopy.

Most of the larvae inoculated with AdhoNPV molted normally until the final instar and were killed 5 to 8 days after the final molt, regardless of the host age and virus dosage. The host age and virus dose did not significantly affect the number of polyhedra produced in virus-killed larvae. The average yield of PIBs was estimated as ca. 10^{10} PIBs/insect. To determine the post-infection increase in virus titer, neonate and newly molted 4th-instar larvae were infected with 2 × the LD_{95} of AdhoNPV. Although the virus titer in larvae infected at the 4th instar increased exponentially until day 8 post-infection, the virus titer in larvae infected at the 1st instar gradually increased. However, the final yield of PIBs in larvae infected at the 1st instar was no different from that in larvae infected at the 4th instar. The potential of AdhoNPV as a biological control agent will be discussed.

POSTER VP30 - Thursday (Viruses)

Expression of viral and cellular PCNAs in Sf9 cells infected with *Autographa californica* nucleopolyhedrovirus

Satoko Iwahori, Motoko Ikeda and Michihiro Kobayashi

Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Proliferating cell nuclear antigen (PCNA) has been shown to be a multifunctional protein that is involved in a variety of cellular functions including DNA replication, cell cycle control, and DNA repair. Previous studies have demonstrated that certain nucleopolyhedroviruses (NPVs) including *Autographa californica* NPV (AcNPV) possess *pcna* gene encoded on their genome. To explore possible functions of AcNPV *pcna* in viral replication, expression profiles of viral and cellular *pcna* were comparatively examined in Sf9 cells infected with AcNPV.

Northern blot analysis showed that AcNPV *pcna* was expressed as a single transcript of approx. 1.9 kb that was first detected at 4 h postinfection (pi), increased to a maximal level by 8 h pi, and then decreased gradually to a negligible level at 72 h pi. On the other hand, an approx. 1.6-kb single transcript assigned to Sf9 cell *pcna* was present in abundance in mock-infected Sf9 cells but decreased abruptly in AcNPV-infected Sf9 cells from 4 h pi. Western blot analysis was conducted with antibodies PC10 (Santa Cruz Biotechnology) and aAcpcna-N that was raised against a fusion protein expressed from pET expression vector with a 364-bp AcNPV *pcna* fragment. In AcNPV-infected Sf9 cells, antibodies PC10 and aAcpcna-N preferentially reacted with 37K and 33K proteins, respectively, thus indicating that the 37K and 33K proteins corresponded to Sf9 cell PCNA and AcNPV PCNA, respectively. The AcNPV PCNA was localized exclusively in nucleus of infected cells while Sf9 cell PCNA was present in both nucleus and cytoplasm. Temporal analysis showed that AcNPV PCNA was first detected at 8 h pi, peaked at 24 h pi, and then decreased very slowly. Cellular PCNA in the nucleus of infected Sf9 cells decreased abruptly at 24 h pi and remained at a negligible level throughout the experiment up to 96 h pi.

Experiments with Sf9 cells infected with *ts*8, a temperature-sensitive mutant defective in viral DNA replication at nonpermissive temperature, further showed that changing profiles of viral and cellular PCNAs at permissive temperature (25oC) were similar to those in wild-type AcNPV-infected cells while those profiles at nonpermissive temperature (33oC) differed strikingly from those in cells infected with wild-type AcNPV. Such characteristic PCNA profiles observed in AcNPV-infected Sf9 cells suggest strongly the participation of AcNPV PCNA in viral replication

POSTER FP3 - Tuesday (Fungi)

Liquid culture production of blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus* using portable fermentation equipment

Mark A. Jackson[1] and David A. Odelson[2]

[1]USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Fermentation Biochemistry Research Unit, Peoria, IL, USA; [2]EcoSoil Systems, Inc., San Diego, CA, USA

A prerequisite for the commercialization of microbial bioinsecticides is the development of cost-effective production and stabilization techniques. The on_site production of a bioinsecticidal propagule using portable fermentors would not only provide an optimally effective, freshly-produced propagule but also would eliminate the costs and product losses associated with the stabilization, storage and transportation of these living microbial agents. The fungus *Paecilomyces fumosoroseus* has shown excellent potential as a bioinsecticide for various soft_bodied insects including whiteflies, thrips and aphids. In this study, methodologies were evaluated and optimized for the liquid culture production of blastospores of *P. fumosoroseus* using portable fermentation equipment. The portable fermentation equipment used in this study allows for aseptic, disinfected, growth conditions. Significant technical constraints to the use of on-site fermentors for the production of fungal spores include requirements for medium concentration, inoculum stabilization, reduced fermentation times and reductions in the growth of unwanted fungal and bacterial contaminants. Results from these studies demonstrated that inoculations of as little as 1 X 10^6 spores/mL yielded high concentrations of blastospores (5-10 x 10^8/mL) of *P. fumosoroseus* in 48 hour fermentations with very low levels of bacterial contamination. The use of a pH adjusted, relatively rich complex medium provided an environment deleterious to bacterial growth yet conducive to rapid sporulation by *P. fumosoroseus*. These studies have demonstrated that potential exists for the on-site production of high concentrations of blastospores of the bioinsecticidal fungus *P. fumosoroseus* using disinfected, portable fermentation equipment.

SYMPOSIUM II - Tuesday, 9:15 (Bacteria)

Ecology of *Paenibacillus popilliae* and *Serratia* spp., pathogens of soil dwelling scarabaeid larvae

Trevor A. Jackson and Maureen O'Callaghan

AgResearch, PO Box 60, Lincoln, New Zealand

Milky disease caused by *Paenibacillus popilliae* and amber disease caused by certain strains of *Serratia* spp. are unique diseases of soil dwelling Scarabaeidae (Coleoptera). To cause disease, bacteria must be ingested by larvae while feeding. The soil, which is rich in microorganisms, is a reservoir for these pathogenic organisms. *P. popillae* (Bacilliaceae) multiplies through growth of vegetative cells in the insect haemolymph. Prior to insect death, bacteria sporulate, with spores and paraspores enclosed within a thick sporangium. Such bacteria are highly resistant to adverse conditions and can survive in the soil until stimulated to germinate by conditions in the insect gut. While incidence of the disease is usually low, favourable conditions can lead to epizootics. *Serratia* spp. (Enterobacteriaceae), in contrast, are nonsporeforming and numbers can fluctuate widely over time. *Serratia* spp. are common inhabitants of grassland and pasture soils. However, only two species *S. entomophila* and *S. proteamaculans* are known to cause amber disease in the New Zealand grass grub. Once ingested, grass grub pathogenic *Serratia* strains cause a cessation of feeding and multiply in the gut. After starvation and death of the insect host, there is a further stage of multiplication in the cadaver before bacteria are released into the soil. While numbers of pathogenic bacteria in the soil are generally related to cycles of amber disease, there is some evidence of saprophytic growth of these bacteria that can lead to enhanced levels of biocontrol.

POSTER FP16 - Thursday (Fungi)

The efficacy of nematophagous fungi against potato cyst nematodes in field soil.

Helen Jacobs[1,2], Simon N. Gray[2] and David H. Crump[1].

[1]Department of Entomology & Nematology, IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, UK.
[2] Faculty of Science, Technology and Design, University of Luton, Park Square, Luton, Beds., UK.

The efficacies of three nematophagous fungi, *Plectosphaerella cucumerina*, *Paecilomyces lilacinus* and *Verticillium chlamydosporium*, against potato cyst nematodes (PCN) (*Globodera* sp.) were tested in field soil from Jersey, UK. Trials were conducted in pots outside, using soil collected from a field that had been monocropped with potatoes for a number of years and contained a mixed population of PCN (*G. pallida* and *G. rostochiensis*) and an indigenous range of micro-organisms, and in the field in Jersey.

Plectosphaerella cucumerina and *P. lilacinus* were formulated into alginate pellets using bran as a nutrient source. In the pot trial, addition of 15 to 100 g of pellets per pot of either species of fungus reduced the Pf/Pi (ratio of final to initial population, indicating the degree of multiplication) of PCN by up to 15-fold compared to untreated controls. A greater reduction in Pf/Pi was observed when greater quantities of pellets were added. However, addition of 100 g of pellets resulted in lower tuber yields compared to 30 g of pellets. Addition of 15 g of alginated *P. cucumerina* in combination with 15 g of alginated *P. lilacinus* did not give improved control of PCN compared to addition of 30 g of either species alone, indicating that there is no synergistic effect on control of PCN between these two fungi. Application of 30 g per plant of alginate pellets containing either *P. cucumerina* or *P. lilacinus* was highly effective, resulting in a three-fold reduction of PCN multiplication compared to the untreated control.

Verticillium chlamydosporium chlamydospores were applied as a post-harvest soil drench, since a survey of fungal infection of PCN females in Jersey had shown that *V. chlamydosporium* infected the nematodes later in the season, when soil temperatures were higher. Soil sampled from both the field and from the out-door pot trial was inoculated with chlamydospores and incubated at 20°C. The number of viable PCN eggs in soil taken from the pot trial were significantly reduced by this treatment compared to the untreated control, but the treatment showed no effect in the soil taken from the field. This may have been due to a difference in the proportions of *G. pallida* and *G. rostochiensis* present in the samples, as *G. rostochiensis* is more likely to hatch spontaneously than *G. pallida* and is therefore more susceptible to infection by *V. chlamydosporium*.

CONTRIBUTED PAPER - Monday, 15:45 (Fungi II)

Effect of exogenous nutrients on conidial germination of two hyphomycetes and infectivity of germinated conidia

R. R. James

USDA-ARS Kika de la Garza Subtropical Agricul. Research Center
Weslaco, TX

The effects of three different sugars (sucrose, trehalose, and melezitose) and two sources of protein (peptone and yeast extract) on the germination rate of conidia from *Beauveria bassiana* and *Paecilomyces fumosoroseus* were tested. Conidia were either suspended in sterile liquid solutions or placed on solid agar containing only the tested nutrients. Germination was monitored every 2 hrs for 16 hrs. In liquid culture, sugars induced 5-27% germination for *B. bassiana*, and less than 11% for *P. fumosoroseus*; where as, yeast extract and peptone solutions induced 71-95% germination, depending on the concentration. The effects of peptone and yeast extract were very similar. For liquid culture, the maximum germination rate that was achieved in sterile water was less than 5% for both fungi. In contrast, *B. bassiana* had a germination rate of ~50% on water agar. Greater germination rates on agar may be due to a higher availability of oxygen, trace contaminants in the agar, or an ability of the fungus to utilize agar. When spores that had been germinated in yeast extract solutions were applied to 3rd instars of the silverleaf whitefly (*Bemisia argentifolii*), a greater proportion of insects became infected, and mortality occurred more rapidly than when ungerminated spores were applied. Soaking spores in water or adding yeast extract to ungerminated spores just before application did not have the same effect as applying pregerminated spores. An exogenous source of protein appears to be more important in initiating spore germination than sugars for these two fungi, at least for the two strains tested. Results concerning the effect of whitefly nymphal cuticle on germination of *B. bassiana* conidia will also be presented.

POSTER VP4 - Tuesday (Viruses)

Searching for nuclear polyhedrosis viruses in the natural populations of *Malacosoma neustria* L. (Lasiocampidae)

Jankevica L.[1], Kropa M.[1], Jankevics E.[2]

[1]Department of Experimental Entomology, Institute of Biology, University of Latvia, Miera street 3, Salaspils, LV 2169, Latvia, [2]LU Biomedical Research and Study Centre, Ratsupites street 1, Riga, LV 1067, Latvia

In nature, nucleopolyhedroviruses (NPV), which are frequently associated with outbreak or declining populations of Lepidoptera, cause diseases of insects and can control the population size of their hosts. Virus may persist within the host insect population in an occult or latent state. Generally natural epizooties caused by NPVs have been observed in most areas were host populations reach high density. The European tent caterpillar *Malacosoma neustria* L. is widely represented in the biocenosis of apple-gardens in Latvia. Few isolates of NPVs have been isolated from *M. neustria*, here in Latvia. The aim of our studies was to observe *M. neustria* populations, to search for NPV and to increase knowledge concerning their occurrence in insect populations. We have studied the possible ways of the spreading viral infections in the pest population area depending on pest population density, the ratio between infected and non-infected individuals. We used a sensitive technique of DNA amplification by the PCR usable for detecting DNA, and developed a PCR-based method that can detect the presence of polyhedrin-specific Mn NPV DNA, in the extracts of *M. neustria* larvae or imago. Monitoring of *M. neustria* populations in the South -Western part of Latvia near the Baltic see were done from 1995 till 2000. Five observation stations were placed in sites where population density of *M. neustria* was high. The population density of *M. neustria* was higher in 1995. In year 1996 density decrease for 27 %, and in next year the population density decrease drastically (79%). Therefore *M. neustria* population density in years 1998 and 1999 was very low. The imagoes collected from natural habitats and by suction traps were checked for presence the NPV. We found in year 1998, that 86-96 % of tested insects (collected in 5 monitoring stations) was NPV positive. Our observations showed that *M. neustria* populations in Latvia are infected with NPV.

CONTRIBUTED PAPER - Tuesday, 8:15 (Fungi III)

Screening Deuteromycete Fungi for the Control of Larval Fleas (Siphonaptera)

Stefan T. Jaronski[1], Rex Thomas[2], and Rebecca Lutz[1]

[1] Mycotech Corporation, Butte MT 59701, [2] Heska Corporation, Ft. Collins CO 80521

Deuteromycete fungi from larval flea habitats and culture collections were screened for commercial potential as mycoinsecticides for the environmental control of larval dog and cat fleas. Isolates were primarily *Beauveria bassiana*, but *Metarhizium anisopliae*, *Paecilomyces farinosus*, and *P. lilacinus* were also represented. All fungi were first passed through and reisolated from larval fleas. Ninety-one isolates were evaluated in terms of spore production in a bench-scale version of Mycotech Corporation's solid substrate production system. Spore production ranged from $1x10^{11}$ to $2.25x10^{13}$ conidia per Kg substrate under identical fermentation conditions. All isolates were also assessed in a two-tier bioassay, in comparison with Mycotech's Strain GHA. The bioassay consisted of exposing flea larvae to fungal conidia incorporated into Heska's proprietary larval flea medium. Mortality was assessed after 10 days. The first tier consisted of two doses, $5x10^7$ and $4x10^8$ conidia/gram larval substrate, which approximately represented the LC_{10} and LC_{50} of strain GHA. Each isolate was bioassayed at least twice. The best 10 isolates, based on their spore production and virulence, were further bioassayed three times using five doses straddling the LC_{50} of each isolate. The LC_{50}'s of these 10 isolates ranged from $4.5x10^7$ to $1.9x10^8$ conidia per gram of medium. A subset of 24 isolates, representing satisfactory spore production and virulence were also evaluated for shelf life of unformulated conidial powders in plastic vials held at 30, 35, and 40° C. Half-lives of these isolates ranged from 23 to >500 days at 30° C. 15-246 days at 35° C., and 7-164 days at 40° C. Two soil-derived *B. bassiana*, SC19 and SA8, were identified as potential commercial candidates based on a combination of virulence, spore production, and shelf life.

POSTER FP4 - Tuesday (Fungi)

Direct Spore Counts vs. Colony Forming Unit Counts As Methods for Quantifying Viable *Beauveria bassiana* Conidia

Stefan T. Jaronski and Judi Liebman

Mycotech Corporation, Butte MT 59701

Two different methods are generally used to quantify spores in fungal preparations. One technique is based on colony forming units (CFU) resulting from the plating of diluted spore suspensions on some agar medium. The second method uses direct counts of conidia in a diluted spore suspension with a hemocytometer. The counts are then adjusted by conidial viability determined by the percent spore germination after a standard incubation time on an agar medium. The purpose of this study was to determine the relative accuracy and precision of the two methods in estimating the viable conidial count of Mycotech technical grade *Beauveria bassiana* spore powder (TGAI) and two end product formulations, a wettable powder (Mycotrol 22WP) and an emulsifiable suspension (Mycotrol ES). Direct conidial counts, adjusted for conidial viability, were compared with a colony forming unit (CFU) method. Three replicate determinations were performed on each of three replicate samples of each formulation.

CFU-based data consistently underestimated the true viable conidial count of all three materials. Underestimates were as low as 16% of the direct count methods (TGAI suspended in 0.05% Tween 80). With 22WP and ES the CFU method underestimated true count by 58% and 50%, respectively. In addition, the precision and reproducibility of the CFU method, as indicated by the Coefficient of Variation of replicate determinations and replicate samples, was poorer for the CFU method, than for the adjusted direct count. Based on our observations we strongly advise against the use of CFU methodology to estimate viable conidial count, such as in shelf life studies or quality control. If circumstances require the CFU method, then allowance should be made for considerable underestimation and reduced precision.

CONTRIBUTED PAPER - Monday, 12:00 (Viruses I)

Genomic heterogeneity of *Cryptophlebia leucotreta* granulovirus is caused by intragenomic recombination of short sequence repeats (SSR)

Johannes A. Jehle

State Education and Research Centre Neustadt/Wstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany

Genetic heterogeneity within wildtyp baculovirus populations have frequently been reported. We have investigated in detail the genomic heterogeneity of three different genotypes of *Cryptophlebia leucotreta* granulovirus (CrleGV), namely that of CrleGV-CV3, CrleGV-CV3.1 and CrleGV-CV4. Aside from minor insertions and deletions these, genotypes differ in a highly variable region within restriction fragment *Eco*RI-H.

Complete sequencing of CV3-*Eco*RI-H revealed the presence of two large open reading frames, a homologue to PE-38 and another isoleucine/leucine-rich zinc finger protein, as well as a highly repetitive sequence stretch. This repetitive sequence consisted of short sequence repeats (SSR) of 27 nt length which are concatamerized in a head-to-tail manner. The repeats are neighboured by an AT-rich region. These SSRs do not resemble the well characterized baculovirus *hr* regions but appeared to be unusual and unique to baculoviruses. In ClGV-CV4 similar repeats but in differing grade of concatamerizations were found within EcoRI-H. In ClGV-CV3.1 a complete repeat structure of about 950 nt is duplicated. Further expansion of these repeat sequences within the ClGV genome were demonstrated by Southern hybridization. We propose that intragenomic recombination events lead to the observed repeat structures and the genome heterogeneity.

POSTER PP2 - Tuesday (Protozoa)

Molecular and morphological investigations on a microsporidium infecting the grape berry moth *Lobesia botrana*

Johannes A. Jehle[1], Charles R. Vossbrinck[2], Regina G. Kleespies[3]

[1]State Education and Research Center Neustadt/Wstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany; [2] Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06504, USA; [3] Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany

We have isolated a microsporidium of a laboratory stock of the grape berry moth, *Lobesia botrana*. Screening of the insect stock showed an infection in more than 90% of the individuals, whereas larvae collected from three different sites in Rhineland-Palatinate (Germany) did not demonstrate any signs of infection.

Light and electron microscopic investigations of infected insects showed that gross pathology, morphology and ultrastructure of the microsporidia are similar to those of *Pleistophora legeri* described earlier by Lipa (Bull. l´Acad. Pol. Sci. 1982, 29(7-8), 305-310). After isolating and purifying the microsporidia using a sucrose gradient, a PCR amplification of 16S rDNA sequences was performed. Sequencing and phylogenetic analysis using most parsimony revealed that our isolate was most closely related to *Endoreticulatus schubergi* and *Vitaforma corneae* (formerly *Nosema corneum*).

Based on our morphological and molecular investigations we will discuss to rename this species. (This investigation was supported by the MWVLW Mainz, Germany).

SYMPOSIUM - Thursday, 8:30 (Microbial Control)

Delivery of Biocontrol Technologies to IPM Farmers: Opportunities and Constraints

Nina Jenkins, Belinda Luke, Janny Vos, Stephanie Williamson, B Ali, D. Raj and Gary Hill.

CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berks, SL5 7TA, UK

The development of practical and cost effective biocontrol technologies (biopesticides, macrobiological control agents) for pest management has progressed more slowly than anticipated. Whereas the international commitment to IPM was expected to lead to a growing demand for biologicals for pest management, technology specific constraints, underdeveloped production and distribution chain and unfavourable regulatory procedures limit their market penetration.

The multinational crop protection industry, to which many looked for new biocontrol technologies, has not found these economic to develop. Consequently, it is the small to medium public and private sector enterprises that specialise in the production of biocontrol technologies. These enterprises are, however, faced with a number of problems. For example, inaccurate assessment of market demand resulted in the creation of excess capacity in some cases. In addition, for the majority of biopesticides, there are technology specific constraints such as lower speed of kill, costly storage and distribution and, complex application procedures, the critical benchmarks for evaluating biologicals against chemical pesticides. These factors have to a large extent contributed to the observed low levels of market penetration among the biopesticides.

A critical assessment of the constraints facing biocontrol strategies in order to identify the more rational ways of producing and delivering them is thus necessary. Towards this end, a study was undertaken by CABI Bioscience programme and UNEP in India, Nicaragua and Vietnam to identify the full range of limiting factors experienced by small scale biocontrol production and to consider ways in which these constraints and barriers to successful delivery to IPM farmers can be removed. A multidisciplinary team approach was employed to provide an appraisal of three representative pest, biocontrol manufacture and delivery systems. Each team included an in-country counterpart, a technical expert (biopesticides or macro-biological augmentation), a socio-economist and a farmer (IPM) trainer in a case study approach. The systems selected for study included fungal biopesticides for control of insect pest in Nicaragua, *Trichoderma* (a biopesticide – an antagonist) for the control of plant diseases in Vietnam and *Trichogramma* a parasitoid for the control of Lepidopterous insect pests in India.

Results indicate that although biocontrol strategies exist in all the three countries, the uptake is limited to farmers already trained in IPM methodologies. Local production units are mainly limited to the Universities and Research organisations. Available biocontrol products are of varied quality and clear quality control procedures are generally lacking. The need for cold chain delivery system in some cases raises the cost of biopesticides substantially. Also, the stringent registration requirements applied to chemical pesticides, although not strictly enforced in the three countries, are a potential hindrance to the uptake of biocontrol strategies. More farmers should be trained in the use of biocontrol through farmer participatory training, technical, start-up capital and training support required for the production and distribution units.

POSTER BP39 - Thursday (Bacteria)

Potentiation of *Bacillus thuringiensis* toxins by a metabolite from *Bacillus pumilus*.

Desmond R. Jimenez, Denise C. Manker, and Lori J. Lehman

AgraQuest Inc. 1530 Drew Avenue, Davis, CA 95616

Bacillus thuringiensis (Bt) is known to produce a variety of insecticidal toxins. Among them the crystalliferous ä-endotoxins, or Cry proteins, are the most widely studied. Approximately 70 different classes of Cry proteins have co-evolved toxicity toward insects yielding several dozen Bt-based biopesticides, a few registered recombinant Bt strains, and a number of transgenic crops. Combinations of Cry proteins, vegetative insecticidal proteins (VIP), Bt spores, and secondary

metabolites have shown synergy against some insect pests. The benefit of such complex mixtures offers intriguing advantages relative to resistance, persistence, and non-targets issues. The ureido amide Zwittermicin is a recognized metabolite that enhances the activity of a number of Cry toxins. Zwittermicin is present in many commercial Bt-based bioinsecticides and it has been shown to reduce the $LC_{50/90}$ resistance ratios in diamond back moth when present at high titer (Manker D.C., Lidster W.D., Starnes R.L. and Macintosh S.C. (1994) Potentiator of *Bacillus* pesticidal activity. Patent WO 94/09630). As the result of our search for insecticidal metabolites at AgraQuest, a water soluble, small molecular weight Bt enhancer has been isolated from the supernatant of a bacteria identified by 16S RNA as *Bacillus pumilus*. Whole broth supernatants have shown synergy with Bt (Javelin®) and reduced time to kill when tested against *Spodoptera exigua* and other lepidopteran pest species. In addition to the potentiation of Bt-based bioinsecticides, active fractions have shown potentiation with lyophilized plant tissue expressing transgenic Cry proteins.

POSTER VP8 - Tuesday (Viruses)

Sequence and organization of the *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (CfMNPV) genome

Jondavid de Jong[1], Clifford N. Dominy[1], Basil M. Arif[2], Hilary Lauzon[2] and Peter J. Krell[1]

[1]Department of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
[2]Canadian Forest Service, Sault. Ste. Marie, Ontario, P6A 5M7, Canada

The *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (CfMNPV) is an ideal candidate as a bioinsecticide to control eastern spruce budworm (*C. fumiferana*) outbreaks due to its narrow host-range. The CfMNPV genome was shotgun cloned and sequenced by primer walking. The 131 kb genome contains 137 putative open reading frames (ORFs) of greater than 150 bp, 10 of which have no previously reported baculovirus homologues. The putative protein products of the CfMNPV unique ORFs range in size from 103 to 303 amino acids with pI values between 6.8 and 12.1 and some may be involved in viral host specificity. CfMNPV has 93% of its genes in common with OpMNPV, 85% with AcMNPV and 61% with LdMNPV, indicating a relatedness to the group I baculoviruses. Genome parity analysis of CfMNPV compared to five sequenced baculovirus genomes (OpMNPV, AcMNPV, BmMNPV, LdMNPV and SeMNPV) indicates that the gene order of CfMNPV is collinear with the *Orgyia pseudotsugata* MNPV (OpMNPV) genome. Two areas of gene inversion were identified relative to AcMNPV. Although genome parity analysis and phylogenetic studies using conserved viral proteins indicates a closer relation to OpMNPV, the CfMNPV genome contains an LdMNPV enhancin-like ORF and remnants of the AcMNPV He65 ORF, both of which are not found in OpMNPV. Four homologous repeat regions (*hr*s) were identified throughout the CfMNPV genome, however CfMNPV appears to lack the non-homologous regions identified in other baculoviruses (OpMNPV, AcMNPV, SeMNPV and SpliMNPV). Each *hr* contains 4-9 copies of a conserved 27-30 bp palindromic sequence which demonstrates homology with the gene sequence for amino acids 38-48 of IE-2 (immediate early-2).

CONTRIBUTED PAPER - Thursday, 17:30 (Microbial Control I)

Storage of insect pathogenic nematodes of the genus *Heterorhabditis* at different pH-values and the influence on selected quality characteristics

Kerstin Jung

Federal Biological Research Centre, Institut for Biological Control, Heinrichstr. 243,
D-64287 Darmstadt, Germany

Infective juveniles (IJs) of heterorhabditid nematodes could outperform IJs of the related genus *Steinernema* against many economic important pests, like the black vine weevil. However, steinernematids are more often used in practice, because they are easier to produce and store than heterorhabditids. One of the major constraints in commercialisation of heterorhabditids is their bad storability. Existing storage techniques, suitable for steinernematids (e.g. immobilization in gels or clay), cannot avoid that heterorhabditids loose their ability to control insects (= quality). Some characteristics of the IJs are highly correlated with their quality and thus, may serve as a measure to control quality.

In two consecutive experiments, the influence of storage at different pH-values on selected characteristics of the nematodes was investigated. First, *Heterorhabditis megidis* (NL-H-F85) was stored at concentrations of 4 000, 8 000, 12 000 and 16 000 IJs/ml in potassium-phosphate buffer solutions of pH 2.5, 5, 7.5 and 10. In the second experiment, different isolates, representing different taxonomic groups [*H. megidis* (NL-H-F85, GB-H-Uk211, IRL-H-K122), *H. zealandica* NZH3 and *H. bacteriophora* HI82], were stored in potassium-phosphate buffer solutions of pH 5 and 10. The IJs were monitored in weekly intervals during 7 and 12 weeks, respectively at 20 °C. Percentages dead, dark (ample foodreserve present), ensheathed and active IJs were assessed visually. Also neutral lipid and glycogen content of the nematodes as well as the number of symbionts in the gut of the IJs were determined. Only during the second experiment, infectivity of IJs was assessed right after production, after 4 weeks of storage and at the end of the storage period using a bioassay with *Galleria mellonella*. The first experiment revealed that storage at pH 2.5 is detrimental to the IJs (≥ 75 % dead after 3 weeks). After 7 weeks, the lowest percentages dead IJs were generally achieved at either pH 5 or at a concentration of 12 000 IJs/ml. The second experiment showed that nematodes of different taxonomic groups respond differently to storage at both pH 5 and 10. In general, after storage at pH 5 more energy reserves were present, less IJs had exsheathed, infectivity was higher and the number of bacteria per IJ was lower. The results are discussed with respect to practical implications.

CONTRIBUTED PAPER - Monday, 14:30 (Fungi II)

First experiences with the development of a myco-insecticide based on *Beauveria brongniartii* against the field and forest cockchafer and related species

Kerstin Jung and Gisbert Zimmermann

Federal Biological Research Centre, Institut for Biological Control, Heinrichstr. 243,
D-64287 Darmstadt, Germany

Entomopathogenic fungi are potential candidates for biological control of scarabs, which are difficult to control with conventional methods. The economic importance of damage to various cultures (e.g. forests, apple orchards, nursery stocks) caused by the scarabaeid species, *Melolontha melolontha*, *M. hippocastani* and *Amphimallon solstitiale* is increasing in Europe. Therefore, there is a growing demand for efficient and environmentally friendly control measures. Within the framework of a European concerted action, the so called BIPESCO-Project (financed by the FAIR-program of the EU), first experiences with the development of a product based on the fungus *Beauveria brongniartii* were made.

Starting with a screening for virulent isolates, experiments for the optimisation of the production in liquid culture were performed and different formulations and drying techniques have been tested. The screening revealed that two isolates, currently available on the European market as products on barley kernels, are highly virulent agains melolonthids with 72 and 90 % mortality after three weeks in a bioassay. Additionally, new isolates with a promising high killing capacity (96 % mortality of grubs after three weeks) could be collected. On liquid media already approved of production of either *Beauveria brongniartii* and *B. bassiana*, practically feasible numbers of spores ($>1x10^9$ blastospores/ml) could be reached. Generally, freeze-drying, spray-drying and fluid-bed-drying of blastospores of *B. brongniartii* is possible. Survival depends on the addition of protecting agents. More results from the screening to the formulated pre-product are presented and discussed with respect to their transfer into practice.

STUDENT POSTER BP40 - Thursday (Bacteria)

Mechanism for high levels of resistance against *Bacillus thuringiensis* δ-endotoxins in *Heliothis virescens*.

Juan L. Jurat-Fuentes[1], Fred L. Gould[2], and Michael J. Adang[1]

[1]Department of Entomology, University of Georgia, Athens, GA, 30602, USA; [2]Department of Entomology, North Carolina State University, Raleigh, NC, 27607, USA.

Insecticides based on the parasporal insecticidal Cry proteins synthesized by the bacterium *Bacillus thuringiensis* (Bt) are considered the most important alternative to traditional synthetic insecticides. These Cry toxins posses a unique mode of action. After ingestion the toxins are solubilized and activated by midgut enzymes. Active toxins bind and insert on the membrane of the columnar cells of the midgut epithelium forming pores that result in impaired permeability and cell lysis due to osmotic shock.

One of the major concerns regarding the use of Bt insecticides is the observation that target insects can become resistant. Resistance to Bt insecticides has been reported to occur in wild populations as well as in laboratory selected insects. Although several mechanisms of resistance have been proposed, the alteration of binding to the specific receptors in the midgut is the most common.

Heliothis virescens is an important pest in the US, causing extensive damage to cotton and other crops. To control this insect, in 1996 transgenic cotton expressing Bt Cry1Ac toxin was introduced in the US.

A *Heliothis virescens* strain (YHD2) under laboratory selection against Cry1Ac (the most active Bt toxin against this insect) developed high levels of resistance to Cry1Ac and high levels of cross-resistance to other toxins including Cry1Aa, Cry1Ab and Cry1Fa. This resistant strain has been continuously selected with Cry1Ac toxin since 1994, and the levels of resistance have increased through this time.

We show that a dramatic decrease in toxin binding and pore formation to resistant BBMV are responsible for the high levels of resistance and probably cross-resistance observed in this strain. However, the same binding molecules for Cry1A toxins on BBMV from both strains were identified in ligand blots and affinity purification.

The molecular mechanism involved in the reduction of toxin binding is being investigated.

POSTER VP5 - Tuesday (Viruses)

Development of *Hyphantria cunea* nucleopolyhedrovirus for microbial control: cloning and biological characterization

K. Kamiya[1], N. Okimoto[2], N. Shirata[2], S. Kawamura[1], M. Ikeda[2], & M. Kobayashi[2].

[1]Gifu Prefectural Institute for Bioindustrial Technology, Minokamo, Gifu 505-0004, Japan; [2]Laboratory of Sericulture and Entomoresources, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-0814, Japan.

The fall webworm, *Hyphantria cunea* is a serious insect pest of roadside trees and some fruit tree. The use of *H. cunea* nucleopolyhedrovirus (HcNPV) as a control agent for *H. cunea* larvae is considered. Twenty HcNPV clones were derived, by plaque assay on SpIm (*Sphilosoma imparilis*) cells, from an uncloned population of HcNPV isolated in the field in Japan. Analysis of genomic DNA by restriction endonucleases (REN) revealed that nineteen among the twenty clones were different in their REN profiles, suggesting the presence of a very wide genotypic heterogeneity of HcNPV in the field. Four clones (N4, N6, N7 and N9) were selected on the basis of BV productivity, and compared their biological activity, polyhedrin synthesis and viral DNA replication. The peroral bioassay for *H. cunea* larvae showed that N9, which had the highest BV productivity among the four clones, exhibited the highest pathogenicity. Southern blot and Western blot analyses showed that both viral DNA and polyhedrin were detected earlier in N9 than in the other HcNPV clones. These results suggested that the efficient accumulation of viral DNA and polyhedrin observed in the infection with N9 was reflected in the pathogenicity and BV productivity. We select HcNPV N9 as the most distinctive strain for microbial control agent. The proliferation of N9 in suspension culture was investigated. SpIm cells were adapted to suspension condition with 200-ml Erlenmeyer flask. SpIm suspension cultures in different growth phase were respectively infected with N9. The yield of polyhedra examined at 7 days postinfection showed that the yield dramatically decreased, as the phase of inoculation were delayed. When the suspension cultures were infected at varying initial cell densities, it was found that the yield of polyhedra was higher at low cell density ($2.5X10^5$ cells/ml) than at high cell density ($7.5X10^5$ cells/ml). These results suggested that the reduction of polyhedra productivity observed in the infection at the mid- or late-exponential growth phase was caused factor due to high cell density.

STUDENT PAPER - Monday, 16:30 (Bacteria II)

Ionic selectivity of the pores formed by the *Bacillus thuringiensis* insecticidal toxins Cry1Aa and Cry1Ac in midgut brush border membrane vesicles

Martin Kirouac[1], Vincent Vachon[1], Jean-François Noël[1], Frédéric Girard[1], Jean-Louis Schwartz[1,2] and Raynald Laprade[1]

Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1] and Biotechnology Research Institute, National Research Council, Montreal, Quebec[2], Canada

The pores formed by *Bacillus thuringiensis* toxins in the midgut apical membrane of susceptible insects are generally thought to be cation-selective. They have nevertheless been shown to allow the diffusion of a variety of monovalent cations and anions and neutral solutes accross the membrane. To further characterize the selectvity of these channels, the membrane permeability induced by Cry1Aa and Cry1Ac to divalent cations (Mg^{2+}, Ca^{2+} and Ba^{2+}) and anions (SO_4^{2-} and HPO_4^{2-}) and to a variety of amino acids (Asp, Leu, Ser, His, Lys and Arg) was analyzed, at pH 7.5 and 10.5, with the use of midgut brush border membrane vesicles, isolated from *Manduca sexta*, and an osmotic swelling assay based on light-scattering measurements. Membrane permeality to $CaCl_2$, $BaCl_2$ and K_2SO_4, whose ionization state is independent of pH, was similarly high at both pH values, indicating little effect of pH on the properties of the pores formed by both toxins. In the presence of either toxin, membrane permeability was higher for the chloride salts of divalent cations than for the potassium salts of divalent anions, in agreement with a cation selectivity of the channels. In general, shifting pH from 7.5 to 10.5 increases greatly the proportion of the more negatively-charged species for the amino acids and phosphate ions. All amino acids diffused well across the toxin-induced pores, at both pH values, although the permeability to the larger amino acids (His, Lys and Arg) was somewhat lower at the higher pH. These results indicate that the cation selectivity of the channels is relatively weak. They also allow a reevaluation of previously reported differential effects of *B. thuringiensis* toxins on the K^+-dependent transport of different amino acids into midgut membrane vesicles which suggested a direct inhibitory interaction of the toxins with amino acid co-transporters in the midgut apical membrane.

POSTER BP15 - Tuesday (Bacteria)

Analysis of the properties of *Bacillus thuringiensis* insecticidal crystal toxins using a potential-sensitive fluorescent probe

Martin Kirouac[1], Vincent Vachon[1], Sébastien Rivest[1], Jean-Louis Schwartz[1,2] and Raynald Laprade[1]

Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1], and Biotechnology Research Institute, National Research Council, Montreal, Quebec[2], Canada

A potential-sensitive fluorescent probe, diS-C_3(5), was used to analyze, at pH 7.5 and 10.5, the effects of *Bacillus thuringiensis* toxins on the membrane potential generated by the efflux of K^+ ions from brush border membrane vesicles purified from the midgut of the tobacco hornworm *Manduca sexta*. Fluorescence levels were greatly influenced by the pH and ionic strength of the media. The effect of these factors were taken into account by maintaining the ionic strength relatively high, constant and equal on both sides of the membrane. Addition of valinomycin greatly increased the potential generated by the diffusion of K^+ ions although membrane permeability to the other ions used to maintain the ionic strength constant was clearly not negligible. In the absence of valinomycin, the toxins had little effect on the small membrane potential generated and thus little influence on the ionic selectivity of the membrane. On the other hand, in the presence of valinomycin, active toxins efficiently depolarized the membrane. These results clearly demonstrate that *B. thuringiensis* toxins not only increase membrane permeability to K^+, but also to other ions. The activity of Cry1Aa, Cry1Ab, Cry1Ac, Cry1B and Cry1E correlated well, at both pH values, with their pore-forming ability evaluated using an osmotic swelling assay.

However, the decrease in pore-forming ability observed for Cry1C at high pH, using the osmotic swelling assay, was not observed with the fluorescence assay. This result strongly suggests that the effect of pH on toxin activity could be influenced by other factors such as membrane potential and ionic strength.

SYMPOSIUM I - Monday, 15:00 (Viruses)

Actin rearrangement during baculovirus infection: The role of Arif-1

Dagmar Knebel-Mörsdorf

Max-Planck-Institute for Neurological Research and Department of Neurology, University of Cologne, Cologne, Germany

In cell culture cells the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcMNPV) induces differential rearrangement of the actin cytoskeleton during the infection cycle. Studies on the functional role of the viral-induced changes suggest the early actin cables to be involved in the transport of the nucleocapsids to the nucleus, and nuclear F-actin to form a scaffold for nucleocapsid assembly. We have identified an early gene of AcMNPV, designated actin rearrangement inducing factor 1 (arif-1), that is involved in the remodelling of the actin cytoskeleton prominently at 6 h p.i. The causal link between Arif-1 expression and actin rearrangement at 4 to 10 h p.i. has been demonstrated by AcMNPV recombinants that express mutated forms of the Arif-1 protein. Infection with the arif-1 mutant viruses leads to the loss of actin accumulation at the plasma membrane in TN-368 cells, although in the course of infection, early actin cables and nuclear F-actin are observed as in wild-type virus infected cells. By immunofluorescence studies, we have shown the localization of Arif-1 at the plasma membrane , and confocal imaging reveals the co-localization to F-actin. Accordingly, the ~ 47 kDa Arif-1 protein is detectable exclusively in membrane fractions prepared at 4 to 12 h p.i. with a decrease at 24 h p.i. Phosphatase treatment suggests modification of Arif-1 by phosphorylation. Further studies provide evidence that Arif-1 is phosphorylated at tyrosine residues from 4 to 12 h p.i. and that additional phosphorylation might occur during the late phase of infection. We assume that Arif-1 is not only tyrosine-phosphorylated but that Arif-1 expression induces tyrosine-phosphorylation of cellular proteins. These results lead us to suggest that Arif-1 is involved in a signal transduction pathway that results in actin rearrangement.

Since the arif-1 mutant viruses are viable in cell culture, the infection cycle of budded viruses in permanent cell lines is not impaired. The phenotype after infection of Heliothes virescens larvae with the mutant viruses will be discussed.

(supported by the Deutsche Forschungsgemeinschaft and the EC-Biotech Program)

STUDENT POSTER PP10 - Thursday (Protozoa)

Preliminary results on the occurrence of microsporidia in the pine bark beetles *Tomicus piniperda* and *Tomicus minor* (Coleoptera, Scolytidae)

B. Kohlmayr*[1], R. Wegensteiner*[1], J. Weiser*[2] and Z. Zizka*[3]

Institute of Forest Entomology, Univ. BOKU-Vienna*[1]
Institute of Entomology, CAV Ceske Budejovice*[2]
Institute of Microbioloy, CAV Prague*[3]

The pine bark beetles *Tomicus piniperda* and *Tomicus minor* are major tree pests, particularly of *Pinus sylvestris* (Scots pine). They are found troughout Europe, from the far north (Finland, Norway, Sweden) to the Mediterranean (France, Greece, Italy, Spain and North Africa). In the 1980[ies] *T. piniperda* was introduced to North America. So the whole European and North American continent as well as China has varying degrees of problems with these bark beetle species in Scots pine.

The main injuries caused to pines by these two insect species are due to maturation feeding by young beetles in one-year-old twigs, and regeneration feeding by adult beetles in two-year-old twigs. These activities result in twig death and weaken the trees, so that they become susceptible to stem invasion by these bark beetles for breeding. *T. piniperda* and *T. minor* construct their galleries in the bark of the stem after boring an entrance hole to the surface of the sapwood. The construction of egg galleries by adults, together with subsequent larval feeding, girdles and kills host trees.

Starting in 1999, bark beetles were sampled in managed forests from 14 stands in eastern Austria, from five stands in the Czech Republic, from two stands in Greece, from two stands in the USA, and in addition from one stand in Finland, Italy and Poland. Logs of densely infested trap trees were incubated in breeding chambers in the laboratory (24 ±1°C, L:D = 16:8). All emerging beetles were removed daily and stored at 15°C (at maximum one week) until they were inspected microscopically. With exeption of the North American beetles, only living adults were dissected. Pathogen occurrence was investigated in all tissues: native, as well as after fixation with Methanol and staining with Giemsa´s dye under normal light microscope.

The study revealed the evidence of microsporidia in *T. piniperda* from the Czech Republic, Finland, Poland, USA and also from Austria. Masses of microsporidian spores were found free in the haemolymphe, in the fat body, in the cells of the midgut epithelium and in some cases in the gonads. Infection rates varied from 2,2 to 32,7%. Furthermore a *Gregarina* species was found in *T. minor* from Lower Austria in the former part of the midgut lumen (0,3% infection).

SYMPOSIUM II - Tuesday, 10:50 (Bacteria)

***Bacillus thuringiensis, Bacillus cereus* and *Bacillus anthracis*: one species based on genetic evidence**

Anne-Brit Kolstø, Erlendur Helgason, Ole Andreas Økstad, Dominique Caugant and Ida Hegna.

The Biotechnology Centre of Oslo and Institute of Pharmacy, University of Oslo, and National Institute of Public Health, Oslo, Norway

Bacillus thuringiensis, *B. cereus* and *B. anthracis* are Gram positive bacteria. Their 16S rRNA is identical or differ by only a few bases – yet these species have widely different phenotypes. *B. thuringiensis* is the most widely used biopesticide due to its production of insecticidal Cry toxins, and the genes for these toxins are usually present on plasmids. *B. cereus* is a common soil bacterium and a common contaminator in dairies and in hospitals. More than half of the strains produce one or more enterotoxins that cause diarrhoea in man, and some strains appear to be the cause of more serious infections in humans. *B. anthracis* is the cause of anthrax in man and animals, and two large plasmids (pXO1 and pXO2) are essential for the high toxicity of this species.

In order to increase our understanding of the genetic relationship of these species, we have analysed strains by multilocus enzyme electrophoresis, determining the allozyme pattern of 13 housekeeping genes. A dendrogram has been prepared showing the relationship between the strains. In addition, we have sequenced nine genes, selected because they are located in different parts of the chromosome. The sequencing has been performed for 5 strains from one cluster (one *B. anthracis*, 2 *B. cereus* and 2 *B. thuringiensis* strains).

The results clearly show that *B. thuringiensis* strains are often more closely related to *B. cereus* than to other *B. thuringiensis* strains. *B. anthracis* strains are highly related to each other, but they all cluster within a cluster of *B. cereus* and *B. thuringiensis* strains. One *B. anthracis* strain is more related to some *B. cereus* and *B. thuringiensis* strains than to the strains within the *B. anthracis* cluster. We conclude that *B. thuringiensis*, *B. cereus* and *B. anthracis* are one species.

The consequences of these results will be discussed.

SYMPOSIUM - Tuesday, 11:20 (Nematodes)

Interactions between entomopathogenic nematode species and other agents

Albrecht M. Koppenhöfer

Department of Entomology, Rutgers University, New Brunswick, NJ 08901

Control of soil dwelling insect pests by entomopathogenic nematodes (EN) may be improved if they are combined with compatible control agents. (1) Combinations of two EN species usually result in additive pest mortalities. The nematode can co-infect a host but only one nematode's symbiotic bacterium will colonize the host, usually excluding

development of the other nematode. Even if the other nematode can develop on the other nematode's bacterium, its infective juveniles will not be able to retain the bacterium. (2) EN and entomopathogenic fungi exclude each other's development within the same host individual. Unless the fungi infect the host several days before the addition of EN, the EN exclude the fungi. EN tend to avoid fungus-infected hosts. (3) EN can infect virus-infected hosts. However, in the case of a granulovirus infection, EN progeny numbers are reduced due to resource competition. In the case of a nucleopolyhedrovirus infection, the fragile host integument ruptures before the nematodes can complete development thus exposing them to desiccation. When applied simultaneously, EN and NPV produce additive mortality in *Spodoptera exigua* larvae in laboratory and field studies. (4) EN and *Bacillus thuringiensis* (*Bt*) co-infect hosts but EN development is adversely affected and usually no EN progeny is produced. In 3rd-instar white grubs, *Bt* Buibui strain and EN produce synergistic grub mortality, especially if applied against early 3rd instars. (5) White grubs infected with *Paenibacillus* (*Bacillus*) *popilliae* (milky disease) show a synergistic increase in EN susceptibility, at least in part, because of accelerated EN penetration through the midgut. (6) EN interact synergistically with the insecticide neonicotinoid imidacloprid in 3rd-instar white grubs. The degree of interaction varies with EN species. The major factor responsible for this synergism is reduced grub activity after exposure to sublethal imidacloprid concentrations, which facilitates EN host attachment and penetration. Imidacloprid has no negative effect on EN reproduction and fitness of the progeny. (6) EN and the pyrethroid insecticide tefluthrin produce synergistic corn rootworm mortality in the laboratory.

SYMPOSIUM IV - Friday, 9:05 (Bacteria)

The role of *Bacillus sphaericus* in vector control programs

Steve Krause

Valent BioSciences Corporation, 870 technology Way, Libertyville, Il 60048, USA

Bacillus sphaericus is a gram-positive, endospore-forming bacteria that possesses effective insecticidal activity against some species of mosquito larvae. *B. sphaericus* produces protein crystals during sporolation which are activated in the larval mosquito midgut, often resulting in rapid feeding cessation and death within 48 hours. It is particularly effective in providing extended residual control of *Culex* spp. in highly organic waters. However, exclusive use of *B. sphaericus* in relatively isolated mosquito populations can induce resistance that may limit the utility of this bacteria in vector control programs. A risk-benefit analysis for *B. sphaericus* to vector control programs, gaps in our research knowledge, and regulatory issues affecting biological larvicides will be discussed.

SYMPOSIUM II - Monday, 17:10 (Viruses)

The spatial and temporal dynamics of the prevalence of nuclear polyhedrosis in a field population of the mulberry tiger moth, *Spilosoma imparilis* (Lepidoptera: Arctiidae)

Yasuhisa Kunimi

Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan

The spatial and temporal dynamics of the prevalence of a nucleopolyhedrovirus (SpimNPV) in a field population of the mulberry tiger moth, *Spilosoma imparilis*, were investigated on Hachijo Island, Japan. Investigation of the seasonal change in prevalence in this species showed that overwintered old larvae infesting plants near the surface of the soil were highly infected with SpimNPV, while pre-overwintered larvae (1st to 5th instar) fed on arboreal leaves were rarely infected with SpimNPV. The soil collected from the habitat of this species contained nuclear polyhedra capable of infection. Modeling suggested that the foliage of plants near the ground could be contaminated when soil containing polyhedra was splashed onto leaves by rain and wind. The prevalence of SpimNPV in overwintered larvae was significantly different among the local populations. The susceptibility of larvae in local populations to SpimNPV was roughly equal, as was the infectivity of SpimNPV derived from each local population. The prevalence of SpimNPV in overwintered larvae also changed from year to year. The prevalence increased in proportion to the population density of overwintered larvae. Horizontal transmission of SpimNPV from infected larvae to healthy larvae occurred with cannibalism of dead larvae by healthy larvae. Models showed that the transmission rate increased with the initial larval density. Based on these results, population density and habitat selection of larvae seem to be important factors affecting the temporal and spatial dynamics of the prevalence of SpimNPV in natural populations of *S. imparilis*.

POSTER NP5 - Tuesday (Nematodes)

Development of entomopathogenic nematodes for control of codling moth in orchards and fruit bins

Lawrence A. Lacey, Thomas R. Unruh, and Richard Chauvin

Yakima Agricultural Research Lab, USDA-ARS, Wapato, WA 98951 USA

Entomopathogenic nematodes (EPNs) have potential as biological control agents of overwintering codling moth larvae under orchard conditions. The tree trunk habitat of prepupae offers a protected environment in which infective nematodes can better survive while searching for and infecting cocooned codling moth larvae.

Fruit bins comprise a significant source of codling moths in the Pacific Northwest. Treatment of bins for control of overwintering codling moth is not currently done. Our research demonstrates that entomopathogenic nematodes can provide effective and affordable control of codling moth larvae in fruit bins.

The most important factors that influence efficacy of EPNs for codling moth control include the species of EPN, temperature and critical exposure time. *Steinernema feltiae* and *S. carpocapsae* are among the most active against codling moth larvae. Treated larvae are controlled when moisture is maintained for 8 h or longer. Temperature below 15ºC reduces the larvicidal activity of *S. carpocapsae*, but *S. feltiae* remains active below 10°C.

Orchard trials of *S. carpocapsae* were conducted under various moisture conditions in 1997-1998. Application of 10^6 IJs/tree using a hand-gun sprayer provided 95% mortality when trees were wetted before application of nematodes and at regular intervals for 6 hours after treatment. Field trials in 1999 compared the efficacy of *S. carpocapsae* and *S. feltiae* using an airblast sprayer and hand-gun sprayer for application of 2×10^6 IJs/tree. Good control was obtained using *S. feltiae* with both sprayers and fair to moderate control was obtained with *S. carpocapsae*. Trials were also conducted in September and October using a back pack sprayer. Moisture was maintained as in the summer trials. Both species performed optimally in September when temperatures were between 18 and 23°C during the six hours following treatment. However, in October temperatures were significantly lower and *S. feltiae* was more efficacious than *S. carpocapsae*.

Bin tests. Immersion of bins in suspensions of *S. carpocapsae* ranging from 5 to 100 infective juveniles/ml of water resulted in 68-100% mortality. Comparable control was obtained with the two nematodes species.

SYMPOSIUM - Thursday, 11:30 (Microbial Control)

The use of a baculovirus (*Phthorimaea Operculella* granulosis virus) in rustic storage facilities, to manage the potato tuber moth: Case studies.

Abdelaziz Lagnaoui

International Potato Center (CIP), Apartado 1558, Lima 12 – Peru.

Potato tuber moth, *Phthorimaea operculella* (Zeller), is the most economically important potato pest in developing countries where adequate cold storage facilities are lacking. The problem is more serious in warmer climates where this pest can cause up to 100% loss in traditional stores. Potato grower response is unilateral. Frequent insecticide applications is a routine operation, resulting in 4 to 6

applications over the growing season and 2 applications over the storage season.

The present problems with Insecticide resistance and residues in the environment have encouraged pest management specialists to seek new approaches to potato tuber moth management. The International Potato Center developed an Integrated Pest Management strategy to control this pest in several developing countries. The management strategy includes the use of the Baculovirus PoGV in storage facilities. In this paper, we will outline the past, present and future status of the potato tuber moth granulosis virus (PTM-GV) as a microbial insecticide and as a component of an integrated pest management strategy. The discussion of the potential role of the virus is based on its pathogenicity, specificity, safety and compatibility with other control components. The Baculovirus was introduced as an alternative to pesticide use for potato tuber moth control. The Baculovirus is currently being produced in cottage-type enterprises in Peru, Bolivia, Colombia, Tunisia and Egypt.

CONTRIBUTED PAPER - Monday, 14:00 (Fungi II)

Developing *Metarhizium anisopliae* for termite control in Africa: Strain selection and first field trial results

J. Langewald [1], A. Bokonon-Ganta [2], W. Gitonga [3], C. Kooyman [4], J. Maniania [5]

IITA (PHMD) Benin, B.P. 08-0932, Cotonou, Benin[1]; 2 Service de Protection des Végéteaux, Porto Novo, Benin[2]; Kenya Agricultural Research Institute, Nairobi, Kenya[3]; CABI Bioscience, Africa Regional Centre Nairobi, Kenya[4]; International Centre of Insect Physiology and Ecology, Nairobi, Kenya[5]

Samples from soil, and from termite mounts were collected in Benin and Kenya. Several strains of *Metarhizium anisopliae* were isolated and their virulence against two termite species compared in bioassays. The novel strains were compared with three well-known standard strains from Kenya, the U.S. and Australia. None of the novel strains was significantly more virulent than the Kenyan standard strain. First field trials started in maize fields during this summer's growing season, using formulations of the Kenyan standard strain as seedbed treatments to explore the repellent action of the fungus against termites. Infecting termite colonies directly, by blowing dry spores into the center of the mound gave promising results and reduced termite activity.

POSTER PP3 - Tuesday (Protozoa)

Free radicals generation in the hemolymph of *Anopheles albimanus* and their effect against *Plasmodium berghei* ookinetes.

Humberto Lanz–Mendoza, Salvador Hernández, Magdalena Ku, Avigail Gil, Uzziel Roman, María del Carmen Rodríguez and Mario H. Rodríguez.

Centro de Investigaciones sobre Enfermedades Infecciosas. Instituto Nacional de Salud Pública. Cuernavaca, Morelos. México. CP 62508.

The mechanisms of *Plasmodium* elimination in resistant mosquitoes are not completely understood. The arrest of parasite development has been related to the production of nitric oxide (NO) and melanization. Some resistant strains of anophelines are able to melanized *Plasmodium* in the midgut .Given the fact that quinoid compounds are known to be potent catalysts for free radicals (FR) generation and since these radicals can be generated in association with melanogenesis, it is probable that they play an important role in the elimination of *Plasmodium.* In the present study we analyzed the production of FR in the hemolymph of *Anopheles albimanus* female mosquitoes and their cytotoxic effect on *Plasmodium bergehi* ookinetes. Inoculation of ookinetes in the hemocel of *An. albimanus* mosquitoes were melanized in 1 h. The parasites were covered by melanine and then encapsulated by hemocytes. The presence of superoxide anion (O^{-}_{2})in hemolymph was verified by the reduction of MTT. Elevated levels of O^{-}_{2} were produced in the hemolymph and this reaction was inhibited by superoxide dismutase (SOD). *P. berghei* ookinetes were killed in vitro by hemolymph. After 24 h parasites exposed to hemolymph were lysed and the culture medium became dark. These results suggest that *An. albimanus* hemolymph produces FR and they may be important to limit *Plasmodium* parasite development.

CONTRIBUTED PAPER - Monday, 17:15 (Bacteria II)

Differential effects of pH on the pore-forming properties of *Bacillus thuringiensis* insecticidal crystal toxins

Raynald Laprade[1], Le Binh Tran[1], Vincent Vachon[1] and Jean-Louis Schwartz[1,2]

Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1], and Biotechnology Research Institute, National Research Council, Montreal, Quebec[2], Canada

The insecticidal crystal toxins produced by *Bacillus thuringiensis* act by forming pores in the midgut luminal membrane of susceptible insects after proteolytic conversion of the crystal proteins to their toxic form and binding to specific receptors. Although the high pH found in the midgut of lepidopteran insects is thought to play a critical role in the activity of the activated toxins, most studies on different aspects of the mode of action of *B. thuringiensis* toxins, including receptor binding and pore formation, have been carried out at near neutral pH. The effect of pH on the pore-forming ability of two *B. thuringiensis* toxins, Cry1Ac and Cry1C, was therefore examined with midgut brush border membrane vesicles isolated from *Manduca sexta* and a light-scattering assay. In the presence of Cry1Ac, membrane permeability remained high over the entire pH range tested (6.5-10.5) for KCl and tetramethylammonium chloride, but was much lower at pH 6.5 than at higher pH values for potassium gluconate, sucrose and raffinose. On the other hand, the Cry1C-induced permeability to all subtrates tested was much higher at pH 6.5, 7.5 and 8.5 than at pH 9.5 and 10.5. These results indicate that the pores formed by Cry1Ac are significantly smaller at pH 6.5 than under alkaline conditions, whereas the pore-forming ability of Cry1C decreases sharply above pH 8.5. The reduced activity of Cry1C at high pH correlates well with the fact that its toxicity for *M. sexta* is considerably weaker than that of Cry1Aa, Cry1Ab and Cry1Ac. However, Cry1E, whose toxicity is comparable to that of Cry1C, formed channels as efficiently as the Cry1A toxins at pH 10.5. These results strongly suggest that, although pH can influence toxin activity, additional factors also modulate toxin potency in the insect midgut.

POSTER VP9 - Tuesday (Viruses)

Gene Organization and Sequencing of the CfDEFNPV genome, a defective Nucleopolyhedrovirus of *Choristoneura fumiferana*

Hilary A.M. Lauzon[1], Peter B. Jamieson[1], Peter J. Krell[2] and Basil M. Arif[1]

[1]Canadian Forest Service, Great Lakes Forestry Centre,1219 Queen St. E., Sault Ste. Marie, Ontario, Canada, P6A 5M7
[2] Department of Microbiology, University of Guelph, Guelph, Canada, N1G 2W1

Two distinct nucleopolyhedroviruses of the eastern spruce budworm, *Choristoneura fumiferana*, exist in a symbiont-like relationship. One virus, designated CfDEFNPV, is incapable of passing through the basal lamina and is therefore, unable to infect larvae by the *per os* route. We have previously shown that in the presence of CfMNPV, the defective CfDEFNPV is able to pass through the basal lamina and synergizes CfMNPV so that the latter is always the predominant progeny. The present study was conducted in order to characterize the genome of CfDEFNPV and shed light on its potential defect and interaction with CfMNPV. It involved total sequencing of the CfDEFNPV genome, characterization of gene organization and comparisons to other baculoviruses. The DNA of CfDEFNPV was cut with *Hin*dIII, *Eco*RI, *Xba*I and *Bam*HI, shotgun cloned and sequenced by primer walking. The sequencing data were used in conjunction with double digests and hybridizations to confirm mapping. The 131.3 kb genome of CfDEFNPV contains homologs to136 ORFs identified in other baculoviruses, 86 of which are conserved ORFs identified in other sequenced genomes (OpMNPV, AcMNPV, BmMNPV, LdMNPV and SeMNPV). Gene parity plots of CfDEFNPV relative to these sequenced genomes showed that CfDEFNPV maintained the general colinearity of ORFs found in group one baculoviruses. CfDEFNPV contained one identified inversion relative to CfMNPV, one relative to OpMNPV and three relative to AcMNPV. Pairwise comparisons of the amino acid identity encoded by putative CfDEFNPV ORFs with CfMNPV ORFs present in genbank and

ORFs in the five fully sequenced baculovirus genomes, showed the Choristoneura CfDEFNPV to be closest to OpMNPV. Interestingly, the closest amino acid identity was to a region in the vlf-1 to gp41 region of AgMNPV with an average amino acid identity of 95.2% for the five CfDEFNPV homologs in this area (78.9% for OpMNPV). Homologous repeat regions (*hr*s) were identified in eight locations on the CfDEFNPV genome, usually near areas of major rearrangement, insertions or deletions relative to other baculoviruses. We showed that CfDEFNPV by intra haemocoelic injection infects a wider range of lepidopterans than expected. We postulate that CfDEFNPV may be a helper virus located in other wild type virus populations and was first identified in the eastern spruce budworm by chance.

CONTRIBUTED PAPER - Monday, 18:15 (Bacteria II)

Expression of *Bacillus thuringiensis* Cry1Ac toxin domain III

Hyun-Ku Lee[1*], Daniela I. Oltean[2], Fred L. Gould[3] and Sarjeet S. Gill[2]

[1*]Department of Entomology & [2]Environmental Toxicology Graduate Program, University of California Riverside, Riverside CA, [3]Department of Entomology, North Carolina State University, Raleigh NC, [*]Department of Immunology, The Scripps Research Institute, La Jolla CA, USA

Bacillus thuringiensis, a Gram-positive spore-former, produces parasporal crystal proteins known as Cry delta-endotoxins during sporulation that are toxic to a variety of lepidopteran, coleopteran and dipteran insects. The three-dimensional structure of the protein reveals a three-domain composition, however the exact function of each domain is not fully understood. In this study Cry1Ac toxin domain III was cloned and expressed in *E. coli.* The purified Domain III protein was used for binding analyses, which indicates domain III alone can bind purified brush border membrane vesicle (BBMV) proteins from *Heliothis virescens* and *Manduca sexta*. For *H. virescens* this domain binds the 120- and 170-kDa aminopeptidases and additional BBMV proteins. Ligand blots were additionally performed with partially purified BBMV proteins from a Cry1Ac resistant *Heliothis virescens* strain, YHD2.

POSTER FP20 - Thursday (Fungi)

Occurrence of *Batkoa* sp. and *Furia* sp. on spittlebugs pests of pasture in eastern São Paulo State, Brazil.

Luis G. Leite[1,2,4], Sérgio B. Alves[3], Hélio M. Takada[1], Antonio Batista Filho[1] and Donald W. Roberts[2]

Instituto Biológico, Centro Experimental do Instituto Biológico, C.P. 70, Campinas, SP, 13001-970, Brazil[1], Department of Biology, Utah State University, Logan, UT, 84322-5305, USA[2], Escola Superior de Agricultura Luiz de Queiróz, Universidade de São Paulo, Departamento de Entomologia e Fitopatologia, C.P. 9, Piracicaba, SP, 13418-900, Brazil[3].

Spittlebugs are the most important pests of pasture in Brazil. Farmers usually recognize spittlebugs attacks only after seeing large areas of grass completely destroyed. Nymph behavior, i.e. locating in the soil, makes their control with insecticides difficult. The fungus *Metarhizium anisopliae*, one of the most used control measures against these pests, also is somewhat inefficient against the nymphal stages. Although Entomophthorales occasionally have been found at epizootic levels since the 1970s, they have not been cultured, incidence levels determined, nor evaluated for pest control potential. The research reported here aimed at evaluating the occurrence of Entomophthorales species on spittlebugs pests of pasture in Pindamonhangaba county, São Paulo State, Brazil. Evaluations were carried out in 2 fields located next to each other, with *Brachiaria decumbens* and *Pennisetum purpureum* grasses, respectively, and a total area of 10 ha. Evaluations were done each 4 days during January to February by capturing spittlebugs adults on leaves with a 30 cm diameter entomological net. There were 50 sweeps per replication, and 4 replications per evaluation. Samples of insects were kept frozen for later evaluation. Insect abdomens were dissected and observed by microscopy for the presence of hyphae and resting spores. Insect cadavers with sporulating fungus were collected in the field and taken to the lab to isolate the pathogen. *Furia* sp. was found at epizootic levels on *Deois schach* in the *Brachiaria* pasture, reaching 80% infected followed by a crash in the insect population. *Batkoa* sp. was found at enzootic level (< 10% infected) on *Mahanarva fimbriolata* in the *P. purpureum* pasture. *Furia* sp. also was found to infect another important spittlebug, *Deois flavopicta*, indicating it to be a good agent for investigation as a bioinsecticide.

[4]Fellowship received by CAPES – Brasilia/Brazil. Research supported in part by grants from CAPES and Utah State University.

STUDENT POSTER FP5 - Tuesday (Fungi)

Effect of salts, vitamins, sugars and sources of nitrogen on the growing of three Entomophthorales species: *Batkoa* sp., *Furia* sp. and *Neozygites floridana*.

Luis G. Leite[1,2,4], Sérgio B. Alves[3], Antonio Batista Filho[1] and Donald W. Roberts[2]

Instituto Biológico, Centro Experimental do Instituto Biológico, C.P. 70, Campinas, SP, 13001-970, Brazil[1], Department of Biology, Utah State University, Logan, UT, 84322-5305, USA[2], Escola Superior de Agricultura Luiz de Queiróz, Universidade de São Paulo, Departamento de Entomologia e Fitopatologia, C.P. 9, Piracicaba, SP, 13418-900, Brazil[3].

Entomophthorales fungi have strong relationships to their insect and mite hosts, which makes it possible for these organisms to cause epizootics. On the other hand, these close relationships also make the Entomophthorales much more difficult to develop as biopesticides. Studies on their production are advancing quickly, allowing the in vitro growth of some species only recently referred to as obligate. However, less expensive and less complicated media are needed. The research reported here was designed to evaluate the effects of salts, vitamins, sugars, amino acids and other sources of nitrogen on in vitro growth of three fungi: *Batkoa* sp. and *Furia* sp. infecting spittlebug pests of pasture, and *Neozygites floridana*, a pathogen of mites. Centrifuge tubes (50-ml) containing 8 ml of liquid were use for *Batkoa* sp. and *Furia* sp., and screw-cap tubes (1.5 x 12 cm) containing 2 ml of medium for *N. floridana*. Replications were 6 for *Batkoa* sp. and *Furia* sp., and 5 for *N. floridana*. Shaking was 200 rpm at approximately 25°C. The number of hyphal bodies /ml was counted each 2 or 4 days for *N. floridana*, and weight of dried biomass was measured at 7 days for *Batkoa* sp. and *Furia* sp. Initially, media containing groups of salts (6), vitamins (10), amino acids (14) and the combination of all together were compared with basic medium containing common components for all treatment (0.33% lactalbum hydrolyzate, 0.33% yeastolate, 2.66% sucrose, 0.1% glucose and 0.7% fructose). Secondly, sucrose (2.66%), glucose (2.66%) and the combinations of sucrose (2.66%) plus glucose (0.1%), plus fructose (0.1%), plus maltose (0.1%) and plus inositol (0.1%) were compared in media with 0.33% lactalbum hydrolyzate (LH), 0.33% yeastolate (Y) and the group of salts. Thirdly, media containing combinations of 4 sources of nitrogen, prepared with glucose (2.66%) and the group of salts, were compared with the best medium from the second experiment. Grace's insect tissue culture (supplemented with LH and Y) was used as the control for all experiments. Salts significantly increased the production of all the fungi, especially when mixed with amino acids. The fungal production was much higher (2-4 times) in media with glucose (2.66%) as the sole source of carbon, indicating all of the fungi metabolized sucrose poorly. Yeast extract (1%) was the best source of nitrogen for *Batkoa* sp., increasing production more than 2 times compared with Grace's. The combination of yeast extract (0.33%), beef extract (0.33%), and skim milk (0.33%) was the best for *Furia* sp., increasing production 4 times. The combination of beef extract (0.33%), peptone (0.33%), and skim milk (0.33%) was the best for *N. floridana*, allowing the same rate of growth and production as Grace's, but at considerably less expense. Accordingly, simple, low-cost media were developed for all 3 fungi.

[4]Fellowship received by CAPES – Brasilia/Brazil. Research supported in part by grants from CAPES and Utah State University.

STUDENT POSTER FP6 - Tuesday (Fungi)

Coating Spores of *Metarhizium flavoviride* for UVB-Protection and Effects on Virulence to African Desert Locust (*Schistocerca gregaria*) Forskal

Jarrod Leland[1], Donald Mullins[1], Herman Warren[2], Larry Vaughan[3], Jacques Fargues[4], Nathalie Smits[4],

[1]Department of Entomology, [2]Department of Plant Pathology and Weed Science, and [3]Office of International Research and Development Virginia Tech, Blacksburg, VA, 24061
[4] Institut National de la Recherche Agronomique, 34982 Montferrier-sur-Lez, France

With funding from the Africa Bureau of the US Agency for International Development, Virginia Tech, in collaboration with seven other US and international partners, is working to develop and improve biopesticides for grasshopper and locust control in Sub-Saharan Africa. One important limitation to the use of biopesticides in environments with high insolation, such as Sahelian Africa, is the persistence of the active agent until infection of the target can be initiated. Exposure to UVB-radiation is detrimental to spores of entomopathogenic fungi. Biopesticide formulations that provide protection from ultraviolet radiation may permit smaller effective application rates or greater efficacy, but coating spores with protective materials may inhibit infectivity of spores. A formulation for coating spores of *Metarhzium flavoviride* has been developed that appears to protect spores produced in submerged liquid culture from UVB radiation. Germination tests of spores of the LUBILOSA isolate IMI 330189 demonstrated improved spore resistance to UVB radiation under simulated Sahelian conditions. Spores coated with the formulation material remained virulent to the African desert locust, *Schistocerca gregaria*.

SYMPOSIUM II - Tuesday, 9:35 (Bacteria)

Control of virulence gene expression in *Bacillus thuringiensis* and *Bacillus cereus*.

Didier Lereclus

Unité de Biochimie Microbienne, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, Station de Lutte Biologique, INRA, La Minière, 78285 Guyancourt cedex, France

Bacillus thuringiensis and *Bacillus cereus* are two closely related species. *B. thuringiensis* is well known for its insecticidal properties and its ability to produce a crystal inclusion during sporulation. *B. cereus* is known as the etiological agent of food-poisoning syndromes. The specific activity of *B. thuringiensis* against insect larvae is due to the crystal proteins (Cry toxins). In addition to these toxins, *B. thuringiensis* (as well as *B. cereus*) produces extracellular proteins which might be involved in the virulence of the bacterium. It is generally admitted that the toxic effect of the Cry proteins either kills or weakens the insect larvae, thus providing favorable conditions for the germination of the spores and the multiplication of the bacteria. The production of extracellular compounds during the stationary phase might contribute to the capability of the bacteria to invade host tissues.

Analysis of phospholipase C gene expression in *B. thuringiensis* led to the identification of a positive regulator (PlcR) which activates the transcription of the *plcA*, *plcB* and sphingomyelinase genes at the onset of the stationary phase. The *plcR* gene was found in all the members of the *B. cereus* group. However, the *plcR* gene from *B. anthracis* is truncated and not functional. A genetic screen and sequencing data allowed the detection of other *B. thuringiensis* and *B. cereus* genes regulated by PlcR. These genes encode exported proteins, including proteases and enterotoxins (Hbl and Nhe). It results that PlcR is a pleiotropic regulator controlling the expression of various potential virulence factors. Disruption of the *plcR* gene in the two Bacilli species reduces the synergistic effect of spores on the insecticidal activity of the crystals, and results in a drastic reduction of the hemolytic and cytolytic properties.

Alignment of the PlcR-regulated promoters reveals the presence of a conserved palindromic sequence presumably required for PlcR activation. The transcription of the *plcR* gene is increased in a *spo0A* mutant unable to initiate sporulation, thus suggesting that Spo0A~P is a repressor of *plcR* transcription. Mutations in the oligopeptide permease genes of *B. thuringiensis* abolish *plcR* expression. This suggests that *plcR* expression may be activated by the uptake of a small peptide.

STUDENT POSTER VP37 – Thursday (Viruses)

Cloning and Expression of the *gp37* Gene of *Spodoptera liture* Nucleopolyhedrovirus and its Implication in the Virus Infection

Chongbi Li[1], Zhaohui Li[1], Ping Zhang[1], Yi Pang[1] and Deming Su[1,2]

[1]State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P. R. China
[2]Virology Research Unit, Fudan University, Shanghai 200433, P. R. China

The *gp37* gene of *Spodoptera litura* multicasid nucleopolyhedrovirus (SpltMNPV) had been cloned, sequenced and expressed. The GP37 product expressed in *E.coli* was fed to the cotton leafworm larvae 30 h in advance of virus infection, resulting a significant reduction in larva mortality. A recombinant AcMNPV super-expressing the GP37 of SpltMNPV was constructed by using the polyhedrin promoter. Bioassay results showed that the recombinant virus caused 26% higher mortality than that of its parent virus against the larvae of the beet armyworm, *Spodoptera exigua*.

CONTRIBUTED PAPER - Monday, 11:30 (Viruses I)

Organization of the *Mamestra configurata* nucleopolyhedrovirus genome.

Qianjun Li[1], Cam Donly[2], Lulin Li[3], Leslie G. Willis[3], David A. Theilmann[3] and Martin Erlandson[1]

[1] Saskatoon Research Centre, AAFC-Saskatoon, SK
[2] Southern Crop Protection and Food Research Centre, AAFC, London, Ont.
[3] Pacific Agri-Food Research Centre, AAFC, Summerland, B. C.

The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae) is an important pest of cruciferous oilseed crops in Western Canada. A series of nucleopolyhedrovirus (NPV) isolates from natural populations of *M. configurata* have been characterized and designated as MacoNPV. Results from laboratory bioassays and preliminary field trials indicate that the virus has good potential as a biocontrol agent for bertha armyworm.

A physical map of the genome of the MacoNPV-90/2 isolate was constructed using cloned fragments from 6 different restriction endonucleases (REN) and the size of the genome estimated at 156 Kbp. Here we report on preliminary sequence analysis from "terminal-sequencing" of REN clones and from complete sequence of randomly generated MacoNPV DNA fragments cloned in pBlueScript vectors. More than 50 open reading frames (ORF) homologous to those from other baculoviruses were identified and their location on the physical map determined. The gene arrangement in the region containing the putative helicase gene, as in other baculoviruses, is highly conserved. The location of other ORF homologues indicate that the MacoNPV genome organization is similar to SeMNPV in many respects. The predicted amino acid sequence from several of the ORF previously used for phylogenetic analysis, including polyhedrin (91 & 97%), egt (74 & 88%), DNA polymerase (65 & 90%) and lef2 (60% with SeMNPV), have a high degree of identity with SeMNPV and MdMNPV proteins, respectively, confirming the earlier designation of MacoNPV as a group II baculovirus. Several regions of unique sequence having no homology to other baculovirus sequences were identified. A gene parity plot analysis was used to explore the relationship between the genome of MacoNPV and other baculoviruses in terms of genome organization.

CONTRIBUTED PAPER - Thursday, 16:45 (Microbial Control I)

Efficacy of bitoxibacillin, a formulation based on *b*- and *d*-toxins of *Bacillus thuringiensis*, for control of suctorial soybean pests

Vladimir Lichovidov, Natalia Lichovidova
State Research Center for Applied Microbiology, Obolensk, Serpukhov region, Moscow area, 142279, Russia

Vladimir Gouli
Entomology Research Laboratory, University of Vermont, Burlington, VT 05405-3400

The red spider mite (*Tetranichus urticae* Koch.) and the onion thrips (*Thrips tabaci* Lind.) cause significant damage to soybean plantings in districts of Russia, Ukraine and Moldova. The Russian bacterial formulation bitoxibacillin (BTB), based on **b**- and **d**-toxins of *Bt* (BT H1, 8000 IU/mg activity), was tested for control of both pests in Moldova. Red spider mite was treated twice with BTB (0.5% suspension) and was compared to a single treatment with the chemical acaricide Acrecs (0.1% conc.). Before treatment, mite density ranged from 16.1-44.2 mites/leaf and leaf damage was rated as 2 on a scale of 8 levels covering 0 to 50% leaf damage. Spider mite mortality was 94% and 96% for BTB and Acrecs, respectively. There was no change in the level of leaf damage. A second experiment was conducted where initial mite density was 18.7-19.9 mites/leaf and the leaf damage rating was 1.3. A single treatment of soybean with BTB (0.5%) provided 82.8% spider mite mortality and no change in the level of leaf damage.

Onion thrips was treated twice with BTB (0.5% suspension) and compared to a single treatment of the chemical insecticide Deltametrin (0.05% conc.). The initial insect density, including larvae and adults, was 10.2-11.4 thrips/leaf. The effectiveness of treatment was 71.0% for BTB and 76.0% for Deltametrin. The level of leaf damage after treatment with BTB and Deltametrin was two times lower than on the untreated control.

Full microbial protection of soybean plantation was evaluated. The most effective scheme included the treatment of seeds and soybean plants with Trichodermin, a formulation based on the antagonistic fungus *Trichoderma lignorum*, Pentaphag, a formulation based on the bacterium *Pseudomonas syringe* pv. Glicinea, and four applications of BTB (0.5% suspension). Microbial options for soybean plant protection provided an additional bean harvest of 78.8%.

CONTRIBUTED PAPER - Thursday, 17:00 (Microbial Control I)

Formulations based on *Bacillus thuringiensis* as effective means for control of beet webworm - *Pyrausta sticticalis* L. on soybean plantations

Natalia Lichovidova, Vladimir Lichovidov

State Research Center for Applied Microbiology, Obolensk, Serpukhov region Moscow area, 142279, Russia

Vladimir Gouli
Entomology Research Laboratory, University of Vermont, P.O. Box 53400, Burlington, VT 05405-3400

Beet webworm, *Pyrausta sticticalis* L., is one of the most destructive insects in southern parts of east Europe. Pandemic outbreaks of this insect take place in cycles of 10-12 years. The pest attacks many annual and perennial plants including major agricultural crops. We conducted a trial of Russian bacterial formulations based on Bt on soybean plantation in different districts of SNG (former USSR) that had high density of beet webworm. The following formulations were tested: Gomelin (*Bt var. thuringiensis*, 16000 IU/mg), Lepidozid (*Bt var. kurstaki*, 16000 IU/mg), Bitoxibacillin (*Bt var. thuringiensis*, 8000 IU/mg), Dendrobacillin (*Bt var. dendrolimus*, 8000 IU/mg) and Insectin (*Bt var. insectus*, 8000 IU/mg). Application rate was 1 kg/ha applied in a suspension at 300 l/ha. Number of larvae before treatment ranged from 80 to 115 larvae/m^2, whereas the economic threshold is 5-10 larvae/m^2. The treatment of soybean plantations was done when approximately 80% of larvae were in instars 1-2. Biological effectiveness after 6 days was: Gomelin - 94%, Lepidozid - 72%, Bitoxibazillin - 79%, Dendrobacillin - 87%, Insectin - 73%. With Lepidozid, Bitoxibacillin and Insectin, repeated treatments were required at a rate of 1.5 kg/ha, 2.0 kg/ha and 2.0 kg/ha respectively, because insect populations included considerable number of middle and full-grown larvae. The second treatment provided an additional 60-69% efficacy. After 12 days from the first application the number of larvae had come down in case of Gomelin - 82%, Lepidozid - 88%, bitoxibazillin - 93%, Dendrobazullin - 82% and Insectin - 92%. The damage to plants was less than 5% in all cases. The number of pests after application of the bacterial formulations did not exceed the economic threshold for the budding and flowering phases of soybean plants. Thus, Bt formulations are the effective means for beet webwoorm control.

POSTER BP18 - Tuesday (Bacteria)

New *Bacillus Thuringiensis* bacteriophage with wide spectrum of action

Vladimir E. Likhovidov , Faskhetdin Sh. Isangalin

State Research Center for Applied Microbiology, Obolensk,region, Moscow area, 142279, Russia

In order to estimate the level of spontaneous isolation of moderate and virulent phages from industrial strains of *Bacillus thuringiensis* SRCAM has specially designed indicator strain *Bacillus thuringiensis* var. Galleria which can be lysed by all the moderate and lysogenic phages of *Bacillus thuringiensis* (*Ât*). The results obtained on the indicator strain showed that all the industrial *Bt* strains have low level of the isolation of moderate phages (10^6-10^7) and do not give lysogenic phages.

The new bacteriophage was isolated from *Bt* var. kurstaki (Í3) (1996, South Vietnam). Preliminary studies of the phage showed that it lyses the strains of most of serotypes of *Bt*. Emission of *Bt* strains Í3 of extremely agressive lysogenic phage is likely to occur under the infleunce of tropical climate of Vietnam.

Preliminary electrone microscopy investigations showed that the new phage belongs to morphotype $Â_1$ according to classification of Ackermann, Dubow (1987). The phage has an icosahedral head, long noncontractable tail and resembles phage $À_1$ *Bacillus sphaericus* and phage SPB *Bacillus subtilis*. The distance between opposite apical tops of its isometric head amounts to 75-80 nm. The phage has a flexible noncotractable tail, the length of which is approximately 370-400 nm.

The new phage does not enter into the classification of known *Bt* phages developed in SRCAM in morphology, DNA composition and proteins. The phage appeared to be very resistant to the factors of the environment: it practically retained the initial titre when stored in chlorophorm solution for 4 months at +30 ^{0}Ñ and then at room temperature for 1 year.

Further studies showed that the new phage lyses not only *Bt* strains, but also a number of *Bacillus cereus* strains and active and vaccine strains of *Bacillus anthracis*, a causative agent of anthrax. At a titre of 10^4- 10^6, the phage showed high lytic activity on capsula-free variant of *B.anthracis* strains and on capsula variants when being grown on poor nutrient media.

POSTER BP17 - Tuesday (Bacteria)

Novel low molecular toxin of *Bacillus Thuringiensis* (H14) strain

Vladimir E. Likhovidov, Faskhetdin Sh. Isangalin

State Research Center for Applied Microbiology, Obolenks, Serpukhov region, Moscow area, 142279, Russia

It is well known that *Bti* toxic for mosquito larvae are water insoluble endotoxin crystals, which in mosquito intestine degrade to several active fragments: Cry4A and Cry4B with a molecular weight of 130000, Cry4C and Cry4D of a molecular weight of 72000 and Cyt with a molecular weight of 25000, under the action of proteases. Since protein component and the active fragments are water insoluble, cultural liquid supernatant does not show activity with regard to mosquito larvae. Laboratory studies of one of the natural strains of *Bti* revealed that the supernatant possessed considerable toxicity for larvae of *Culex, Aedes*.2-3 instar larvae of *Aedes aegypti* died in supernatant after 20-30 min of incubation. Standard nutrient media do not provide sufficient amount of the toxin, so the supernatant toxin was concentrated and partially purified b y HPLC in aluminium oxide. Then is was found that neither supernatant nor the purified toxin affect the larvae of *Musca domestica*, *Galleria mellonella*, *Ochneria dispar*, that is the effect of the toxin is rather selective. The partially purified and concentrated toxin was studied by a number of physico-chemical methods. Ebulioscopic method showed a molecular weight of the toxin to be 600 Da. UV-specter gave absorption at 220-240 nm, that testified the absence of aromatic groups in the toxin structure. ^{1}Í-NMR spectrum showed absorbtion at 1.3-2.7 md that suggested the presence of $ÑÍ_3$ and $ÑÍ_2$ groups and the absorption within 3.5-5.4 md provided the presence of approximately 20 CH groups. Thus, preliminary

results pointed to the fact that water-soluble toxin *Bti* is a single molecule of a liner structure. Relatively high stability of the toxin (it resists to heating up to 80 ^{0}Ñ for 30 min and pH 4.0) confirmed the above statement.

POSTER BP41 - Thursday (Bacteria)

Nematocide Activity of *Bacillus thuringiensis* towards *Meloidogyne incognita.*

Vladimir E. Likhovidov, Vasily S. Perepechko, Larisa I. Volodina, Elena K. Savostianova.

State Research Center for Applied Microbiology, Obolensk, Serpukhov region, Moscow area, 142279, Russia.

According to FAO data, Gallic nematodes of a genus of *Meloidogyne* affect plant growing by 30 milliard dollars annually in the countries of Europe and USA. In this connection development of nematocide preparations, especially biological ones, is an urgent task. Screening of natural isolates of *B.thuringinesis* (*Bt*) resulted in an isolation of a number of strains with nematocide activity. Most of the strains, exhibiting activity towards *M. incognita*, refer to serovariants H4, H20 and H24. Biological and technological properties of the strains were studied. It was found that they differ from each other in productivity, activity and phage resistance. Activity of *Bt* strains (LC_{50}) was found to be 0.1% for 1542 (H4); 0.3% for 1575 (H20); 0.7% for 1535 (H4), 1.0 % for 1579 (H24). Strain 1579 (H24) is the most resistant to the phages. Strain 1535 (H4) is the least sensitive to them. The rest of the strains occupy intermediate position. Strain 1542, *var. sotto*, isolated from larvas of *Elateridae* in Mongolia seems to be the most promising. Laboratory technology for producing preparation Bacnem was developed on the base of this strains. Experimental samples of Bacnem (stabilized cultural liquid) have been tested on cucumbers. 5% Bacnem was tested by three ways: seed treatment, decontamination of soils, and root system treatment. Invasion background amounted to 1200-1500 larvas of *M. ingonita* per a unit of the tested material. All the variants exhibited activity towards the spreading and development of meloidogynesis by 2.5-3.0 times higher than that in control. Biological efficiency (average values) of the preparation upon seeds treatment, soil decontamination and root treatment amounted to 82%, 85% and 80%, respectively. Further investigations will be focused on the studying of the mechanism of the preparation action, development of a technology for production and application of Bacnem.

POSTER FP18 - Thursday (Fungi)

Physiological characterization of *Verticillium lecanii* (Moniliales : Moniliaceae) and preliminary investigation on its virulence to the silverleaf whitefly, *Bemisia argentifolii* (Homoptera:Aleyrodidae)

Chu-Hsu Lin[1], Wen-Feng Hsiao[2], and Roger, F, Hou[1]

Dept. of Entomology, National Chung-Hsing University, Taichung, Taiwan 400
Dept. of Biological Resources, National Chiayi University, Chiayi, Taiwan 60083

Three isolates (#173, #159, and #F113) of the entomopathogenic fungus, *Verticillium lecanii* (Zimm.) Viegas, were cultured on yeast-peptone-dextrose agar to determine their development. The mycelial growth and sporulation were best in #173 followed by #159, and poorest in #F113. The maximum sporulation for #173, #159 and #F113 were determined to be 1.5 x 10^8, 7.9 x 10^7 and 7.7 x 10^7 conidia/ml, respectively, with an inoculum of 5 µl of 10^7 conidia/ml. The mycelial growth and sporulation of both #159 and #F113 were stimulated when cultured under full light. However, at full dark, #173 grew better but the growth of #159 and #F113 were arrested. The results showed that five media, *i.e.*, yeast-peptone-dextrose agar, potato dextrose agar, malt extract agar, Sabouraud's dextrose, and Czapek'-dox agar were better for mycelial growth and sporulation than the others tested. The optimal temperature for *V. lecanii* was found to be at 20-30^{o}C on all media cultured. These isolates could grow at a pH value ranging from 5 to 10; however, they grew better under an acidic condition. The requirements of *V. lecanii* for carbon source varied with substances assayed. Peptone was found to be the best nitrogen source for this fungus and could substitute the technical grade of yeast extract. When assayed with API ZYM system, alkaline phosphatase, esterase, lipase, aminopeptidase, galactosidase, glucosidase, and glucosaminidase were detected in agar plates of these isolates; however, β-glucuronidase, which may cause damage to plants was not detected.

Among 3 isolates, #F113 was the most sensitive one to the fungicides tested followed by #159 and #173 in term of mycelial growth and sporulation. Imazalil, propineb, metalaxyl + mancozab and metalaxyl + copper oxychloride were inhibitory to these isolates, whereas bupirimate showed only slight inhibition. Among insecticides assayed, pirimorb, Applaud, and neem were stimulatory to the sporulation of #173. Most insecticides except cartap were found to be inhibitory to #159 and #F113 but pirimorb was inhibitory to #159 only.

V. lecanii was not virulent to Lepidoptera such as *Plutella xylostella*, *Crocidolomia binotalis*, *Spodoptera litura* and *Pieris rapae crucivora;* Coleoptera such as *Cassida circumdata*, and Homoptera such as *Dysmicocus brevipes* when inoculated to these species. However, it was pathogenic to the silverleaf whitefly, *Bemisia argentifolii.* At 100% RH, the LC_{50} values of isolates #173, #159, and #F113 were determined to be 6.52 x 10^4, 3.57 x 10^5 and 1.65 x 10^5 conidia/ml, respectively, to the 4th instar nymph when the conidial suspension was mixed with a sticker. But the LC_{50} values were 3.78 x 10^4, 8.45 x 10^4, and 3.49 x 10^4 conidia/ml, respectively, without mixing with any sticker. The LT_{50} values of these 3 isolates at a concentration of 1 x 10^8 conidia/ml were 80.1, 89.3, and 86.3 h, respectively, while the LT_{50} values were prolonged at a lower concentration of 10^7, being 90.3, 80.0 and 89.6 h, respectively. However, this fungus was not infective to the whitefly under 90% RH or lower, indicating that high humidity is required for infection of insects with this fungal pathogen.

POSTER VP2 - Tuesday (Viruses)

Stable cell lines expressing baculovirus P35: Resistance to nutrient stress and enhanced production of a secreted reporter protein

G. Lin, G. Li, R.R. Granados, and G.W. Blissard

Boyce Thompson Institute, Cornell Univ., Ithaca, NY 14853-1801;

The AcMNPV P35 protein is expressed early in the infection cycle and is known to inhibit apoptosis triggered by AcMNPV infection of Sf21 cells. To generate cell lines resistant to stressful culture conditions, and to examine the effects of constitutive cellular P35 expression on protein production from baculovirus expression vectors, we generated cell lines expressing AcMNPV P35 or an epitope-tagged P35 protein. Cell lines expressing P35 were first selected using a neomycin resistance gene and G418, then selected again in the presence of actinomycin D, an inducer of apoptosis in Sf9 cells. Several cell lines were cloned and examined for a) resistance to actinomycin D induced apoptosis, b) nutrient deprivation, c) growth in various media, and d) baculovirus expression of intracellular and secreted proteins. When compared with unmodified Sf9 cells, two P35 expressing cell lines, $Sf9^{P35AcV5-1}$ and $Sf9^{P35AcV5-3}$ showed increased resistance to actinomycin D induced apoptosis, and a substantial resistance to nutrient deprivation. When these cell lines were infected with a recombinant baculovirus expressing a secreted glycoprotein (Secreted Alkaline Phosphatase, SEAP), expression of the glycoprotein from these cells exceeded that from unmodified Sf9 cells, and was comparable to expression levels obtained from Tn5B1-4 cells. High level protein production in $Sf9^{P35AcV5-1}$ cells appears to result from a prolonged infection cycle compared with that from AcMNPV infected Sf9 cells. In typical baculovirus expression vector infection of Sf9 or Tn5B1-4 cells, foreign protein accumulation may plateau around 120 h p.i. In contrast, secreted proteins continued to accumulate until 216 h p.i. in both $Sf9^{P35AcV5-1}$ and $Sf9^{P35AcV5-3}$ cells.

SYMPOSIUM III - Tuesday, 18:30 (Bacteria)

Interaction of Cry3A d -endotoxin with model liposomes

O. I. Loseva[1], E. I. Tiktopulo[2], V. D.Vasiliev[2], A. P. Dobritsa[1] and S. A. Potekhin[2]

State Research Center for Applied Microbiology, Obolensk, Moscow Region, 142279[1]

Institute of Protein Research, Russian Academy of Sciences,
Pushchino, Moscow Region, 142292[2], Russia

Interaction of Cry3A and its fragments with phospholipid vesicles was studied using electron microscopy, scanning microcalorimetry, CD spectroscopy and limited proteolysis. It was shown that the intact protein acts in a destructive mode on liposomes. Removal of four N-terminal α-helices and the extreme 56 C-terminal amino acid residues does not eliminate this ability.

The results obtained by limited proteolysis of δ-endotoxin bound to lipid vesicles indicate essential conformational changes in three or four N-terminal helices and in the C-terminal region. The calorimetric method provides a unique possibility for validation of existing models of protein binding and for more accurate determination of the regions where conformational changes take place. It was found that binding of the protein to model liposomes does not alter its structure in the regions leading off with the fourth α-helix of Domain I. This follows from the fact that activation energy of denaturation of the protein remains unchanged upon its binding to the phospholipid membrane. A new structural model has been proposed, which agrees with the data obtained.

Effects of methanol on the structure of δ-endotoxin were examined. The results of a limited proteolysis study show that certain methanol concentrations (20-25%) may cause structural changes similar to those occurring in the molecule upon its binding to the phospholipid membrane. However, further increase in methanol concentrations leads to an absolutely nonfunctional, aggregated fibrillar state. The new structure is different in its cooperativity and the secondary structure content from the membrane-bound protein.

CONTRIBUTED PAPER - Thursday, 10:45 (Viruses IV)

Expansion of the *Autographa Californica* nucleopolyhedrovirus host range to *Spodoptera littoralis*

Liqun Lu, Quansheng Du and Nor Chejanovsky.

Department of Entomology, Inst. of Plant Protection, The Volcani Center,
POB 6, Bet Dagan, Israel

Infection of *Spodoptera littoralis* SL2 cells with the *Autographa californica* nucleopolyhedrovirus (AcMNPV) results in apoptosis, aborting the viral infection. In contrast, the *S. littoralis* nucleopolyhedrovirus (SlNPV) yields a productive infection in SL2 cells. This suggested that SlNPV could provide elements necessary to sustain AcMNPV replication in SL2. Transfection of SL2 cells with wild type AcMNPV DNA and cosmids from a cosmidic library representing the entire SlNPV genome, allowed the isolation of vAcSL2, a recombinant AcMNPV able to replicate and form polyhedra in SL2 cells. Interestingly, the vAcSL2 genome contained a "foreign" DNA insert located between the AcMNPV genes *me53* and *ie0*. SL2 cells infected with vAcSL2 produced high steady state levels of IE1 in contrast to AcMNPV-infected cells.

vAcSL2 budded virus titers in SL2 cells were about 100-fold higher than AcMNPV titers. Moreover, the infectivity of vAcSL2 towards *S. littoralis* larvae was significantly higher than AcMNPV. These results suggest that increasing the level of expression of *ie1* enable the expansion of the host range of AcMNPV to *S. littoralis*.

SYMPOSIUM IV - Friday, 12:35 (Bacteria)

Mosquito Control with Bti: Ecology and Economy

Peter Luethy[1] and Nicola Patocchi[2]

[1]Institute for Microbiology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland
[2]Fondazione Bolle di Magadino, Casa comunale. CH-6573 Magadino, Switzerland

Mosquitoes represent an increasing global threat as vectors of infectious diseases but also as a source of severe nuisance. There are strong indications that the problems caused by mosquitoes will extend from the tropical regions into temperate climatic zones. The main reasons are the global warming, the increasing migration, and the intensified world wide trade of goods by sea and air which provide vehicles for infectious agents and their vectors.

Therefore mosquito control has to become a matter of high priority. The measures include three key factors, which are source reduction, larviciding, and protection of residential areas with adulticides. Source reduction and the control of larval stages are closely interconnected.

According to our experience, mosquito control is on the one hand side labour intensive, on the other side the use of modern equipment, adapted to the ecosystems and the breeding sites is a prerequisite.

Products based on *Bacillus thuringiensis israelensis* (Bti) or *B. sphaericus* in polluted water bodies, should at present always be the first option for the control of mosquito larvae. The delta-endotoxins of Bti are highly effective if delivered into the feeding zones. Bti has an outstanding safety record. Predators and the integrity of the ecosystems are not touched.

Our field work emphasizes the control the flood water mosquito, A. Vexans, in very difficult environments which flat zones flooded frequently at unpredictable intervals during the spring and summer months. The main breeding sites of *A. vexans* are located within a natural reseve or other ecosystems of high value. Access is impeded by dense vegetation. A thorough control of A. vexans is indispensible since the breeding sites are in close vicinity of residential areas with tourist resorts.

Treatments by helicopter represent the backbone of the larviciding actions with Bti. Up to date we have succeeded in keeping the A. vexans population on a tolerable level. No signs of resistance have appeared.

The economy of Bti applications is an important aspect. However, the factors, which determine the cost-benefit ratio are hard to comprehend. The standard of quality of life or the potential prevention of a transmission of an infectious disease can not be expressed in dollars and cents.

Our long-term experience for more than a decade with Bti in the control of the flood water mosquito, *Aedes vexans*, allows now a reliable judgment of the cost-benefit factors. In all instances the benefit has exceeded the costs.

STUDENT PAPER - Monday, 11:15 (Fungi I)

Storage compatibility of *Metarhizium anisopliae* var. *acridum* with other pesticides used for locust and grasshopper control.

B. Luke and R. Bateman

CABI Bioscience, Silwood Park, Ascot, SL5 7TA UK.

The short to medium term viability and growth of *Metarhizium anisopliae* var. *acridum* conidia was investigated when combined with other pesticides, used in locust control. Viability of the conidia was measured by percentage germination tests. All of the pesticides used in this study were suitable for immediate spraying with *M. anisopliae* var. *acridum* conidia except for the conidia stored in the fenitrothion. The viability assessments showed that neem oil, diflubenzeron and deltamethrin had no significant effect on the germination of *M. anisopliae* var. *acridum* conidia. Conidia suspended in the 100% fipronil formulation had a low germination of 13.6% after 28 days compared to 69.7% for the control. Conversely, the 10% triflubenzeron treatment lost viability relatively quickly with 9.9% germination after 28 days compared to 42.6% and 42.8% of the 100% and 50% treatments, respectively. Conidia suspended in 10% and 50% fenitrothion formulations resulted in germination 12-13% and 30-35% lower than the controls, respectively. Germination of conidia suspended in 100% fenitrothion formulation followed a similar pattern but by day 84 viability had dropped to 13.7%.

The effect on growth of the fungus was measured as colony diameter grown on SDA plates. Samples of the conidial suspensions were taken on 0, 28, 56 and 84 days after being stored in the pesticides at 30°C. Colony diameter was measured along two axes of the colony after ten days growth. Conidia stored in fenitrothion had approximately the same colony diameter at the different storage times. There was a general decrease in growth the longer the conidia were stored in the pesticides, with significant ($P<0.05$) differences between the first and last sampling dates. Conidia stored in triflubenzeron and fipronil had significantly ($P<0.05$) less colony growth compared to most of the pesticides after 56 and 84 days in storage, respectively.

POSTER VP12 - Tuesday (Viruses)

Sequence analysis of the *Cydia pomonella* granulovirus genome

Teresa Luque[1], Ruth Finch[2], Doreen Winstanley[2] and David O'Reilly[1]

[1]Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK
[2]Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

The nucleotide sequence of the DNA genome of *Cydia pomonella* granulovirus (CpGV) was determined and analysed. The genome is composed of 123,424 bp and has a G+C content of 45.3%. It contains 148 ORFs of 150 nucleotides or larger that show minimal overlap. 40 ORFs are unique to CpGV, 98 have homologues in *Xestia c-nigrum* GV (XcGV) and 70 in *Autographa californica* nucleopolyhedrovirus (AcMNPV). These homologues show on average 41% amino acid sequence identity to those from XcGV and 34% to those from AcMNPV. A total of 64 ORFs are conserved among all seven baculovirus genomes sequenced so far and thus, they are likely to be essential genes. The CpGV gene content was compared to other baculoviruses and several genes reported to have major roles in the baculovirus biology were not found in the CpGV genome. It lacks a homologue of the major budded virus glycoprotein gene *gp64* and several genes encoding proteins involved in DNA replication and transcription, including *lef-3, -6* and *-7*. In addition, the CpGV genome lacks a homologue of the *enhancin* gene. However, several genes not present in the AcMNPV genome but present in other baculoviruses have been identified, including ORFs encoding the large subunit of ribonucleotide reductase and two copies of the small subunit, a second helicase with similarities with the pif-1 yeast mitochondrial helicase and a DNA ligase. Furthermore, the CpGV genome contains three inhibitor of apoptosis (*iap*) genes and two protein tyrosine phosphatase genes, which are homologous to Op-ptp-2 and Se-ptp-2.

SYMPOSIUM - Friday, 10:45 (Fungi)

Production and application of entomopathogenic and antagonistic fungi in Cuba.

Mercedes Lujan Macias, O. Fernández Larrea, Esperanza Rijo and Ofelia Milán

Instituto de Investigaciones de Sanidad Vegetal. Calle 110 Nº 514. Playa,
Ciudad Habana, Cuba

In the last 20 years the production and use of entomopathogenic and antagonistic fungi in Cuba has increased and diversified. Production is achieved using different methods, although the most widespread systems are small scale and use agricultural biproducts, eg from the sugar industry, as growth media. There are 220 production centres (CREE) in Cuba, and their products have been registered under the company name, BIASAV. Formulated products of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii* for control of arthropod pests in different crops, in urban situations, and for the regulation of populations of mammalian and avian ectoparasites have all been produced. Formulated products of *Paecilomyces lilacinus* and *Trichoderma harzianum* are also available for control of plant parasitic nematodes and plant pathogenic fungi respectively. Currently research is focusing on methods for the industrial production of these fungi by solid and submerged fermentation and the development of effective and stable formulations. Quality control of these products is guaranteed by means of state regulation where parameters of quality are specified for each product type. The intensive and continued use of fungi for pest control in Cuba has led to a considerable decrease in the use of pesticides, with increasing benefits for the environment which is maintained in ecological harmony with the natural biological regulators while still reducing economic damage to crops.

POSTER NP3 - Tuesday (Nematodes)

Mortality of larvae and pupae of *Galleria mellonella* treated with bacterial symbiont from entomopathogenic nematodes

Ali N. Mahar, Sami A. Elawad, Simon, R. Gowen and Nigel M.G. Hague

Department of Agriculture, The University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT, U.K.

It is a widely held belief by those who research on (EPNs) that bacterial symbionts (*Xenorhabdus* and *Photorhabdus*) can only cauase death if carried delivered into the haemocoele of insect by the nematode vector. The experiments described in the poster show conclusively that larvae and pupae of *Galleria mellonella* exposed in sand (20% MC) to both *Xenorhabdus nematophilus* bacteria (*S. carpocapsae*) and *Photorhabdus luminscens* bacteria (*H. bacteriophora*) are susceptible to infection by the bacterial symbionts. Since oral and anal infection by the bacteria has been shown to be non-pathogenic it is concluded that penetration to the haemoceole is via spiracles. Questions arise how long do these bacteria can survive and remain pathogenic outside the vector and the host? Do these bacteria exert control of insects in the soil phase of their life cycle? What therefore are the ecological consequences of applying large number of pathogenic bacteria in the soil environment?

CONTRIBUTED PAPER - Thursday, 10:30 (Viruses IV)

Complementation of a gp64null mutation in AcMNPV with the Vesicular Stomatitis Virus G protein

J. Mangor[1], S. A. Monsma[2], and G. W. Blissard[1]

[1]Boyce Thompson Institute, Cornell University, Ithaca, NY 14853
[2]Novagen, Inc., 601 Science Drive, Madison, WI 53711

GP64 is an essential AcMNPV virion protein that is involved in receptor binding and membrane fusion during virus entry. Previous studies have shown that an AcMNPV virus containing a *gp64* deletion (vAc^{64-}) is unable to move from cell to cell due to a defect in the assembly and production of budded virions (BV). To examine the requirements for virion budding, we asked whether a heterologous viral envelope protein, the vesicular stomatitis virus (VSV) G protein, was capable of complementing the deletion of *gp64* in the gp64null virus, vAc^{64-}. To address this question, we generated and characterized a stably transfected insect Sf9 cell line ($Sf9^{VSV-G}$) that inducibly expresses the VSV G protein upon infection with AcMNPV. $Sf9^{VSV-G}$ and Sf9 cells were infected with vAc^{64-} and cells were monitored for infection and movement of infection from cell to cell, using plaque assays. vAc^{64-} formed plaques on $Sf9^{VSV-G}$ cells but not on Sf9 cells. However, plaques formed on $Sf9^{VSV-G}$ cells were observed only after prolonged periods. The gp64null virus was propagated and amplified on $Sf9^{VSV-G}$ cells, but could not be similarly propagated on Sf9 cells. Pseudotyped virus particles produced in $Sf9^{VSV-G}$ cells contained the VSV G protein. Although cell-to-cell propagation of the gp64null virus was delayed in comparison to wt AcMNPV propagation in the $Sf9^{VSV-G}$ cells, growth curves showed that pseudotyped virions were generated at titres of approximately 10^6 to 10^7 infectious units (IU)/ml, compared with titres of approximately 10^8 IU/ml for wt AcMNPV in the same cells. While titers of vAc^{64-} pseudotyped with VSV G protein were lower than those produced by wt AcMNPV, the propagation and amplification of pseudotyped virions in $Sf9^{VSV-G}$ cells suggests that the VSV G protein possesses the necessary signals for baculovirus BV assembly and budding at the cell surface.

POSTER VP31 - Thursday (Viruses)

Multiplex PCR and quality control of *Epap*GV production

M. A. Manzán[1]; E. M. Aljinovic[3]; A. Sciocco-Cap[1,2]; P. D. Ghiringhelli[3] and V. Romanowski[1,3]

[1]IBBM, Facultad de Ciencias. Exactas, UNLP, Calle 49 y 115, 1900-La Plata; [2]IMYZA, INTA-Castelar, [3]Dto. de C. y Tecnol. - CEI, UNQ, Argentina.
(e-mail: alemanza@nahuel.biol.unlp.edu.ar or victor@nahuel.biol.unlp.edu.ar)

A system based on multiplex PCR was developed as a method for the specific, rapid, and highly sensitive quality control of the viral DNA during *Epinotia aporema* granulovirus (*Epap*GV) production. Usually, the design of truly specific primers requires the knowledge of the complete nucleotide sequence. When this condition is not met, alternative approaches must be used for the primer design. At the beginning of this work only the 2.3% of the *Epap*GV genomic sequence was known (*granulin* and *egt* loci). To increase the availability of specific DNA information, the sequence of the ends of a series of selected clones of *Epap*GV genomic libraries was determined using the dideoxy method. These data comprised 8.4 % of the total EpapGV DNA sequence and corresponded to regions distributed throughout on the genome. Based on this information, a set of 32 primers was designed, complying with the criteria of a theoretical maximum of specificity and minimum probability of random hybridization.

The information theory was used as the theoretical basis for the design. Briefly, the user defined the length of the desired oligonucleotide and each sequence was scanned for all the possible, partially overlapping, "words" of this length moving one residue at the time (total of words = length of sequence - length of word + 1). *A priori* all the words are considered as putative primers, and are scored on the basis of three parameters: information complexity, GC content and presence of nucleotide runs; the last parameter has an additional differential penalty based on the 3' end proximity of the run. Each pair of designed primers was initially tested in individual PCRs to assess the correct size of the expected product and the sensitivity of the amplification. The specificity was verified in multiplex PCRs, using 1 to 3 subsets of primers and *Epap*GV DNA from different sources and degrees of purity. The results indicate that the multiplex PCR could be used for quality control in the bioinsecticide production, as well as in other applications such as the detection of possible latent infections of *E. aporema* colonies, and studies related to virus distribution and persistence in the field.

CONTRIBUTED PAPER - Thursday, 11:15 (Viruses IV)

A Host Midgut Cell-Binding Envelope Protein of *Spodoptera litura* Nucleopolyhedrovirus

Wenfu Mao[1], Xiaoguang Ouyang[1], Yi Pang[1], Jiang Zhong[1,2] and Deming Su[1,2]

[1]State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P. R. China
[2]Virology Research Unit, Fudan University, Shanghai 200433, P. R. China

Baculoviruses initiate their infection after the host ingests the polyhedra and polyhedron-derived virions (PDVs) were released. However, the molecular mechanism of virus infection of midgut cells is not known. In this investigation we have identified a host midgut cell-binding envelope protein from *Spodoptera litura* nucleopolyhedrovirus (*Splt*NPV). PDVs were liberated from *Splt*NPV polyhedra by dissolution with the carbonate solution. Peroral infection assay showed that the virions were highly virulent. A total of 16 envelope proteins with their molecular weights ranging from 14.4 to 66 kDa were detected by SDS-PAGE and silver staining. Protein components extracted from viral envelope were labeled with ^{125}I by the chloramine-T method, and were allowed to react with *Spodoptera litura* brush border membrane vesicles (BBMV) freshly prepared *in vitro*.The envelope proteins bond with BBMV were separated by ultracentrifugation from the other free envelope proteins. By binding kinetics assay it was shown that when the reaction temperature was above 4º, the binding rate reached its peak within 5 mins, then dropped low and was in fluctuation. Long term stable conjugation would be obtained at 0º. The envelope proteins bound with BBMV was resolved by SDS-PAGE followed by autoradiography. A distinct single labeled protein of 15 kDa bound to BBMV was identified. We suggest that the 15 kDa protein could be a key protein involved in the first step of PDV infection.

POSTER VP13 - Tuesday (Viruses)

Toxic effects of *Invertebrate iridescent virus 6* in mosquitoes

C. F. Marina,[1,2] J. I. Arredondo-Jiménez,[2] I. Fernández-Salas[3], J. Ibarra[4] & T. Williams[1].

[1]ECOSUR, AP 36, Tapachula 30700, Chiapas, Mexico
[2]Centro de Investigación de Paludismo, AP 537, Tapachula 30700, Chiapas, Mexico
[3]Fac. Ciencias Biológicas, Univ. Auton. Nuevo León, AP 109-F, Monterrey, Mexico [4]CINVESTAV-IPN, AP 629, Irapuato 36500, Guanajuato, Mexico.

Invertebrate iridescent viruses [IIVs] (*Iridoviridae*) are dsDNA viruses that infect invertebrates, particularly insects in humid and aquatic habitats. IIVs cause obvious, lethal infections in which the host becomes an iridescent blue or purple colour and later dies. Inapparent, non-lethal infections have also been reported from natural populations of blackflies and in laboratory studies of mosquitoes. Such covertly infected mosquitoes show reduced fecundity, longevity and smaller body size compared to uninfected conspecifics. However, the cause of these sublethal effects is uncertain. Viruses of this family possess a common protein of 12.5 kDa that shows amino acid sequence homology to a neurotoxin present in the venom of a Brazilian armed spider. In this poster we present the results of a study on the effects of the toxic IIV protein on selected demographic parameters of *Aedes aegypti*. Groups of 4th instar *Ae. aegypti* larvae were exposed to one of the following treatments for a period of 6 h: infective IIV-6; UV-inactivated IIV-6; heat-inactivated IIV-6; nothing (controls). Mosquitoes were subsequently reared until adult emergence, blood-fed and allowed to oviposit. The presence of infective IIV in experimental insects was determined post-mortem by a sensitive insect bioassay using *Galleria mellonella*. Results to date indicate that control insects pupate before IIV-exposed insects ($F_{3,1553}$=12.429, P< 0.001) and control adults emerge before virus-exposed conspecifics ($F_{3,1526}$=12.465, P<0.001). A full analysis of the results of this experiment will be presented in an attempt to distinguish the toxic effects of IIV proteins from the pathological effects of IIV replication.

POSTER BP42 - Thursday (Bacteria)

Production and evaluation of cultures of *Bacillus thuringiensis* (Berl.) with effect on *Polyphagotarsonemus latus* (Banks) (Acarina: Tarsonemidae)

María Elena Márquez Gutiérrez, Orietta Fernández-Larrea, Lérida Almaguel.

Plant Health Research Institute
Street 110 #514 e/5ta and 5ta F. Playa, Ciudad Habana. Cuba
E.mail: inisav.ceniai.inf.cu

The use of *Bacillus thuringiensis* (Berliner) (*Bt*) for mite control, mainly against *Polyphagotarsonemus latus (Banks*, (P.latus), a nocive pest of potato crop, plays an important rol to reduce the chemical acaricides which produce disorders in the agricultural ecosystems. An isolate from the collection of the Plant Health Research Institute (INISAV), denominated LBT-13, was selected for its lethal action on *P. latus.* It was reproduced in a fermentator with effective capacity of 10L in four culture media with different composition, during 24, 48 and 72h. The higher yield of spores and delta endotoxin crystals, were found after cultivation of 72 hours in a culture media with Torula yeast, rice starch and salts. The best combination time/culture medium was used to calculate the LC 50 and LC 95; they were $1.1X10^7$ and $4.4X10^9$ spores and crystal/ml respectively on populations of the mite under laboratory conditions.To optimize the cultivation conditions, the inoculum, agitation and aereation were adjusted by using an orbital shaker with controlled temperature of 29± 1 °C. The inoculum in 5% v/v logarithmic phase, a high agitation and a relationship between volume media and flask of 1/10, increased the yields of spores and delta endotoxin crystals. The best reproduction medium obtained by submerged cultivation were also tested by the method of static cultivation and the maximum yield of biomass was reached after 7 days of incubation. Cultures obtained by both methods were centrifuged and the supernatant was heated to 120° C degrees for 15 minutes to check the presence and the effect of thermostables exotoxins produced during the growth. There were little

differences in the control of *P. latus* between the complete cultures of *Bt* and the variant with exotoxins alone.

SYMPOSIUM I - Monday, 15:20 (Bacteria)

Bacillus toxin* (*Bt*) susceptibility and resistance in *C. elegans

Lisa Marroquin[1], Dino Elyassnia[1], Joel Griffitts[1], Jerald Feitelson[2], and Raffi V. Aroian[1]

Department of Biology[1], University of California, San Diego, La Jolla, CA 92093-0349; Akkadix Corportation[2], La Jolla, CA 92037

The protein toxins produced by *Bacillus thuringiensis* (*Bt*) are the most widely used natural insecticides in agriculture and have been expressed in transgenic corn, potato, and cotton to provide organic crop protection against insect pests. Despite successful and extensive use of these toxins, little is known about toxicity and resistance pathways in target insects since these organisms are not ideal for molecular genetic studies. To address this limitation and to investigate the potential use of these toxins to control plant-parasitic nematodes, we are studying *Bt* toxin action and resistance in the nematode *Caenorhabditis elegans*. We demonstrate for the first time that a single *Bt* toxin can target a nematode. The *Bt* toxin we chose is Cry5B since it is approximately 24% identical with Cry1A toxins across the entire toxin domain. When fed Cry5B, *C. elegans* hermaphrodites undergo extensive damage to the gut, a decrease in fertility, and death, consistent with toxin effects in insects. We have screened for and isolated 46 recessive mutants that resist the toxin's effects on the intestine, on fertility, and on viability. These mutants define five genes, indicating that more components might be required for *Bt* toxicity than previously known. We find that a second, unrelated nematicidal *Bt* toxin may utilize a different toxicity pathway. Our data indicate that *C. elegans* can be used to undertake detailed molecular genetic analysis of *Bt* toxin pathways and that *Bt* toxins hold promise as nematicides. Recently, we have cloned one of these resistance genes by rescuing the mutant with a 4.3 kb genomic fragment that contains a single predicted open reading frame (ORF). This ORF is likely to be present in insects since the gene is highly conserved in *Drosophila*. Once confirmed by sequencing of mutant alleles, this would result in the first definitive identification of a gene required for *Bt* toxin action in any organism.

STUDENT POSTER BP11 – Tuesday (Bacteria)

***Bacillus thuringiensis* Cry proteins activity against the Andean potatoe weevil *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae)**

Martinez, W.; Grosso, V.; Uribe, D.; J. Cerón

Instituto de Biotechnología, Universidad Nacional de Colombia. A.A. 14490, Santefe de Bogotá, Colombia

Colombia is one of the main potatoes producing and consuming latinamerican country. This crop is strongly affected in field conditions by the coleopteran *Premnotrypes vorax* Hustache (Andean potatoe weevil), a pest whose control is based in highly toxic and residual chemical insecticides.

In searching for biological control strategies against Andean potatoe weevil a *B. thuringiensis* proteins evaluation methodology was developed. First instar larvae were used in natural diet based bioassays. *B. thuringiensis* Cry3Aa, Cry3Bb, Cry3Ca and Cry7Aa, were evaluated as crystal-spore mixtures and as soluble proteins at two alkaline and acid pH conditions.

In order to evaluate binding behaviour of above mentioned proteins to the insect gut. It was also standardized the *P. vorax* brush border membrane vesicles (BBMV) purification method, using whole last instar larvae.

Results of this evaluation are presented and discussed.

SYMPOSIUM - Tuesday, 10:30 (Nematodes)

***Photorahbdus and Xenorhabdus* Genes for Transgenic Plants**

Thomas Meade, Scott Bintrim , Donald J. Merlo ,, Jon Mitchell and Jean L. Roberts

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

Nematophilic bacteria from the genera *Photorhabdus* and *Xenorhabdus* produce orally active, insecticidal proteins active against a wide range of insect pests including economically important Coleoptera, Lepidoptera, Diptera, and Acarina. These proteins, and their corresponding genes, are distinct from the *Bacillus thuringiensis* proteins and genes that are currently deployed in transgenic plants. As such, transgenic plants containing genes from these bacteria represent exciting new tools for insect pest management. The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, a serious pest of maize in the USA, is the target of current efforts to produce transgenic maize expressing a gene from *Photorahabdus.*

SYMPOSIUM - Thursday, 11:00 (Microbial Control)

Bioinsecticide Marketing Aspects in Mexico

H. Medrano-Roldán [1] and C. García-Gutiérrez [2] .

[1] Instituto Tecnológico de Durango, Felipe Pescador 1830. Tel. y Fax. 01 (18) 18-69-36
[2] CIIDIR-IPN Unidad Durango, Sigma s/n Fracc. 20 de Nov. II 34220 Durango, Mex.
Tel y Fax. 01 (18) 14-20-91

The bioinsecticide marketing in Mexico is incipient, in contrast with the national marketing of pesticides. These products have been acceptable as alternative to use them in management integrated pest control programs (MIP), in vegetables, corn, sugar cane, sorghum and cotton. The bioinsecticides marketing is present in national factories for supply regional trades, in the five last years many products have been commerced. The bacterium *Bacillus thuringiensis* (*B.t*) is the most important bioinsecticide with potential marketing of 100,000 ha in corn, 174,000 ha in cotton, and 200,000 ha in vegetables, each year. Actually, national products sales is around of 150,000 ha. In Mexico there is technology to produce *B.t*, although the bacterium marketing is dominated by foreign companies; the average cost of a dosage was $19/ha, this price is still competitive compared with chemical insecticides cost. In this case, the bacterium toxicity has been successful which has based in high capability to kill insects in field of many important pests insects in Guanajuato and La Laguna region in Durango and Coahuila, as well as the Culiacan valley in Sinaloa State. The entomopathogenic fungus is produced to local use for *Aenolamia* sp. control of sugar cane in Colima; in Oaxaca *Beauveria bassiana* is used to pest control of coffe borer *Hipothenemus hampeii.* In Culiacan, Sin., Agrobionsa factory are producing *Metarhizium anisopliae, B. bassiana* and *Paecilomyces fumosoroseus* to pest control insects of vegetables, corn and soybean. The trade size is more than 100,000 ha. Biological products elaborated with *Tagetes* sp. and *Chrysanthemum* sp. which have been recommend for use in organic agriculture. The annual sales are estimated in $30,000.00 each year, the price of the product is $ 8.0/l o kg. However, these products not achieve an important impact in traditional agriculture. The nematode *Romanomermis* spp. is produced, at laboratory level, in a government institution in Oaxaca, for *Anopheles* sp and *Culex* sp control. Other products, as nematodes pheromones, virus and azadirachtina have low trade position. This situation indicates that the technology for production of *B.t* and entomopathogenic fungus production are adequated. However, the formulation, synergist action, and field evaluation of bioinsecticides studies are not enough. In relation to economic and commercial aspects, the bioinsecticides are even more expensive than many chemical insecticides and have stability problems yet; for these reasons, the bioinsecticides have low grade of acceptance in national trade, so the possibilities to increase the marketing bioinsecticide and industry development in Mexico will depend of many aspects as the plaguicide resistance in insects, the MIP advances are permitting the biologic insecticide use, as well as, progress in manufacture (fermentation and formulation), and commerce strategies; these aspects are very important to improve the biological bioinsecticide position. The globalization trade of bioinsecticides will depend of the efficiency of those products, earnings and competitions in the development of news products, as well as, a strong support of private sectors and government institutions efforts.

CONTRIBUTED PAPER - Tuesday, 8:30 (Viruses II)

The AcMNPV *pe38* gene is not essential but affects DNA synthesis and budded virus production.

Maynard L. Milks, Leslie G. Willis, and David A. Theilmann

Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B. C., Canada V0H 1Z0,

AcMNPV PE38 is a transcription factor that has been shown to activate baculovirus genes such as p143 (*helicase*). In addition, transient replication assays have shown that PE38 augments viral DNA replication. Despite these important functions homologues of *pe38* have not been identified in the completely sequenced genomes of LdMNPV, SeMNPV, and XcGV, suggesting that it may not be an essential gene. To address this question, we successfully isolated *pe38* deletion viruses that replaced the wild type gene with the gene encoding Green Fluorescence Protein. The *pe38* knock out viruses were assayed in *Sf9* cells and in *Trichoplusia ni* larvae. No differences were observed in total protein synthesis, but levels of viral DNA synthesis and budded virus production of *pe38* deletion viruses in *Sf9* cells were decreased significantly. Interestingly, bioassays revealed no differences in LD_{50}, LD_{95}, and LT_{50} of *pe38* deleted and wild type viruses in *T. ni* larvae did not differ. Scanning and transmission electron microscopy revealed no structural differences between the wild type and *pe38* deletion viruses.

STUDENT POSTER BP43 - Thursday (Bacteria)

Intramolecular cleavage of Cry1Ab by midgut proteases.

Raúl Miranda and Alejandra Bravo

Instituto de Biotecnología. UNAM. Apdo. Postal 510-3, Cuernavaca 62250, Morelos, México raulm@ibt.unam.mx

Activation of Cry toxins is performed by midgut proteases and is important for toxicity and in some cases for specificity. The proteolytical processing generates a 50-60 kDa protein by the digestion of the N- and C-terminal ends to produce the so-called toxic fragment.

There is an increasing list of Cry toxins that also suffer intramolecular digestion. Cry11A is proteolytically processed by *Culex quinquefasciatus* midgut proteases between β5-β6. Cry3A is digested by chimotrypsin, producing peptides of 11, 49 and 6 kDa. One cleavage site is between α3−α4 in domain I. Chymotrypsin digestion enhances the solubility of this toxin at the pH of the host lumen midgut. Cry4A is nicked intramolecularly by *Culex pipiens* midgut proteases to produce two peptides of 20 and 45 kDa. When cloned and expressed separately, these peptides do not show any activity. However, when they are coexpressed and tested, the toxic activity is similar to the 60 kDa non-cleaved toxin. In all of the previous examples, the intramolecular cleavage do not result in the separation of the products as they are coeluted in size exclusion or affinity chromatography and processing does not cause a drop in toxicity.

To date there are no examples of intramolecular processing in Cry1A toxins. However, there are evidences that at least in artifitial membranes, the channel activity of the δ-endotoxin depends on the method of activation. In the current work, we show intramolecular processing of Cry1Ab with midgut proteases of *Manduca sexta* larvae.

Activation of Cry1Ab with trypsin produces the toxin fragment of 60 kDa. We found that the processing of the Cry1Ab protoxin with *M. sexta* gut juice produces a 30 kDa band. After feeding 5th instar *M. sexta* larvae with Cry1Ab protoxin it was observed the *in vivo* production of the 30 kDa fragment. This peptide binds to BBMV isolated from *M. sexta*. The toxicity of gut juice or trypsin activated toxin was similar as seen in bioassays against 1st instar *M. sexta*. In addition, pore formation activity in BBMV remains unaltered. Thus, digestion of this protoxin with gut juice does not result in a lose of function.

The 30 kDa band coeluted in a size exclusion chromatography with the 60 kDa monomer suggesting that the gut juice treatment does not destroy the actual structure of the protein but only makes a nick. We tried unsuccessfully to separate the 30 kDa proteins by ion exchange chromatography. Nevertheless, the Western blot analysis of the different fractions, performed with a monoclonal antibody which recognizes an epitope of domain I, showed that there are at least two different peptides of 30 kDa, and only one of them contains domain I. On the other hand, a polyclonal antibody raised against the 60 kDa Cry1Ab toxin recognized both 30 kDa fragments.

POSTER GP1 - Thursday (General)

Development of aseptic rearing system for the brown-winged green bug on semi-artificial diets

Koji Mishiro, Fumio Ihara and Takeru Sato

National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305-8605, Japan

Aseptic rearing system for the brown-winged green bug, *Plautia stali* SCOTT (Hemiptera: Pentatomidae), was developed to clarify the functions of intestinal bacterial flora especially their symbionts. Newly developed semi-artificial diets were used in this experiment. Peanuts, soybeans, brown rice and vitamin mixtures were used as basal diets, and amino acids and sterols were added to them as suppliments for some diets. Sterilized eggs, diet and water were kept in a sterilized glass plant culture flask (55mm diameter at the bottom and 90mm hight). Silicon grease was smeared at 2/3 height of inside of each flask in a belt to keep nymphs on the bottom. Nymphs were reared under 22.5 degrees and 16L8D conditions and their developments were compared. Emerged adults were individually tested for contamination. After all nymphs emerged or died, each flask was also tested. No contamination was confirmed, then emerged adults were decided as aseptically reared ones. On the diets without amino acids and sterols, growths of hatched nymphs were not synchronized in each flask and nymphal periods prolonged, but a few numbers of small adults emerged. On the other hand, on the diets with amino acids and sterols, growths of hatched nymphs were improved, and on the certain diet, more than 30% of individuals emerged. Their body sizes were as large as adult stinkbugs reared in our laboratory fed on raw peanuts and soybeans. Consequently, this method will be applicable to the aseptic rearing system for the stinkbugs.

CONTRIBUTED PAPER - Thursday, 12:00 (Viruses IV)

Efficacy of the *Helicoverpa armigera* nuclearpolyhedrovirus (HaNPV) against *H. armigera* Hübner (Lepidoptera: Noctuidae) on citrus

Sean D. Moore[1], Timothy M. Pittaway[1], Jeremy G. Fourie[1] and Gustav Bouwer[2]

[1]Outspan Citrus Centre, P.O. Box 12531, Centrahil 6006, Port Elizabeth, South Africa; e-mail: sean_moore@capespan.co.za
[2]Department of Microbiology, University of the Witwatersrand, Johannesburg 2050, South Africa

Chemical sprays directed against citrus thrips and the bollworm, *Helicoverpa armigera*, applied in spring are disruptive to the biological control of California red scale and citrus mealybug. As an alternative to organophosphates and pyrethroids, a locally isolated nuclearpolyhedrovirus (HaNPV) was tested against *H. armigera*. In laboratory bioassays HaNPV caused 98% mortality of second instar *H. armigera* larvae within 11 days. The virus was mass produced *in vivo* in the laboratory. In a pilot trial various concentrations of HaNPV suspension were sprayed on tomato plants, artificially infested with *H. armigera*. Two concentrations (7.26×10^5 and 1.15×10^6 OBs/ml) were subsequently selected to apply against *H. armigera* on navel orange trees at two field trial sites. These were applied at rates of between 3.35×10^{12} and 7.50×10^{12} OBs/ha. Both concentrations at both sites caused 100% mortality within 14 days after application or slightly longer. HaNPV was significantly more effective than a commercial *Bacillus thuringiensis* insecticide, a neem kernal extract and mevinphos, which were applied at the same time. Despite the protracted killing time, fewer fruit were damaged and downgraded from the HaNPV treatments than from any of the other treatments. At one site only 0.4% of HaNPV treated fruit were graded non-exportable, compared to 10.0% of untreated fruit. During a second season, HaNPV was applied on navel orange trees at a further two sites at concentrations of 3.63×10^5 and 7.26×10^5 OBs/ml. These were applied at rates of between 1.06×10^{12} and 2.42×10^{12} OBs/ha, both with and without the addition of a mineral oil. The oil appeared to improve

efficacy, although no differences were significant. HaNPV reduced infestation to significantly less than in the untreated control, however not as dramatically as during the previous season. The less impressive results may be attributed to the lower rates at which HaNPV was applied, and to spraying being conducted when mature eggs were observed rather than young larvae, as was the case during the previous season. Despite this, HaNPV was one of only two treatments for which no fruit were graded non-exportable.

WORKSHOP I - Thursday, 9:15 (Bacteria)

The Mexican experience with insect resistant (Bt) transgenic crops

Ariel Alvarez Morales

Cinvestav, IPN. Irapuato Unit. Department of Plant Genetic Engineering, Apdo. Post. 629, Irapuato, Gto. Mexico 36500

The first transgenic materials with the phenotype of insect resistance were tested in Mexico in 1992. This was a Bt-tomato aimed to be resistant to pin-worm infestation. Since then, a total of 45 permits have been issued which include field trials with maize, cotton, tomato and potato, and one field trial with a modified strain of Bacillus thuringiensis. Bt-Cotton (Bollgard) from Monsanto has been the most widely tested Bt-crop in Mexico. It has been grown in the north of Mexico as a pre-commercial release, which means that it is subject to strict monitoring to ensure that the guidelines and procedures, required to minimise the risk of accelerating the appearance of insects resistant to the bioinsecticide, are being properly implemented and thoroughly followed. Insect populations have also been screened, by independent researchers as well as by Monsanto, and up to this date, no resistance has been reported. The total area that has been planted to Bt-cotton has been around 22,500 hectares in the years 1997-1999.

Although most of the permits were issued to allow field trials with Bt-maize, 24 out of the total of 45, these never included large trials nor pre-commercial releases, and field trials of transgenic maize from the biotechnology industry were stopped since 1998 (there was only one permit granted, to Pioneer Hi-Bred, that year). The reason for this "non official moratorium" on transgenic maize is a lack of a suitable legal framework to address many of the issues posed by this crop in Mexico, as well as fears, real and imagined, of potential damage of the ancestors of maize Ð teocintle - the land races, and biodiversity as a whole. The legal topics are now being addressed by a completely new legal system which approaches the issues of release, importation, deregulation, labelling, etc., from a intersecretarial and multisciplinary point of view. The problems of evaluating the risks involved in the release of transgenic maize still requires data that must come from research aimed at understanding the biology of teocintle, the relationship between teocintle, the land races, and the hybrids. Social scientists are also assessing the potential benefits that this technology could bring to the poor farmers.

STUDENT POSTER GP2 - Thursday (General)

The discovery of late male-killing in the oriental tea tortrix, *Homona magnanima* (Lepidoptera: Tortricidae)

Sayaka Morimoto, Madoka Nakai, and Yasuhisa Kunimi

Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan

A sex ratio distortion towards females was found in the oriental tea tortrix, *Homona magnanima* (Lepidoptera: Tortricidae) in Tsukuba, Ibaraki, Japan. There was no difference in the mean egg hatchability between the all-female strain and the normal strain. More than 50% mortality was observed in the all-female strain during the larval and pupal stages, suggesting that the production of females only was the result of late male-killing. The female-biased sex ratio was maternally inherited and was maintained in a backcross with normal strain males. Therefore, a cytoplasmic parasite was thought to be the causative agent. When normal strain larvae were inoculated with a homogenate of dead male larvae of the all-female, this male-killing trait was transmitted to the next generation, suggesting that the agent could be horizontally transmitted.

SYMPOSIUM IV - Friday, 12:10 (Bacteria)

Field efficacy of *Bacillus thuringiensis israelensis* (Bti) against aquatic midges of public health importance

M. S. Mulla

Department of Entomology, University of California, Riverside, CA 92521 USA

Aquatic midges in the family Chironomidae constitute a major taxon of nemotocerous diptera. They are closely related to mosquitoes, but incapable of piercing and sucking blood. These insects propagate in shallow to deep waters and stagnant to fast flowing water. The immature stages are either attached to algae, rocks, trailings, macrophytes or reside in muck and other substrates in the bottom of lakes, ponds, reservoirs, creeks, streams and small to huge rivers. Most of the aquatic midge species serve a useful purpose in the recycling of organic wastes or providing an ample source of food for fish, waterfowl and other birds. At times in some communities, huge numbers of adult midges emerge which pose a serious health and economic threat to the people living close to water habitats. There is ample evidence that dead midges and their parts are the cause of atopic allergy in humans. Asthma and rhinitis symptoms have been attributed to abundance of adult midges. Most often the midge swarms pose a serious threat to outdoor living and sporting activities. They also enter dwellings, attracted to lights causing a great deal of annoyance. On account of this serious problem, aquatic midges are controlled on an ongoing basis.

Because of the aquatic nature of the midges which are controlled in the larval stage, many synthetic insecticides are contraindicated for use in aquatic habitats, because of their possible adverse impact on fish, birds and wildlife. Organic phosphate insecticides such as malathion, fenthion, chlorpyrifos and others have been recently prohibited from further use for midge larval control in aquatic habitats. The microbial control agent Bti has been found to provide effective control of some groups of chironomid midges in lakes, ponds, sewage oxidation ponds and other. Efficacy of formulations and dosages required for control in various biotopes will be discussed. It should be pointed out that aquatic midges are considered as nontarget biola and the risk/benefit analysis of control programs with Bti will be addressed.

Bti formulations have varied activity against different groups of midge larvae. In general Bti granules (corn grit, sand core) provide excellent control of midge larvae in the subfamily Chironominae, which are highly abundant and annoying to people residing close to lakes, rivers, streams, canals, percolation basins, ponds, reservoirs and storm drains and flood control channels. The dosages required for the control of these midges is higher than the dosage needed for mosquito control. Several groups of midges are not inherently susceptible to Bti formulations. For example the tanopodine, orthocladine and a few other groups are refractory to Bti. Before Bti is recommended for aquatic midges control, it is important that the species composition and seasonality of midges is determined. If chironomine midges contribute the major proportion of the midge fauna, then it would be possible to control with Bti applications

SYMPOSIUM II - Monday, 16:30 (Viruses)

Where and when do nucleopolyhedroviruses matter to insect populations?

Judith H. Myers

Department of Zoology and Faculty of Agricultural Sciences,University of British Columbia, 6270 University Blvd. Vancouver, B.C.,Canada V6T 1Z4

Nucleopolyhedroviruses (NPVs) are an important aspect of the population dynamics of some cyclic populations of forest Lepidoptera, but in others they are rarely observed. Similarly NPVs are associated with some lepidopterans that are agricultural pests, but not with others. What makes the difference between host species for which viral epizootics are a regular occurrence and those for which they are not? Host density, food plant type, number of generations per year, virulence of the virus and geographic location are all features that could influence the potential for NPV persistence and for the occurrence of epizootics. I

will summarize the characteristics of species and populations of Lepidoptera for which epizootics of NPV have and have not been recorded. I will test the hypothesis that NPV is more commonly associated with more northern (colder), cyclic populations of forest caterpillars and more southern populations of agricultural pests that are characterized by multiple generations a year.

POSTER BP19 - Tuesday (Bacteria)

Screening Of Tropical Strains Of *Paenibacillus popilliae* Against Two Mexican White Grub Species.

Najera-Rincon, Miguel R.[1], E. Hidalgo[2], P.J. Shanon[3], and L. A. Rodriguez del Bosque[4]

[1]National Research Center for Sustainable Production, CENAPROS-INIFAP. PO BOX: 7-116. Morelia, Michoacan. 58260. Mexico. miguel_najera@infosel.net.mx

[2]Tropical Agricultural Research and Higher Education Center (CATIE), Costa Rica

[3]Natural Resources Institute, United Kingdom,

[4]North East Regional Research Center, Mexico

Twelve entomopathogenic bacteria *Paenibacillus* (*Bacillus*) *popilliae* isolations from Central and Norh America pathological potential was evaluated on two "white grub" species of *Phyllophaga* (COLEOPTERA: Melolonthidae) genus, which are economically very important pests in Mexico. Inoculations were done topically (*per os*), and through a water spore suspension at $1.25X10^6$ spore/larva dosis on L3 *P.ravida* (Blanch) produced at the laboratory, and *P. vetula* (Horn) collected in crop fields. Ten isolations infected larvae, and nine caused mortality with Bp symptoms at least to one of the two *Phyllophaga* species. Of those isolations, four caused the highest infection percentajes when they were inoculated through injection to both *P. vetula* and *P. ravida* (33.3%) during the first selection stage. These stocks produced lower infection levels (9.9%) when they were applied via *per os* (natural infection via) to *P. vetula* larvae. However, with both infection metods a large asymptomatic mortality percentages occurred. This phenomenon should be studied deeply to determine the real bacteria control effect under field conditions, as well as the optimal infection levels with sympton development to assure an efficient diffusion of the disease.

POSTER BP32 - Thursday (Bacteria)

A novel *Bacillus thuringiensis* delta-endotoxin Cry1 hybrid protein with high activity against Colorado potato beetle

Samir Naimov,[1] Mieke Weemen-Hendriks,[1] Elena Ouzounova,[2] Stefan Dukiandjiev,[2] Dirk Bosch,[1] and Ruud A. de Maagd[1]

[1]Business Unit Cell Cybernetics, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[2]Department of Plant Physiology and Molecular Biology, University of Plovdiv, 24 Tsar Assen Street, Plovdiv, Bulgaria

In order to identify and study *Bacillus thuringiensis* delta-endotoxin features that determine general activity (or the lack thereof) against classes of insects such Lepidoptera and Coleoptera, we have made a number of Cry1/Cry3A-hybrids, as well as Cry1B/Cry1I-hybrids. Cry3A is the most active known natural toxin for the Coleopteran Colorado Potato Beetle (CPB). Cry1Ba and Cry1Ia. are most active against several lepidopteran larvae altough, in contrast to other Cry1 toxins for these two weak activity against CPB has been reported as well. Recombination between either Cry1Ia or Cry1Ba and Cry3A gave only a few soluble events resulting in soluble protoxins which could be studied further. Hybrids of Cry3A with (a larger part of) domain I replaced by that of Cry1B or Cry1I are about 5 times less active against CPB, suggesting that domain I of Cry3A has some role in specificity. A hybrid in which Cry3A has obtained the protoxin-specific C-terminal part of Cry1B is (as a solubilized protein) twice as active as Cry3A on a molar basis.

Both Cry1Ba and Cry1IA as solubilized protoxin were found to be slightly active against CPB larvae (2 and 6% of Cry3A activity, on a molar basis). However, a hybrid of Cry1Ba with its first two domains replaced by those of Cry1Ia had CPB activity higher than that of either parent (20% of Cry3A activity), and a mosaic consisting of Cry1Ba with only its second domain replaced by that of Cry1Ia had 45% of Cry3A activity. Together with its potential for anti-lepidopteran activity the latter hybrid could be interesting for applications were both activities are required.

CONTRIBUTED PAPER - Tuesday, 11:30 (Viruses III)

The effect of entomopoxvirus infection on the development and endocrinology of *Mythimna separata* larvae

Madoka Nakai, Akane Ohba and Yasuhisa Kunimi

Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology

The entomopoxviruses (EPV) belong to the Poxviridae, which is a large family that infects vertebrates and invertebrates. Some EPV-infected lepidopteran larvae are reported to exhibit an increased larval life span with retarded or no pupation. However, the physiological mechanism that prevents the pupation of infected lepidopteran larvae in these chronic infections is not known. This study examined the pathological features and physiological alteration of *Mythimna separata* larvae infected with *Mythimna separata* EPV (MyseEPV), in order to elucidate the mechanism of host regulation.

Mythimna separata larvae did not pupate and were killed during the final instar when they were inoculated with 10 $\times$ the LD_{95} (10^6 OBs/ larva) of MyseEPV at the fourth instar. The development time of larvae inoculated with MyseEPV at the fourth instar was longer than that of healthy larvae, and the weight gain by inoculated larvae was significantly less. However, when sixth (final) instar larvae were inoculated with 10 $\times$ the LD_{95} (10^7 OBs/ larva) of MyseEPV, they pupated, but did not emerge as adults. The development time and weight gain of larvae inoculated with MyseEPV at the sixth instar were not significantly different from those of healthy larvae. The final yield of occlusion bodies in larvae infected at the fourth instar was no different from that in larvae infected at the sixth instar. The ecdysteroid UDP-glucosyl transferase (EGT) assay did not detect EGT activity in hemolymph from MyseEPV-infected larvae. The 20 OH-ecdysone titer and activity of juvenile hormone esterase in hemolymph from larvae inoculated with MyseEPV at the fourth instar remained at a low level compared to the levels in healthy larvae. However, the prothoracic gland from MyseEPV-infected larvae was not infected with MyseEPV and had normal viability by trypan blue staining.

CONTRIBUTED PAPER - Friday, 8:45 (Microbial Control II)

Electronic measurements of insect feeding effects caused by biopesticides

Navon[1], V. Alchanatis[2], J. Grinshpun[2], I. Glazer[1]

[1]Institute of Plant Protection, [2]Institute of Agricultural Engineering, ARO The Volcani Center, POB 6, Bet Dagan 50250, Israel

The video-imaging technology provides useful means to quantify and analyze at real time feeding behavior of insect larvae exposed to peroral biopesticides. Image processing algorithms were developed to segment the leaf from the background and estimate the consumed leaf area in each image.The feeding rhythm was then characterized by: rate of leaf defoliation, meal size, inter-meal time and number of meals per time unit. The feeding rhythm was measured in mature larvae of *Spodoptera littoralis* (Lepidoptera), prefed with the entomopathogenic nematode *Steinernema riobrave* in an alginate gel carrier. The mealsize/h and the number of meals/h in the nematode treated larvae was lower than in the control treatment. The same system was used to test Bt-transgenic cotton fed to *Helicoverpa armigera* larvae. Bt- transgenic cotton reduced mealsize/h and increased intermeal time. This feeding profile suggests that the Bt toxic protein in the plant suppressed feeding. The feeding effect was characterized with longer periods of feeding arrest, probably expressing larval recovery from the Bt toxicity. Biting force of mandibles in lepidopterous larvae was measured by another electronic system. In this system, the mature larva bit two metal beams causing them to deflect. This deflection was sensed by a string gauge-tension sensors which transferred the signals to an analog/digital converter mounted on the

computer bus. Forces of mandible adduction and frequency of biting were measured with this gauge. Uses of the electronic systems for evaluating feeding effects in lepidopterous larvae as a diagnostic tool in developing microbial control programs will be described and discussed.

SYMPOSIUM IV - Friday, 10:55 (Bacteria)

Resistance in larvae of *Culex pipiens* complex mosquitoes to *B. sphaericus:* mechanisms, genetics and management

C. Nielsen-LeRoux[1], J. F. Charles[1], M. H. Silva-Filha[2], L. Regis[2], C. M. F. Oliveira[2]
C. Chevillon[3], N. Pasteur[3], G. F. Pei[4], Q. X. Cai[4], and Z. M. Yuan[4]

[1]Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France; [2] FIOCRUZ Centre de Pesquisas Aggeu Malgalhães P.O., 7472, 50670-420 Recife, PE, Brazil; [3] University of Montpellier II, 34095 Montpellier, France; [4] Wuhan Institute of Virology, Wuhan, 430071, China.

*Bacillus sphaericus (*Bs) is a spore forming aerobic bacterium, several strains of which are pathogenic to mosquito larvae. The main larvicidal activity is due to the production of a binary toxin (51 kDa/42kDa) protein complex.

Resistance to *Bs* has occurred both under laboratory and field conditions but only in the *Culex pipiens* complex. All commercialized strains (2362, 1593, C3-41) contain the same binary crystal toxins (Bin 2).

Recent reports from China and from France (Southwest) have shown that resistance occurred in areas which have been treated for a long period. The level of resistance is variable and can reach more than 50,000 fold. The genetic background of the mosquito populations and the conditions for selection of resistance are important for the levels of resistance. In all studied cases, the resistance is due to one major recessive gene, but ongoing investigations in Brazil, China, France and India may change this view.

Two different mechanisms of resistance have been reported, one in which the initial midgut toxin-receptor binding step is interrupted, while the second still remains unknown. There is no evidence for change in the proteinase activity of the larval gut juice. The presence of the binary toxin receptor molecule, a 60 kD alfa-glycosidase, is investigated by partial purification based on CHAPS solubilized BBMF from several resistant *Culex pipiens* complex colonies.

To avoid appearance of resistance, the presence and susceptibility of the treated mosquito populations have to be monitored. Moreover integrated mosquito control methods should be used in general. There is no cross-resistance to *Bti* within the *Bs*-resistant populations, there is even evidence for increased *Bti* susceptibility and recently it was found that some other *Bs* strains are toxic to *Culex* larvae resistant to the commercialized *Bs* strains. In this regard, investigations have been carried out with the *B. sphaericus* strain IAB-59, which exhibits very low cross-resistance. Laboratory selection for resistance to this strain is ongoing in Brazil and China, indicating that resistance can occur, but its development is slower and to a much lower level than to the commercialized strains. The mechanisms of resistance are investigated for these selected colonies.

POSTER BP20 - Tuesday (Bacteria)

Various levels of Cross-resistance to *Bacillus sphaericus* strains in four *B.sphaericus* resistant *Culex pipiens* (Diptera : Culicidae) mosquito populations

Christina Nielsen-LeRoux [1]*, D. Raghunatha Rao[2], T.R. Mani[2], Sylvianne Hamon[1], Jittawadee Rodcharoen[3] & Mir.S. Mulla[3]

[1] Bactéries et Champignons Entomopathogènes, Institut Pasteur, 28, Rue du Dr. Roux, 75724 Paris Cedex 15, France, [2] Centre for Research in Medical Entomology I.CM.R.) Post Box 11, 4 Sarojini Street, Chinna Chokkikulam Madurai- 625 002, India. [3] Department of Entomology, University of California, Riverside,CA 92521-0314, U.S A.

Four *Culex pipiens* complex populations resistant to *Bacillus sphaericus* strain 2362 and 1593 serotype (H5a5b) have been tested for cross-resistance to three other highly toxic *B. sphaericus* strains (IAB59 (H6), IAB881 (H3) and IAB872 (H48).The two field-selected highly resistant populations originating from India (KOCHI) and France (SPHAE) and a high-level resistant laboratory selected population from California (GeoR), showed strong cross-resistance to strain IAB-881 and IAB872 but very low to IAB59. For the lab-selected low level resistant population from California (JRMMR) cross-resistance was found to all three *B. sphaericus* strains.This demonstrates that within the mosquitocidal strains of *B. sphaericus* it is possible to find some which can overcome the acquired resistance to the commercialised strains 2362 and 1593. It is possible that the origin and mechanism of resistance can influence the extent of cross-resistance. These results have important implications for developing resistance management strategies while using *B. sphaericus* in area-wide mosquito control programs.

POSTER BP21 - Tuesday (Bacteria)

Isolation and identification of a putative *Bacillus sphaericus* strain producing Cry-like proteins

M. Eugenia Nuñez-Valdez, F. Javier Villalobos, Marco A. Barreda, Angel Romero, Luciano Hernandez[‡] and Alejandra Bravo

Instituto de Biotecnología, Cuernavaca, Mor. and [‡]Facultad de Química, D.F. Universidad Nacional Autónoma de México

The Cry protein family contains more than 168 hundred different insecticidal proteins organized in 28 groups according to their DNA secuences. The Cry proteins or δ-endotoxins show specific toxic activity against a wide range of insect orders and families. These proteins are mainly produced by strains of *Bacillus thuringiensis*. Two other *Bacillus* spp different to *B. thuringiensis* have been reported containing *cry* genes: *Bacillus popilliae* containing *cry18Aa* and *Clostridium bifermentans* containing *cry16Aa* and *cry17Aa*. There are not previous reports showing strains *of Bacillus sphaericus* producing Cry-like proteins. Some strains of *B. sphaericus* are known for their ability to produce a binary toxin of 52 and 42 kDa with toxic activity against mosquitos. We report the isolation and identification of a *Bacillus* spp. (strain IM22a) active against *Anomala donovani* and *Phyllophaga blanchardi* (Coleoptera:Scarabaeidae) larvae. This strain was isolated from a dead *A. donovani* larva. Phenotypic tests used for *Bacillus* spp taxonomy, identified this isolate as a *B. sphaericus* strain. Molecular characterization by PCR analysis showed that this strain may harbor *cry8* and *cry9* type genes. SDS-PAGE analysis of the crystals produced by this strain showed the production of two proteins of estimated MW of 128 and 147 kDa. The solubilized proteins are processed to trypsin resistant proteins of ~66 kDa. This is the firts report of a *B. sphaericus* strain producing Cry-like proteins. This strain has potentialities to be developed as bioinsecticide for the control of Scarabaeidae pests.

CONTRIBUTED PAPER - Monday, 16:45 (Bacteria II)

Analysis of mutants in some conserved residues of helix α-5 from *Bacillus thuringiensis* Cry1Ab δ-endotoxin

M. Eugenia Nuñez-Valdez, Jorge Sanchez, Laura Lina, Leopoldo Güereca and Alejandra Bravo

Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ap. Postal 510-3, Cuernavaca 62250, Mor., México

Bacillus thuringiensis produces insecticidal crystal proteins known as Cry toxins or δ-endotoxins. It is proposed that the mechanism of action of these toxins is based on three main steps: i) solubilization and proteases activation of the protoxin in the larvae midgut; ii) binding of the toxin to specific gut receptors tiggering a conformational change and iii) toxin insertion, oligomerization and pore formation leading to cell lysis and eventual dead of the insect. The δ-endotoxins are formed by three protein domains: Domain I is a seven-α-helices bundle having the helix α5 in the center, which is thought to be involved in pore formation; domain II is a greek key β-barrel participating in receptor binding, and domain III is a β-sandwich that stabilizes the protein structure and also participates in receptor binding. It has been proposed that domain I helices α4 and α5 might be transmembrane segments lining the pore. We hypothesized that highly conserved residues of α5 could play an important role in toxin insertion, oligomerization and/or pore formation. Functional analysis of a

total of 15 Cry1Ab site-directed mutants located in the highly conserved residues Y153, Y161, H168, R173, W182 and G183 within helix α5 and in the loops linking this helix with α4 and α6 was done. Then, their effect on binding, pore formation activity and toxicity against *Manduca sexta* larvae was compared. Results provide direct evidences that some residues located within α5 have an important role in stability of the toxin in the insect gut, while some others have also a important role in pore formation.

CONTRIBUTED PAPER - Thursday, 16:45 (Bacteria V)

Dynamics of *Serratia* spp. pathogenic to the New Zealand grass grub, *Costelytra zealandica*

Maureen O'Callaghan, Trevor A. Jackson, Sandra D. Young and Steven Dodd

Biocontrol and Biosecurity, AgResearch, PO Box 60, Lincoln, New Zealand

The bacteria *Serratia entomophila* and *S. proteamaculans* (Enterobacteriaceae) cause epizootics of amber disease in the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabideae), resulting in collapse of grass grub populations. The disease is encoded on a large self-transmissible plasmid (pADAP). Both pathogenic (plasmid-bearing) and non pathogenic forms of the two species are often found coexisting in pasture soils, where they are capable of saprophytic growth. Laboratory experiments have shown that plasmid transfer occurs readily within and between the two species, with strains becoming pathogenic after a single conjugation event. Thus the *Serratia*/grass grub system provides an opportunity to examine the evolutionary advantage of pathogenicity and the role of plasmid transfer in determining the competitiveness of the pathogenic strain. Survey of *Serratia* populations in the field shows that while non-pathogenic *Serratia* of at least one species can be isolated from almost all sites, plasmid-bearing pathogenic strains are restricted to pastures infested with grass grub. At the peak of an epizootic, plasmid-bearing strains predominate, following multiplication in the diseased insects. Following decline of grass grub populations, non-pathogenic strains are more commonly isolated, suggesting that pathogenic strains are less competitive in the absence of resources provided by its host. It seems likely that pathogenic and non-pathogenic strains will be competing for similar resources in the absence of the host and in this situation, plasmid-bearing strains may be disadvantaged by the metabolic burden of maintaining the large plasmid in the population. While both pathogenic and non-pathogenic forms can reproduce saprophytically in the absence of insects, laboratory experiments with paired strains have shown that non-pathogenic *Serratia* strains are maintained at higher levels in the soil. The extent to which the two forms compete may influence the efficacy of *Serratia* as a biocontrol agent. While plasmid transfer between *Serratia* strains occurs readily *in vitro*, the frequency of plasmid transfer between *Serratia* strains in insect larvae and soil is unknown and is currently being investigated. These experiments will elucidate the role of horizontal gene transfer in disease evolution and the population dynamics of pathogenic *Serratia* spp.

POSTER GP3 - Thursday (General)

Infectious diseases of the water invertebrates from rivers and lakes on territory bordered with north part of the Black Sea.

Elena Oleinikova

Moldavian Research Institute for Horticulture, Chisinau, Moldova, 2019

The research of infectious diseases of water invertebrates was started around 10 years ago. The study has been more sharply focused on the crustacean invertebrates including *Gammarus lacustris, G. pilex, Dickerogammarus haemobaphes (Crustacea, Gammaridae)* and chironomids (*Diptera, Chironomidae*) - *Chironomus plumosus, Ch. salinarius* and *Ch. reductus*. The pathological material was collected in Simpheropol's storage lake, Salgir river, small lakes near the Black Sea zone (Crimea, Ukraine) and streams fall into the Dniester river, including Dniester estuary.

Etiology and pathomorphology of the diseases were studied on the individuals manifested unusual external symptoms. Light, phase contrast, transmission and scanning electron microscopy were used for the identification of pathogens.

As a result we had observed microsporidiosis between all investigated species of the crustacean invertebrates from small lakes located near the Black Sea. In all cases the microsporidia was identified as *Thelohania sp*. The population of *G. lacustris* was the most intensively infected with *Thelohania.* As a rule, we found from 70% to 95% individuals with typical symptoms of disease. The parasite develops in fat body cells but mature spores we observed in different parts of body including legs and head appendages.

Beside microsporidioses, *G. lacustris* was infected with *Bacillus cereus* (0.5-1.0%) and sometimes we found the resting spores of entomophthorous fungi.

Different type of viral infections and microsporidioses were registrated in the chironomids populations. The typical nuclear polyhedrosis (*Baculoviridae, subgroup* A) and iridovirosis (*Iridoviridae, Iridovirus*) were revealed in *Ch. plumosus* from pathological material collected in Dniester estuary. The level of polyhedrosis was 7%. As regards the iridovirosis we found only several individuals with the typical signs of iridodiseases among one thousand larvae. The larvae of *Ch. salinarius* and *Ch. reductus* were infected with poxviruses (*Poxviridae, Entomopoxvirinae*, genera C). The level of the natural infection forms from 0.5 to 16%.

Microsporidium (Bacillidium) chironomi Voronin, *Thelohania dabaisieuxi* Coste-Mathiez, Tuzet and *Caudospora sp*. were observed in *Ch. plumosus* from Dniester estuary. In all cases the level of infection was within from 0.5 to 1.0%.

The chironomids were identified by Dr. Ion Toderash (Institute of Zoology, Academy of Science of Moldova, Chisinau).

SYMPOSIUM I - Monday, 14:40 (Bacteria)

Insect proteinases and adaptation to *Bacillus thuringiensis*

Brenda Oppert[1], Juan Ferre[2], Salvador Herrero[2], Mike Grove[3], and Fred Gould[4]

[1] USDA Agricultural Research Service, Grain Marketing and Production Research Center, 1515 College Ave., Manhattan, KS 66502, [2]Department of Genetics, University of Valencia, 46100 Burjassot, Valencia, Spain, [3]Department of Entomology, 501 A.S.I. Building, The Pennsylvania State University, University Park, PA, 16802, [4]Department of Entomology, 2301_A Gardner Hall, North Carolina State University, Raleigh, NC 27695

Adaptations in insects that precipitate resistance to *Bacillus thuringiensis* (Bt) toxins have been characterized as a loss of toxin binding or differences in proteolysis of the protoxin or toxin. In the Indianmeal moth, *Plodia interpunctella*, both receptor- and proteinase-mediated mechanisms have been documented. A reduction in the binding of Bt toxin Cry1Ab was first described in a *kurstaki*-resistant strain of *P. interpunctella*. However, other Bt-resistant strains of *Plodia interpunctella* lack a major serine proteinase that has been genetically linked to survival on Bt-treated diets. Additional data support proteinase-mediated resistance to Bt in some strains of *P. interpunctella*, as well as in other species. Altered proteinases in Bt-resistant insects can result in incomplete activation and/or solubilization of the protoxin. Alternatively, serine proteinases in Bt-resistant insects, particularly chymotrypsin, can mitigate toxicity by fostering the elimination of toxin. Toxin elimination by proteinases is not only important in the evolution of insect resistance, but also in determining the natural toxin sensitivity in some insect species. Furthermore, serine proteinases in both lepidopteran and coleopteran insects influence Bt toxicity. Research on the interactions of insect proteinases with Bt toxins will identify modifications in toxins that improve solubility and stability, resulting in insecticidal toxins with increased toxicity. Alternatively, manipulations of insect digestive physiology may increase toxicity and expand the host range of existing Bt products.

CONTRIBUTED PAPER - Tuesday, 11:45 (Bacteria III)

Properties of a recombinant *Bacillus thuringiensis* subsp. *israelensis* IPS-82 that produces Cry11B

Hyun-Woo Park [1], Armelle Delécluse [2] and Brian A. Federici [1]

Department of Entomology, University of California, Riverside, California 92521, USA [1]
Unité des Bactéries Entomopathogènes, Institut Pasteur, Paris, France [2]

The mosquitocidal bacterium *Bacillus thuringiensis* subsp. *israelensis* (Bti) produces four major endotoxin proteins, Cry4A, Cry4B, Cry11A and Cyt1A. This combination of toxins makes Bti the most toxic of the known strains of Bt, with toxicity in the range of many synthetic chemical insecticides. Due to this high toxicity, Bti has been used widely as a major ingredient of bacterial insecticides employed to control mosquitoes and blackflies. Cry11B, the gene for which was recently isolated from *B. thuringiensis* subsp. *jegathesan*, is a close relative of Cry11A, but is approximately 10-fold more toxic to *Culex quinquefasciatus*. To determine whether Cry11B could improve the toxicity of Bti, we used the shuttle vector pHT3101 to clone the gene encoding this protein into two strains of Bti, 4Q7, an acrystalliferous strain, and IPS-82, a derivative of wild-type Bti containing the normal complement of endotoxin proteins. In both strains, *cry11B* was expressed using *cyt1A* promoters combined with STAB-SD mRNA stabilizing sequence. Synthesis of Cry11B in Bti 4Q7 produced crystals that were approximately 40% larger than those produced using its natural promoters without STAB. However, less Cry11B was produced per unit culture medium using *cyt1Ap*/STAB than with the wild-type construct, apparently because the latter construct produced more cells per unit medium. Nevertheless, the Bti IPS-82 strain that produced Cry11B using the *cyt1Ap*/STAB system was 2-fold more toxic to mosquitoes than Bti IPS-82.

CONTRIBUTED PAPER - Tuesday, 11:30 (Bacteria III)

Structural role for Domain I helix α-7 in Cry3A crystallization *in vivo* in *Bacillus thuringiensis*

Hyun-Woo Park [1] and Brian A. Federici [1,2]

Department of Entomology [1] & Graduate Programs in Genetics and Microbiology [2]
University of California – Riverside, Riverside, California 92521, USA

The N-terminal half of truncated Cry1 proteins, which contains the toxin moiety, does not crystallize *in vivo*. In a previous study, we showed that Cry3A Domain I, or Domains I and III together, could be used to crystallize Cry3A-Cry1C chimeras. This suggested that differences between Cry3A and Cry1C in conserved block 2, which spans the junction of Domains I and II, and/or conserved block 3, which spans the junction of Domains II and III, were involved in crystallization. To determine the role of these blocks in Cry3A crystallization, the corresponding blocks of Cry1C were substituted into Cry3A. Cry3A molecules containing Cry1C block 2 failed to crystallize, whereas Cry3A molecules containing Cry1C block 3 produced crystals characteristic of Cry3A. To identify the Cry3A block 2 region responsible for its crystallization, three sets of Cry1C substitution mutants, each spanning about a third of conserved block 2, were constructed. One, which contained most of α-helix 7, did not produce crystals when substituted into Cry3A, whereas the other two produced typical Cry3A crystals. Alignment of Cry3A and Cry1C showed two amino acids in α-helix 7 that differed markedly in side groups. A series of Cry3A mutants identified one of these, Y268L, as important to normal Cry3A crystallization, by its failure to form a typical Cry3A crystal. Selected mutations made in other α-helices in Domain I had no effect on Cry3A crystallization.

POSTER VP14 - Tuesday (Viruses)

Advances in the use of *Spodoptera exempta* nuclear polyhedrosis virus (SeNPV) to control the larvae of *Spodoptera exempta* (East African Armyworm) in Tanzania.

Mark Parnell[1], David Grzywacz[1], Charles Dewhurst [1] and Wilfred Mushobozi[2].

1: Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK.
2: Pest Control Services, PO Box 7473, Arusha, Tanzania.

A project for the control of the East African Armyworm (*Spodoptera exempta*) using the biological agent *Spodoptera exempta* nuclearpolyhedrovirus (SeNPV) was set up in collaboration with the Tanzanian Government. A strain of SeNPV originally isolated from wild *Spodoptera exempta* larvae in 1974 was produced *in vivo* at NRI in the UK and taken to Tanzania under import licence for experimental use in the trials. Virus production was achieved at a rate of 2.70×10^9 polyhedral inclusion bodies (PIBs) per larva in over 53,000 larvae. The main aim of the project was to carry out field trials of the virus to test its efficacy in controlling armyworm on rangeland and pasture land in Tanzania. Trials were carried out in 1999 during the armyworm outbreak season running from late December to May.

The armyworm outbreak that year was particularly severe with the heaviest and most widespread infestations since 1985. The SeNPV was applied using two different methods, an ultra low volume (ULV), oil-based formulation with spinning disk sprayers and a water based formulation using lever operated knapsack sprayers at high volume rates. Four different application rates were tested on plots of $100m^2$. The results of these small-scale trials on pastureland indicated that SeNPV was capable of controlling armyworm on pasture. Most encouragingly, control was achieved with the lowest dose level tested (1.0×10^{11} PIB/ha) on the heaviest armyworm outbreaks experienced for fourteen years. It is believed that such a dose level would make control of armyworm with SeNPV an economically viable concept.

The success of the project under which these trials were carried out has prompted further research into the use of SeNPV for control of the East African Armyworm and the possibilities of SeNPV being developed as an ecologically sound armyworm control agent for the future.

STUDENT POSTER VP32 - Thursday (Viruses)

Baculovirus Infection at the cellular and organismal level: bio-imaging of viral proteins using fluorescent proteins and probes.

A. L. Patmanidi(1,2), C. J. Thomas (1), G. Saville(1), R. D. Possee (2), and L. A. King (1).

(1) School of biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP.
(2)NERC, Centre for Ecology and Hydrology, Oxford OX1 3SR.

The previously hypothesized synergistic action of the baculovirus chitinase and cathepsin genes has provided the stimulus for a new series of experiments and bio-imaging in virus pathogenesis. The exoskeleton of the insect body is made of chitin fibres embedded in a proteinaceous matrix. Both chitinase and cathepsin have been found to play a key role in the terminal stage of virus infection by degrading this layer, which leads to host liquefaction. This final event assists with the transmission of the virus in the environment by physical forces.

Localisation and subcellular co-localisation studies have been carried out to follow the processing and the action of these two enzymes. Initially, the proteins were localised in virus infected cells using immunofluorescence labelling. The availability of different fluorescent proteins and probes encouraged further studies on these and other key viral proteins, such as p10, polyhedrin, gp64, and p35. These genes from Autographa californica nucleopolyhedrovirus (AcNPV)were targeted for a more detailed round of studies. Their protein products were tagged with green and red fluorescent protein analogues to visualise them in living cells, and to be able to record their pathways of action with time-lapse microscopy. We are currently following the processing of the gene products in live cells using state-of-the-art confocal laser scanning microscopy (CLSM). Our studies include the engineering of gene knockout and mutant viruses, in order to account for gene function. We will also apply these techniques on insect larvae tissue sections to observe the gene products that exert effects only on the organismal level.

STUDENT POSTER BP59 - Thursday (Bacteria)

Assigning functional roles for Cry1Ac domains in its toxicity to *Manduca sexta*.

James Pearce, Bill James, Ray Akhurst and David J. Ellar

Department of Biochemistry, University of Cambridge, UK

The work of Carrol and Ellar ((1997). J.Cell Sci. **110**: 3099-3104) revealed that Cry1Ac has two mechanisms of action in *Manduca sexta*. One is inhibited by GalNAc and only operates in the posterior region of the midgut. The other is unaffected by GalNAc and appears to be equally active throughout the midgut. It was also shown that if the GalNAc-inhibitable system was blocked, the second mechanism could still operate. Burton *et al* ((1999) J.Mol.Biol. **287**: 1011-1022) created a triple mutation in the outer sheet of Cry1Ac domain III. This mutant had lost the GalNAc-inhibitable mechanism and thus was only toxic through the GalNAc independent route, suggesting that domain III was responsible for binding to GalNAc. More particularly these GalNAc residues are likely to be on aminopeptidase-N (APN) since binding of Cry1Ac to APN in a ligand blot can be blocked by GalNAc (Knight *et al.* (1994) Microbiol. **11(3)**: 429-436).

Evidence will be presented that suggests that the second, GalNAc-independent mechanism operates through domain II and through a common component of midgut proteins. Work will also be presented that demonstrates that the two mechanisms of action of Cry1Ac can operate independently or in unison in other organisms.

Finally, domain I is considered primarily responsible for pore formation, in which oligomerisation is a critical part. Experiments will be described that investigate whether domain I alone can invoke oligomerisation or whether domains II and or III are also required.

STUDENT POSTER BP46 - Thursday (Bacteria)

Characterization of a *Bacillus thuringiensis* strain active against *Epilachna varivestis* (Coleoptera:Coccinellidae).

Guadalupe Peña and Alejandra Bravo

Instituto de Biotecnología-UNAM. Apartado postal 510-3, Cuernavaca, Mor., México. C P 62250.
e-mail: penacl@cib.uaem.mx

The Mexican bean beetle *Epilachna varivestis* is a mayor pest of beans in Mexico. The adult and larvae feed on the plant's foliage and green beans. The principal control system that has been used against this pest is based on chemical insecticides. An alternative method of control of insect pests is the use of insecticidal proteins produced by the bacteria *Bacillus thuringiensis* that have been widely used in Integrate Pest Management Programs. *B. thuringiensis* is safe to other organisms as plants, animals and humans. It is also environmental safe since is biodegradable and its genes can be introduced into the plant genome. In this work we will present the screening of *B. thuringiensis* strains from the Mexican strain collection developed in the Institute of Biotechnology-UNAM against *E. varivestis*. The bioassays of toxicity were developed by using the leaf contamination procedure. The symptoms that we observed were stop feeding, diarrhea and dead of the larvae. Selected strains were characterized by PCR in order to identify the *cry* genes present in each strain. The crystal inclusion were visualized by phase contrast microscopy and the proteins present in the crystal were analyzed by SDS-PAGE.

We have identified one *B. thuringiensis* strain that has a high insecticidal activity against this pest. The toxin responsible of this activity is a 100 kDa protein that did not react with any of our policlonal antibodies raised against different Cry toxins. PCR analysis with different general primers designed to identify *cry1, cry3, cry4, cry5, cry7, cry8, cry9* and *cry11, cry12, cry13* and *cry14*, indicates that this strain does not contain any of these *cry* genes. The data suggest that this strain may harbor a putative novel *cry* gene that could be used in the control of the Mexican bean beetle.

CONTRIBUTED PAPER - Monday, 17:30 (Bacteria II)

Estimation of the radius of the pores formed by the *Bacillus thuringiensis* Cry1C δ-endotoxin in planar lipid bilayers

Olivier Peyronnet[1], Brian Nieman[1,2], Francis Généreux[1,3], Vincent Vachon[1], Jean-Louis Schwartz[1,4] and Raynald Laprade[1]

Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec[1], University of Waterloo, Waterloo, Ontario[2], Université de Sherbrooke, Sherbrooke, Quebec[3] and Biotechnology Research Institute, National Research Council, Montreal, Quebec[4], Canada

Pore formation constitutes a key step in the mode of action of *Bacillus thuringiensis* δ-endotoxins and many studies have shown that various activated Cry toxins have the ability to form ionic channels in receptor-free planar lipid bilayers, at high concentrations (generally above 80 nM). Multiple conductance levels have been observed with several toxins, including Cry1C, suggesting that the channels result from a multimeric assembly of toxin molecules. In symmetrical 300 mM KCl solutions, Cry1C induces about 12 ohmic conductance levels ranging from 20 to 250 pS. Previous studies have shown that, for each conductance level, the channels have a similar weakly cationic selectivity. Nevertheless, the nature of these multiple conductance levels remains unclear. They could result from the formation of different multimeric pore structures, differing in channel diameter, or from the synchroneous gating of several identical channels forming clusters within the lipid bilayer. The present study attempts to discriminate between these two possibilities by estimating the size of the pores from the effect of poly(ethylene glycol) molecules of various molecular weights, ranging from 200 to 10 000, on individual single-channel conductance levels. Addition of 20% (w/v) poly(ethylene glycol) to 300 mM KCl decreases the bulk conductance of the solution independently of the molecular weight of the non-electrolyte. Single-channel conductance, however, is only reduced when the non-electrolyte molecule is sufficiently small to penetrate into the lumen of the channel, thus allowing channel diameter to be evaluated from the hydrated radius of the smallest poly(ethylene glycol) molecule that is excluded from the channel. Current analyses using this approach suggest that Cry1C could form clusters composed of channels of different sizes, the largest having a maximal pore radius of about 1.5 nm.

CONTRIBUTED PAPER - Tuesday, 9:45 (Viruses II)

***De novo* generation of defective interfering baculoviruses in insect cells.**

Gorben P. Pijlman[1], Erwin B. van den Born[1], Dirk E. Martens[2], Cornelis D. de Gooijer[2] and Just M. Vlak[1]

Laboratory of Virology[1] and Food and Bioprocess Engineering Group[2], Binnenhaven 11 6709 PD, Wageningen University, The Netherlands.

Baculovirus and recombinant protein production in insect cells is hindered by the presence and accumulation of defective interfering viruses (DIs) during infection. They are generated upon multiple passaging in insect cells and are the cause of the so-called 'passage-effect' observed among others in bioreactor configurations. DIs lack considerable parts of the viral genome and interfere with the replication of intact (helper) virus. In the case of baculovirus AcMNPV deletions of up to 43% of the genomic DNA were detected in early passage virus stocks by a PCR-based method. The proportion of DIs increases upon multiple passaging in Sf21 insect cells. Interestingly, these major deletions were also detected in viral DNA derived from polyhedra isolated from infected larvae. This may suggest a biological role for DIs *in vivo.*

We investigated whether DIs dominating the virus population after prolonged passaging are already present in the virus inoculum and prevailed over time or whether they are also rapidly generated *de novo*. We serially passaged a recombinant baculovirus, which was generated from a so-called 'bacmid', in insect cells. Bacmids are baculovirus shuttle vectors that contain a complete baculovirus genome and replicate in *E. coli* as a low-copy plasmid and which are genetically homogeneous. From experiments starting with an AcMNPV 'bacmid' we concluded that DIs are quickly generated upon serial passaging of this 'bacmid'-derived AcMNPV in insect cells. This suggests that *de novo* generation of DIs is an intrinsic property of baculovirus infections in cell culture. The question whether these DIs are also generated *in vivo* remains to be elucidated.

SYMPOSIUM IV - Friday, 11:20 (Bacteria)

Activity of *Bacillus thuringiensis* toxins against *Bacillus sphaericus* resistant mosquitoes

S. Poopathi, T. R. Mani, G. Baskaran, and Lalitha Kabilan

Centre for Research in Medical Entomology (Indian Council of Medical Research)
4 Sarojini Street, Chinnachokkikulam, Madurai-625-002, South India

Biological mosquito larvicides such as *Bacillus sphaericus (Bs)* is a potential control and promising agent which has been advocated in mosquito control programs. However, rapid development of resistance of mosquitoes in some species and some areas to *B. sphaericus* has certainly warranted alternate mosquito control measures. A strain of *Culex quinquefasciatus*, which has been resistant to *Bs* since 1995 is reared in the laboratory and subjected to selection pressure. We have conducted bioassays against larvae of this resistant colony and a susceptible colony by using *B. thuringiensis* H14 based biopesticide (VectoBac® 12AS) to study whether the resistant larvae are tolerant to this biopesticide. We have not observed any significant difference in the mortality of *Bs*-resistant and susceptible larvae after 24 hrs exposure to the *Bti* toxins. The susceptibility levels as shown at the LC50 and LC90 in resistant larvae were 0.123 and 0.427 milligram per litre. Similar values were also observed in *Bs*-susceptible larvae. These results suggest possibility for alternate application of *Bti* based biopesticide for the management of *Bs*-resistance in mosquito control operations.

CONTRIBUTED PAPER - Tuesday, 10:30 (Viruses III)

Characterization of the 122b isolate of LdMNPV

Holly J.R. Popham[1], David S.Bischoff[2], Melissa J. Mercer[1], and James M. Slavicek[1]

USDA Forest Service, Northeastern Research Station, Delaware, OH, 43015, and AgriVax Inc., USC School of Medicine, Los Angeles, Ca 90033

The 122b isolate of the *Lymantria dispar* nuclear polyhedrosis virus has been shown to produce more polyhedra than wild type (wt) virus, to have an apparent increased budded virus (BV) titer like that of an few polyhedra (FP) mutant, and to not accumulate FP mutants during serial passage in cell culture. To determine if isolate 122b produces more BV than wt virus, Ld652Y cells were infected with either 122b or wt virus, BV $TCID_{50}$s were determined, and the amount of BV DNA present was quantified. Infections by 122b yielded about 10-fold higher BV titers than infections by wt virus. However, there was no difference in the amount of BV viral DNA produced by isolate 122b compared to wt virus. This result suggests that the apparent increase in BV titer is actually a consequence of enhanced viral infectivity. To identify the gene(s) responsible for the unique traits of 122b, a cosmid library was generated for the 122b virus and the clones were used in marker rescue experiments. These cosmids were co-transfected individually with the parent 122 virus which does become predominantly FP in *ca*. 3 passes. The recombinant virus from these transfections was then used to infect cells, and this infection was serially propagated 5 times. One cosmid, cosmid 61, repeatedly rescued isolate 122 to generate a virus with greater infectivity than wt virus. This cosmid spans from *ca*. 30-66 kbp of the genome (Kuzio et al., 1999; Virol. 253:17-34). Currently we are working to further subclone and sequence this area to determine what gene is responsible for the increased infectivity of isolate 122b.

POSTER FP7 - Tuesday (Fungi)

Effect of host plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)

T. J. Poprawski, S. M. Greenberg, and M. A. Ciomperlik[1]

Beneficial Insects Research Unit, USDA-ARS Subtropical Agricultural Research Center, and Texas Agricultural Experiment Station, 2413 East Highway 83, Weslaco, Texas 78596;[1] Mission Plant Protection Center, USDA-APHIS Plant Protection and Quarantine, Moore Air Base, P.O. Box 2140, Mission, Texas 78572

Conidial suspensions of *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith were tested for pathogenicity to 3rd-instar nymphs of *Trialeurodes vaporariorum* (Westwood) reared on cucumber and tomato plants. Nymphs were highly susceptible to infection by both fungi following a one-time application of conidia onto cucumber plants. In contrast, insects reared on tomato plants were significantly less susceptible to infection. We hypothesized that the glycoalkaloid tomatine might have been involved in antimicrobiosis on tomato leaves. Tomatine mixed with Noble agar at five concentrations was tested for its effects on germination of conidia of both fungi. Germination of conidia of *B. bassiana* was only slightly affected at the two highest concentrations of tomatine. In contrast, germination of conidia of *P. fumosoroseus* was completely inhibited at 500 and 1000 ppm of tomatine. The *in vitro* tolerance of tomatine by *B. bassiana* contradicted our *in vivo* data. Sequestered tomatine by *T. vaporariorum* nymphs would explain, at least partially, the insect's defense against the pathogens. That little *in vitro* inhibition of *B. bassiana* was found supported the hypothesis that *B. bassiana* was inhibited only *in vivo*, after the penetration process. Inhibition of *P. fumosoroseus* might have occurred on the insect's cuticle before penetration, as evidenced by the complete inhibition of spore germination *in vitro* in the presence of tomatine at 500 and 1000 ppm. An explanation for the differential *in vitro* sensitivity of *B. bassiana* and *P. fumosoroseus* to tomatine is being sought.

CONTRIBUTED PAPER - Tuesday, 11:00 (Viruses III)

Replication of Hz-2V in the reproductive tissues of *Helicoverpa zea*

Christopher P. Rallis[1] and John P. Burand[1,2]

Departments of Entomology[1] & Microbiology[2]
University of Massachusetts-Amherst, Amherst, Massachusetts 01003

Hz-2V, also known as gonad specific virus (GSV) replicates in the reproductive tissues of the corn earworm, *Helicoverpa zea*. Virus replication in these tissues causes what has been described as agonadal pathology in adult moths which results from the malformation of reproductive tissues. Insects infected with Hz-2V emerge as either sterile adults that show the pathology associated with the disease (agonadal) or as fertile asymptomatic carriers of the virus. The effects of virus dose and timing of viral infection on virus replication, pathology and the appearance of agonadal and asymptomatic carrier moths have been examined. In experiments where healthy female moths were injected with varying concentrations of the Hz-2V and then mated with healthy male moths it was found that with decreasing virus concentrations the percentage of resulting agonadal offspring decreases while the percentage of asymptomatic carriers increases. Injections of 5th instar larvae with a high virus dose results exclusively in agonadal moths. Virus injections of 6th instars, prepupae and pupae results in malformation of only some of the adult reproductive tissues and in asymptomatic carrier moths. A detailed study of the reproductive tissues of female pupae revealed that malformation of these tissues in virus infected insects can be detected as early as 48 hours post pupation with evidence of productive virus in these tissues being detected only by about seven days post pupation.

POSTER VP29 - Thursday (Viruses)

Replication of Hz-2V in insect tissues

Christopher P. Rallis[1],Woojin Kim[1], Sarah Carpenter[1,2] and John P. Burand[1,2]

Departments of Entomology[1] & Microbiology[2]
University of Massachusetts-Amherst, Amherst, Massachusetts 01003

The insect virus Hz-2V, also known as gonad specific virus (GSV) is thought to replicate only in the reproductive tissues of the corn earworm, *Helicoverpa zea*. Virus replication in these insects results in sterile, agonadal moths with malformed reproductive tissues. In addition, some virus infected moths are fertile asymptomatic carriers of the virus. A PCR based procedure was developed to detect Hz-2V replication in adult tissues of *H. zea* and to provide a molecular method for detection of asymptomatic carriers. DNA samples were extracted from tissues prepared from adult agonadal and asymptomatic carrier moths by several different methods and the results of PCR analysis of DNA samples prepared using these different methods was compared. The results for individuals tissues varied between DNA extraction methods with viral DNA most often being detected in the eggs, bursas and common oviducts of asymptomatic carriers. Reproductive tissues from *Trichoplusia ni* and *H. virescens* infected with Hz-2V were also examined.

SYMPOSIUM - Thursday, 16:35 (Insect Immunity)

Agglutinins and reactive oxygen and nitrogen intermediates as determinants of **infection of *Rhodnius prolixus* by trypanosomes**

Norman Ratcliffe[1], Miranda Whitten[1], Patricia Azambuja[2], Suzete Gomes[2] and Eloi Garcia[2]

[1] School of Biological Sciences, University of Wales Swansea, South Wales, UK
[2] Department of Biochemistry and Molecular Biology, Fiocruz, Rio de Janeiro, Brazil

Reduviid bugs such as *Rhodnius* are the vectors of two closely related trypanosome parasites, namely, *Trypanosoma rangeli* and *Trypanosoma cruzi*. While the former is non-pathogenic in humans, the latter is responsible for South American trypanosomiasis termed Chagas disease. These two parasites both develop in the gut of the vector insect but only *T.rangeli* subsequently invades the haemocoel before passing into the salivary glands from which it infects the vertebrate host. The fact that these two trypanosomes occupy different niches in the insect provides an unique opportunity to study ways by which they utilise the host and avoid the attentions of the host defence reactions.

The first potential determinants of infectivity in the host, which we have considered, are the agglutinins present in the gut and haemocoel. The titre of the gut lectin against three strains of *T.cruzi* was shown to correlate with levels of infectivity of these parasites in the intestine. In addition, the haemolymph agglutinin potentiates the association of *T.rangeli* with the haemocytes as it enhances the formation of parasite/haemocyte nodules which may be essential for parasite development. This agglutinin is also responsible *in vitro* for killing short epimastigote *T.rangeli* and may therefore enhance the appearance of the long form epimastigotes which then invade the salivary glands.

The second potential determinant of infectivity in the insect vector is the possible role of reactive oxygen (ROS) and reactive nitrogen intermediates (RNI). Preliminary work with *Rhodnius* has shown significant superoxide dismutase-inhibitable superoxide generation in the haemolymph of *Rhodnius* in response to infection by *T.rangeli* and *T.cruzi.* Significantly, only with *T.rangeli* did the parasites continue to differentiate or multiply in the presence of elevated ROS. In addition, NADPH oxidase (enzyme generator of ROS) was detected in *Rhodnius* haemocytes as well as elevated levels of nitrate and nitrite which are indicative of nitric oxide.

The significance of these results is discussed, not only for the *Rhodnius/ Trypanosoma* model but for other arthropod vectors of disease, particularly those with a haemolymph stage in the life cycle.

POSTER BP9 - Tuesday (Bacteria)

Effect of *Bacillus thuringiensis* toxins on the midgut of the nun moth, *Lymantria monacha*

C. Rausell, [1], N. De Decker[2], I. García-Robles[1], B. Escriche[2], E. Van Kerkhove[2], M.D. Real[1] and A.C. Martínez-Ramírez[1]

[1] Dep. Genética, Fac. CC. Biológicas, Universitat de Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia) SPAIN. [2] Department Medische Basis Wetenschappen, Limburgs Universitair Centrum, B-3590 Diepenbeek, Belgium

The mode of action of *Bacillus thuringiensis* (Bt) entomopathogenic toxins has been intensively studied, but it has not been totally clarified yet. The ultimate cause of the insecticidal effect of Bt toxins is an extensive damage of the midgut epithelial cells which is believed to be due to the ability of Bt toxins to produce pores, once they are bound to specific receptors on the apical membrane of epithelial cells.

In the present work, we have been studied the three final key steps of the proposed mode of action of the Bt toxins: binding to midgut brush border membrane, effects on midgut physiology and midgut damage. A forest lepidopteran pest, *Lymantria monacha* (L.), the nun moth, and Cry1Aa, Cry1Ab, Cry1Ac and Cry1B trypsin activated toxins were used.

We demonstrated that only the toxins that caused typical pathological changes in midgut epithelial cells and bound to the midgut brush border membrane were able to drastically reduce the midgut transepithelial voltage of the nun moth.

The present work gives support to the correlation of post-activation events in *Lymantria monacha*, and toxin toxicity, validating the model of mode of action. We found a positive relationship between histological damage, binding and midgut effects: Cry1A toxins, that caused histological damage, did bind to the midgut brush border membrane and changed its electrical properties.

CONTRIBUTED PAPER - Tuesday, 12:15 (Viruses III)

Baculoviruses and tritrophic interactions: The effects of insect host-plant on a baculovirus from the winter moth, *Operophtera brumata.*

Ben Raymond & Rosie Hails

CEH-Oxford, Institute of Virology and Environmental Microbiology, Oxford, OX1 3SR, UK.

Lepidopteran host-plants have been shown to affect baculovirus infectivity and persistence in the laboratory and in the field. As part of a study into the effects of insect host plants on the population dynamics of a baculovirus we investigated the effect of three host plant species on viral infectivity, yield, and time to death of insects for two virus isolates in a bioassay; and the effect of host plant on the persistence of virus particles on host plants in the field. The host plant on which insects were infected *per os*, had no effect on virus infectivity, although there were significant differences between viral isolates. Host plant did significantly affect virus yields, insects infected on *Quercus robur* producing the most virus, although the influence of other host plants were dependent on virus isolate. Host plant also affected the persistence of virus particles over two months in the field. Decline in the infectivity of plant material was significantly faster on heather, *Calluna vulgaris*, than on the other host plants tested. These results are discussed in relation to the ecology of the host insect and the evolutionary history of virus/host plant association.

STUDENT POSTER BP47 - Thursday (Bacteria)

Analysis of the Activity of Cry1Ab1 and Cry1Ac1 toxins on the Insect Cell Line CF1 and isolated midgut cells from *Manduca sexta*.

Mauricio Realpe and Alejandra Bravo

Departmento de Microbiologia Molecular, Instituto de Biotecnología. A.P. 510-3. Cuernavaca, C.P. 62250. Morelos, Mexico. Email: realpe@ibt.unam.mx

The mechanism of action of *Bacillus thuringiensis* (Bt) Cry toxins has been analized by using model membranes, brush border membrane vesicles from larvae midgut and the whole midgut tissue. It involves several steps including proteolytic activation, receptor binding, pore formation and cell lysis. To date, it is not clear if the receptor in cultured cells is the same as in the insect midgut. It is interesting to analize post-binding events in the mode of action of Cry toxins in relation with the source of the cells.

IPRI-CF-1 cell line has been widely used to study the activity of Cry1A toxins, reflecting at a certain degree the same pattern of insect specificity for some toxins, similar toxicity levels, and the same mode of action. This work will show preliminary results of a comparative approach to analize the mode of action of these Bt toxins on the CF1 cell line and on isolated midgut cells from *Manduca sexta*. The correlation between pore formation and viability time-courses will be discussed using both systems.

STUDENT POSTER BP23 - Tuesday (Bacteria)

A study of the factors determining susceptibility to Cry d-endotoxins

Johanna S. Rees[1], Daniel J. Lightwood[1,2], Catherine E. Chambers[1], Eileen J. Bone[1] and David J. Ellar[1]

Deapartment of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, Cambridgeshire, CB2 1GA, UK[1], Celltech Chiroscience plc, 216 Bath Road, Slough, Berkshire, SL1 4EN, UK[2]

For three insects (*Pieris brassicae (Pb)*, *Mamestra brassicae (Mb)* and *Agrotis ipsilon (Ai)*) that vary greatly in their susceptibility to a range of Cry toxins (1Ac, 1C, 1B and 1J) a study has been made of insect-based and toxin-based factors that determine relative potency. For example, the possibility that the peritrophic membranes (PMs) of the relatively resistant insect *Mb* are able to sequester toxins and prevent them reaching the midgut epithelial membrane has been studied. 1Ac has been shown to bind high molecular weight proteins in *Mb* PMs. Preliminary results show that *Ai* PMs have similar high molecular weight proteins. We have also investigated the effect of soluble gut proteases from the different insects on the structure of each of the toxins. Other experiments have uncovered steps in toxin proteolysis that are restricted to the epithelial membrane surface and may be closely associated with the steps of membrane insertion and oligomeric pore formation. These events appear to differ in brush border membrane vesicle preps made form susceptible and resistant insects. By using segment swapping, we have been able to show that the three domains characteristic of the Cry toxin structure play different and important roles in potency and the overall toxic mechanism. For example, domain III plays a more critical role in *Mb* than *Ai*. These results can assist in devising strategies for the design of novel toxins.

POSTER PP8 - Thursday (Protozoa)

Susceptibility of *Aedes aegypti* and *Aedes albopictus* larvae to single and dual infections of the gregarines *Ascogregarina culicis* and *Ascogregarina taiwanensis*

Filiberto Reyes-Villanueva[(1)], James J. Becnel[(2)], and Jerry F. Butler[(1)]

[(1)] University of Florida, Entomol. & Nematol. Dept., Bldg. 970, Hull Road, PO Box 110620, Gainesville, FL 32611. E-mail: frv@gnv.ifas.ufl.edu
[(2)] Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, 1700 S.W. 23rd Drive, Gainesville, FL 32608. E-mail: jbecnel@gainesville.usda.ufl.edu

Ascogregarina culicis and *A. taiwanensis* are parasites of the mosquitoes *Aedes aegypti* and *Ae. albopictus*. These mosquitoes are sometimes sympatric in nature, however, *Ae. albopictus* has been progressively displacing *Ae. aegypti* in parts of the USA. In Florida, there are few surviving populations of *Ae. aegypti*. To determine if the gregarines played a role in displacement of *Ae. aegypti*, we have studied the interactions of these 2 gregarines and their hosts. *Ae. aegypti* (infected with *A. culicis*) and *Ae. albopictus* (infected with *A. taiwanensis*) were collected in Tampa FL and colonies established. Susceptibility to single and dual infections with gregarines was determined in the "wild" mosquito colonies from Tampa, and then compared with USDA colonies in Gainesville, FL. A dose-response experiment with 5 doses ranging from 6 to 100 oocyst/larva, plus a control, was conducted to evaluate infections. In a 2nd experiment, single (one mosquito species) and mixed larval samples (50 *Ae. aegypti* + 50 *Ae. albopictus*) were exposed to individual gregarines and mixtures of the 2 species. Gamont numbers per larva were estimated from ten larvae per treatment by midgut dissection at 6 days post-hatching. According to the linear regression equations computed between average gamonts/larva (Y) and oocyst concentration (X), the *Ae. aegypti* "Tampa strain" was twice as susceptible to *A. culicis* infection while the *Ae. albopictus* "Tampa strain" had the same susceptibility to *A. taiwanensis* as the "USDA strain". Gamont numbers per larva tended to be higher in *Ae. aegypti* than *Ae. albopictus*. For *Ae. aegypti* at 100 oocysts/larva, the average gamonts/larva was 214.8 for the "Tampa strain" and 107.5 for the "USDA strain", while for *Ae. albopictus* the average was 137.90 gamonts per larva for the "Tampa strain" and 93.67 gamonts per larva for the "USDA strain".

Results of the 2nd experiment showed that *Ae. aegypti* larvae (Tampa strain) infected with only *A. culicis* had an average of 202.45 $\pm$ 14.61 gamonts/larva. This was more than twice the number found for *A. taiwanensis* in *Ae. albopictus* ($\overline{X}$ = 87.40 $\pm$ 15.42 gamonts/larva). In dual infections, the average number of gamont/larva of *A. culicis* in *Ae. aegypti* was much lower and averaged 103.85 $\pm$ 12.94. *Ae. albopictus* (Tampa strain) was not infected by *A. culicis*. In contrast, *Ae. aegypti* was susceptible to *A. taiwanensis* either in dual infection experiments ($\overline{X}$ = 2.0 $\pm$ 0.78 gamonts/larva) or single infection experiments ($\overline{X}$ = 5.05 $\pm$ 0.95 gamonts/larva).

POSTER BP58 - Thursday (Bacteria)

***Bacillus thuringiensis* isolates from host plant leaves, guts and fecal pellets of caterpillars: A case study of *Bt* in natural ecosystems**

César Rodríguez[1], Ana Sittenfeld[1], Daniel Janzen[3] and Ana M. Espinoza[1,2]

Centro de Investigación en Biología Celular y Molecular[1], Universidad de Costa Rica, Ciudad de la Investigación, San José, Costa Rica.
Escuela de Fitotecnia[2], Facultad de Agronomía, Universidad de Costa Rica. San José, Costa Rica.
Department of Biology[3], University of Pennsylvania, Philadelphia, USA

Caterpillars are the major herbivores in tropical forests and every leaf they eat contains a diversity of microbes. This innoculum plus potential food material is added into the established microbial community within the caterpillar gut, remains there for a few hours or days and passes on through as fecal pellets that fall to foliage below and to the forest floor. The caterpillar-based microbial community may thus be visualized as a diffuse network of short-lived nodes between which microbes move. The main goal of this work is to determine the presence of *Bacillus thuringiensis* (*Bt*) in the gut, fecal pellets and feeding foliar material of 37 caterpillars collected from natural ecosystems of Costa Rica. Caterpillars from different species and host plant materials were collected in different life zones (tropical dry forest, cloud forest, very wet rainforest) of National Parks in the Guanacaste Conservation Area in the Northern Pacific slopes of the country. Samples from leaves and insect material (guts and fecal pellets) were macerated and incubated in LB broth for an enrichment period of 24 hours at 30°C and plated on LB agar, incubated for 48 hours at 30°C. A total of twenty seven *Bt* strains were isolated: fifteen from leaves (56%), two from guts (7%) and ten (37%) from fecal pellets. These *Bt* isolates constitute a diverse population as revealed by size and morphology of the parasporal crystalline inclusions and the *cry* genes they contained. *Bt* isolated from insect material was observed only when it was also cultured from leaves that caterpillars were feeding on at the moment of collection. In twelve cases (38%) *Bt* was present in the leaves, absent in the guts and recovered in the fecal pellets. Also, in five cases (14%) *Bt* was isolated from leaves but not from insect material. In general, the higher amount and diversity of different bacteria cultured from leaves is contrasting with the very few colony types that were recovered from insect material. These results demonstrated that *Bt* is found in the same habitat of these caterpillars, associated to the leaf material from which these larvae were feeding. Since the gut of caterpillars constitutes a selective habitat for microorganisms, it can be speculated that *Bt* isolates unable to colonize the gut could be transient passengers and as a result are eliminated in the fecal pellets. The retention time depends on the intestinal transit of each caterpillar. We postulate that caterpillars contribute to the dispersion of *Bt* in their natural ecosystems.

POSTER BP44 - Thursday (Bacteria)

Expression of mosquito active toxin genes by a Colombian native strain of *Asticaccaulis excentricus*

Magally Romero, Flor M. Gil, and Sergio Orduz

Biotechnology and Biológical Control Unit, Corporación para Investigaciones Biológicas, Apartado Aéreo 7378, Medellín, Colombia

Mosquito control with biological insecticides, such as *Bacillus* sp. toxins, has been used widely in many countries. However, rapid sedimentation away from the mosquito larvae feeding zone causes a low residual effect. In order to overcome this problem, it has been proposed to clone the *Bacillus* toxin genes in aquatic bacteria which are able to live in the upper part of the water column. Two strains of *Asticaccaulis excentricus* were choosen to introduce the *B. sphaericus* binary toxin gene and *B. thuringiensis* subsp. *medellin cry11Bb* gene cloned in suitable vectors. In feeding experiments with these aquatic bacteria it was shown that *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles*

albimanus larvae were able to survive on a diet based on this wild bacterium. *A. excentricus* recombinant strains were able to express both genes, but the recombinant strain expressing the *B. sphaericus* binary toxin was toxic was to mosquito larvae. Crude protease *A. excentricus* extracts did not degrade the Cry11Bb toxin. The flotability studies indicated that the recombinant *A. excentricus* strains remained in the upper part of the water column longer than the wild type *Bacillus* strains.

CONTRIBUTED PAPER - Friday, 9:30 (Microbial Control II)

Studies in Mexico of *Hirsutella thompsonii* Fisher for use as mycoacaricide.

José Luis Rosas-Acevedo[1,5], Audel Sánchez-Infante[2], Ana Yolanda Rosas-Acevedo[2], Raquel Alatorre-Rosas[3] Salvador Guzmán-González[4] & Laura Sampedro-Rosas[1]

[1]Centro de Desarrollo Regional-UEPI. [2]Preparatoria No. 13. Universidad Autónoma de Guerrero. Apdo. Postal 612 Acapulco, Gro. [3]Colegio de Posgraduados [4]Facultad de Ciencias Biológicas y Agropecuarias. [5]Doctorado en Biotecnología. Universidad de Colima. Apdo. Postal 10. Tecomán, Col. 28100. E. Mail jlrosas71@hotmail.com

The interest for fungi, classified as Deuteromycotina, are increased last years for their potential as microbial control agents for a wide range of plant pathogens, weeds and arthropod pests. For some of these fungi have been developed the production technology and sold commercially Here, we concentrate on results of studies that illustrate 10 years of basic and laboratory, glasshouse and field investigations carried out in Mexico with *Hirsutella thompsonii*. The initial step by mycoacaricide development, it was to have a collection of fungal isolates and screening for virulence (CL_{50} $1X10^5$; $1X10^6$; $1.1.X10^6$ conidia/ml) to target mites (Eriophydae, Tetranychidae and Tenuipalpidae). For laboratory and field studies, mycelia, mycelia-conidia, conidia, was obtained in small quantities and several production methods varied according to the experimental design and just to development methods (liquid culture, mixed culture) by induce conidiogenesis. Laboratory, greenhouse and field tests, have demonstrated their kindness as control agent to selected strains (HtMOR, HtM5 and HnC83) in lemon and coconut orchards, cherry and foliage plants. Likewise we observed with SEM, the development of the disease from a single spore adhered to the mite's body and the sporulation of the fungus once it has come to the surface of the infected mite's body. In adition to develop in large-scale method to induce high sporulation or vegetative growth. Actually, we are starting molecular studies of the infective process, in order to know the enzymatic expression in adhesion, germination and penetration of *Hirsutella spp.*, conidia facing up *Tetranychus urticae* Koch. These could be to enhance their potentialities as control agent, if assuming that enzyme activity associated with *H. thompsonii*, in the phases of interaction of the infectious process against *T. urticae*, as mecanism to manifest their aggressiveness and virulence to the contact with the mite cuticle surface. So, would have elements that are used as a molecular base in order to enhance the ability of infection, and may be a key determinant in regulating the host specificity of this mycoacaricide.

STUDENT POSTER BP48 - Thursday (Bacteria)

Evaluation of toxic activity of native and collection strains of *Bacillus thuringiensis* against larvae of the sugarcane borer *Diatraea* sp.

Ninfa M. Rosas-García[1], Hiram Medrano-Roldán[2], Katiushka Arévalo-Niño[1], Benito Pereyra-Alférez[1] and Luis J. Galán-Wong[1]

[1]Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, U.A.N.L., San Nicolás de los Garza, N.L. México. C.P. 66450,
[2] Departamento de Biotecnología, ITD, Durango, Durango. México.

Diatraea sp. (Lepidoptera: Pyralidae) is one of the major pests of the sugarcane and other economically important crops in Mexico, the larvae of these insects tunnel vertically within stalks producing a hollow cavity, weakening the plant and finally causing death. Because of this problem, the objective of our study is to find a *Bacillus thuringiensis* strain with toxic activity against larvae of *Diatraea* sp. *Bacillus thuringiensis* has been wide used to control a great number of pests, but due to its high specificity, a toxic strain against this insect must be selected to further for development of an efective formulation to be applied on field in sugarcane or corn crops. A reared colony of *Diatraea* sp. was established from pupae obtained from USDA in Weslaco, Texas. The colony was maintained on an artificial diet at 28ºC, ambient humidity between 60-70% and a photoperiod of 14:10 (L:D). The spore-crystal complexes of *Bacillus thuringiensis* were obtained from 12 strains (HD1, HD2, HD9, HD29, HD37, HD59, HD133, HD137, HD551, GM7, GM10, GM34) and mortallity bioassays were done using each one of the complexes with doses of 50µg/ml and 500µg/ml. The strains that caused a mortality of 50% using the dose of 50µg/ml, were selected as toxic strains to *Diatraea* sp.The percentage of mortality for the selected strains is: GM10, 66.67%; GM34, 78.79%; GM7, 50.79%; HD133, 62.71%, HD551, 64.91%. Experiments and PCR analysis are currently underway to further define the highly toxic strain or strains based on the presence of cryI genes.

POSTER NP6 - Tuesday (Nematodes)

Controlling white grubs (*Phyllophaga* spp.) with entomopathogenic nematodes and fungi in Oaxaca, Mexico.

Ruiz V., J[1]., Aquino B., T.[1] and H. K. Kaya[2]

CIIDIR OAXACA, IPN[1] and University of California, Davis[2]
Corresponding author: Jvega@vmredipn.ipn.mx

To evaluate the degree of control with local and introduced entomopathogenic nematodes and fungi on white grubs, laboratory and field experiments were conducted during 1999. In the laboratory, *Steinernema carpocapsae* isolated from the USA and *Heterorhabditis* sp. collected in Mexico provided mortality that was proportional to applied concentration. However, a *Steinernema* sp. From Mexico and *Steinernema glaseri* from the USA showed no defined trend with nematode concentration. The most effective nematodes were *Heterorhabditis* sp from Mexico and *Heterorhabditis bactriophora*, both giving 75 % control. The entomopathogenic fungi, *Beauveria bassiana* and *Metarhyzium anisopliae* were grown separately on rice and tested alone or in combination with nematodes for white grub control. *B. bassiana* was more effective when 2 g of infested rice / larva were applied, but *M. anisopliae* was equally effective at 1 or 2 g of infested rice / larva. White grub mortality was 48 % when 1 g of rice infested with *B. bassiana* was applied, and 95 % when 2 g were used. Total control was obtained with *M. anisopliae* at either dosage. Both fungi required six days to kill 50 % of the larvae (LT_{50}), but when each fungus was combined with nematodes the LT_{50} was reduced in two days, especially with the combination *M. anisopliae* + *Heterorhabditis* sp. In a field experiment carried out in a commercial maize plot, *B. bassiana* was as effective as *M. anisopliae*, but no synergism was observed when these fungi were applied together with S. *carpocapsae*. Under semi-controlled conditions (using 5 liter plastic bags), entomopathogenic fungi alone produced the largest vegetative development in potted maize plants. However, the percentage of white grub control increased when the *Heterorhabditis* sp isolate was used either with *B. bassiana* or *M. anisopliae*. It was concluded that more than 600 nematodes per larva are required and that combining fungi and nematodes, especially *Heterorhabditis* sp, increases their efficacy against white grubs.

CONTRIBUTED PAPER - Tuesday, 12:00 (Viruses III)

Sublethal baculovirus infections in the Indian meal moth, *Plodia interpunctella*: From Individuals to Populations

Steven M. Sait

Population and Evolutionary Biology Research Group, School of Biological Sciences, University of Liverpool, Nicholson Building, Brownlow Street, Liverpool, L69 3GS, UK

If the full potential of baculoviruses as biocontrol agents is to be realised, an understanding of their ecology, both at the individual and population level, is critical. While attention has focussed, naturally, on lethal baculovirus effects, sublethal infections are also acknowledged to be an important component of the insect-pathogen interaction. Many studies have documented the effect of sublethal infections on their hosts, particularly an increase in development time and reduced fertility. However, these infections remain poorly understood, especially their

ecological and evolutionary consequences. The interaction between the Indian meal moth, *Plodia interpunctella*, and its granulovirus (*Pi*GV) has been extensively studied at both the individual and population level. Here, the effects of sublethal infections in this host are detailed. In particular the potential causes and consequences of the reduction in *P. interpunctella* male fertility are described, both in terms of the reproductive investment by sublethally-infected males, and its potential behavioural consequences; evidence exists for a reduction in sperm numbers, which may have an impact on mate selection by healthy females. Finally, these results are linked directly to a comparison of the population dynamics of virus-free cultures of the moth and those that are continually exposed to *Pi*GV in laboratory microcosms; virus-infected populations exhibit both reduced adult numbers and a clear increase in the cycle period of changes in abundance.

Key words: development time, fertility, population cycles, granulovirus, *Plodia interpunctella* , sublethal effects

CONTRIBUTED PAPER - Monday, 12:00 (Bacteria III)

Binding of ICP to the BBMVs and midgut epithelia of *Culex pipiens* and *Bombyx mori* larvae

Hiroshi Sakai[1], Kanao Otake[2], Motoyuki Esaki[2], Tohru Komano[3], and Masashi Yamagiwa[1]

[1]Department of Bioscience and Biotechnology, Okayama University, Okayama 700-8530, Japan, [2]Division of Applied Life Sciences, Kyoto University, Kyoto 606-8502, Japan, [3]Department of Genetic Engineering, Kinki University, Wakayama 649-6493, Japan

When an insecticidal crystal protein (ICP), or a Cry protein, of *Bacillus thuringiensis* is ingested by a susceptible insect, it is solubilized and activated through proteolytic processing in the insect midgut. Binding characteristics of activated Cry4A, Cry4B, Cry1Aa, and Cry1C were analysed using BBMVs and tissue sections from midguts of *Culex pipiens* and *Bombyx mori.*

We examined interaction between Cry proteins of *B. thuringiensis* and brush border membrane vesicles (BBMV) from larval midguts of *C. pipiens* and *B. mori*. In coprecipitation experiments using BBMV from *C. pipiens*, it was shown that Cry4A, a dipteran-specific insecticidal protein, was associated with the BBMV. However, Cry1Aa, a lepidopteran-specific insecticidal protein was not associated with the BBMV. In similar experiments using BBMV from *B. mori*, Cry1Aa, Cry1C, and Cry4A were associated with the BBMV.

Binding of the Cry proteins to epithelial cells of the larval midguts from *C. pipiens* and *B. mori* was examined with immunocytochemical analyses using midgut tissue sections. It was shown that the Cry proteins that were toxic to *C. pipiens* were bound to epithelial cells of the larval midgut of *C. pipiens*. However, no binding of Cry1Aa was observed. On the other hand, Cry1Aa, Cry1C, Cry4A and Cry4B were bound to epithelial cells of the larval midgut of *B. mori*.

These results suggested that, in the case of *C. pipiens*, characteristics of initial binding of the Cry proteins to the midgut epithelia corresponded to their insecticidal specificities. On the other hand, the results with *B. mori* suggested that characteristics of the initial binding of the Cry proteins was not necessarily correlated with their insecticidal specificities, and, therefore, that another process is involved in determining them.

STUDENT POSTER VP36 – Thursday (Viruses)

Characterization of three temperature-sensitive mutants of *Autographa californica* nucleopolyhedrovirus defective in budded virus production and polyhedra formation

Makiko Sakurai, Yoshitaka Sano, Tsuguo Matsumoto, and Yoshifumi Hashimoto

Department of Applied Biology, Faculty of Textile Science Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

A number of baculovirus genes have been identified by characterization of temperature-sensitive virus mutants. We report in this paper the isolation and characterization of three ts mutants of AcMNPV, ts345, ts375 and ts455, which have been generated by mutagenesis with BrdU. Phenotypic characterization of these mutants showed that all of them normally synthesize viral DNA, but possess a defect in budded virus (BV) production and polyhedra formation at non-permissive temperature of 33^{O}C. At permissive temperature of 25^{O}C, they normally produced BV and polyhedra, although polyhedra produced by ts455 showed altered morphology such as cubic shape and bigger size. Marker rescue test using cosmid clones of AcMNPV DNA demonstrated that ts345, ts375 and ts455 possessed ts mutation genes within genome regions of 13.7 to 38.0 m.u. (cosmid clone pWA22), 43.7 to 67.7 m.u. (pWA25) and 88.5 to 10.3 m.u. *via* 0 m.u. (pWA23), respectively. In terms of late expression factor (*lef*) genes, some of which include post-viral DNA synthesis events, pWA22 contains *lef*-6, 8, 9, 10, 11, *p47* and *39K*, pWA25 contains *lef*-4 and 5, and pWA23 contains no *lef* genes. The genes defining ts phenotype of ts345, ts375 and ts455 are currently studied by marker rescue test using plasmid clones of the AcMNPV genome.

POSTER FP23 - Thursday (Fungi)

Evaluation of several supports for the air-drying method of blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) produced in two different liquid media

Carlos F. Sandoval-Coronado[1], Hugo A. Luna-Olvera[1], Katiushka Arévalo-Niño[1], Mark A. Jackson[2], Tadeusz j. Poprawski[3] and Luis J. Galán-Wong[1].

[1] Dept. de Microbiología, Fac. de Ciencias Biológicas, UANL, Cd. Universitaria. A. P. 414. San Nicolás de los Garza, N. L., México. 66450.
[2] USDA-ARS-NCAUR, 1815 N University St. Peoria, IL, USA. 61604-3999.
[3] USDA-ARS-Biological Control of Pests Research Unit, 2415 East Highway 83, Weslaco, TX. 78596.

Formulation matrices can play an important role in improving the storage survival and biocontrol efficacy of microorganisms that are used for the control of pest insects. In this study, blastospores of ***Paecilomyces fumosoroseus*** produced in liquid culture medium, were formulated with different inert and organic materials prior to air-drying. The initial and long-term survival of these air-dried blastospores was evaluated after storage at 4 and 28°C. *Paecilomyces fumosoroseus* cultures were produced in two different liquid media, a basal salts medium supplemented with Casamino acids and glucose (LM1), and a complex medium containing peptone of collagen and glucose (LM2). Blastospores obtained from culture supernatants of the two test media, were formulated with different materials at three concentrations: 2.5, 5 and 7%. The formulation matrices used were: cornstarch, rice flour, various talcs, Mexican limes, calcined kaolin clay and diatomaceous earth. Results demostrated that initial blastospores´ viability was affected by both, the formulation material and the blastospore production medium. Highest blastospore survival after drying was obtained with calcined kaolin clay formulations, regardless of clay concentration However, higher clay concentrations were shown to improve blastospore storage survival at 4°C and 28°C. Blastospores produced in the LM 1 medium showed better long-term storage survival compared to blastospores produced in the LM 2 medium, regardless of the formulation tested. In all formulations tested, spore survival over time was reduced for blastospore formulations stored at 28°C rather than 4°C. In this study, the best long term survival (70% after 45 days of storage at 4°C), was obtained with blastospores produced in LM 1 media and formulated with Surround at 2.5 and 5%.

POSTER VP15 - Tuesday (Viruses)

Replication of nuclear polyhedrosis viruses under high temperature *in vitro*

Takeru Sato

National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305-8605, Japan

Some baculoviruses have been tested the possibilities to use in fields as biological control agents, and few of them have been succeeded to replicate in insect cell culture systems. While, it is said that they do not replicate under the mammalians' basic temperature, and this point is an advantage for safety consideration of the viruses as biological control agents. In this study, replication of nuclear polyhedrosis viruses *in vitro*

at the nonpermissive temperature were examined by using high temperature adapted insect cells. Four nuclear polyhedrosis viruses, AgNPV isolated from *Anticarsia gemmatalis*, AsNPV from *Agrotis segetum*, BmNPV from *Bombyx mori*, and SeNPV from *Spodoptera exigua*, were used for the experiments. The first inocula of the NPVs were prepared from the infected hemolymph collected from the diseased larvae infected with homologus combination of the viruses and their susceptible insects. Four cell lines, FTRS-ANGEL/TR derived from the velvetbean caterpillar *Anticarsia gemmatalis*, FTRS-AgsL-4F/TR from the common cutworm *Agrotis segetum*, FTRS-BmX/TR from the silkworm *Bombyx mori* and Se301A/TR from the beet armyworm *Spodoptera exigua*, were used for the inoculation tests. Cells were cultured in modified Goodwin's IPL-41 medium (named as IPL41S) supplemented with 10% fetal bovine serum and were adapted to 35-37°C as incubation temperature. High temperature resistant cell lines were inoculated with infectious media, and were maintained at 37°C except for BmX/TR of 35ºC. After the inoculation of the viruses, appearance of polyhedra in the cell nuclei was checked under a phase contrast microscope as an indicator of virus replication in the cells. Both AgNPV and SeNPV did not produce polyhedra in the inoculated cells even though they were adapted at the temperature. While, AsNPV and BmNPV replicated in the adapted cells, however formation of polyhedra is very scarcely in these cells. From above-mentioned experiments, it would be concluded that some NPVs could be replicated under a high temperature around 37°C, but the continuous formation of viruses' polyhedra at the temperature would be fairly difficult.

STUDENT POSTER VP16 - Tuesday (Viruses)

Deletion of the KDEL ER-retention motif promotes secretion of chitinase from AcMNPV infected insect cells.

G. Saville[1], C.J.Thomas[1], S.Mann[1], R.D.Possee[2] and L.A.King[1].

[1]School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, OX3.
[2]NERC-CEH, Oxford OX1 3SR.

Baculoviruses are insect specific pathogens which have long been used as natural pest control agents. Characteristic larval liquefaction during the terminal stages of wild-type virus infection is associated with genes encoded by the virus. The type member *Autographa californica* nucleopolyhedrovirus (AcMNPV) encodes two genes, chitinase and cathepsin, involved in the liquefaction associated with wild-type infection.

AcMNPV encodes a single chitinase gene able to hydrolyse a broad range of chitin substrates, whose deletion has no effect on viral replication and ultimate larval death but abrogates liquefaction. Most of the chitinase activity remains intracellular despite the presence of a cleavage signal peptide at the N-terminus of the gene. Targeting of chitinase to its correct location within the cell involves recognition of signals encoded in the amino acid sequence. We have shown that a tetrapeptide signal, KDEL, present at the carboxyl terminus of chitinase, results in the retention of the enzyme in the lumen of the endoplasmic reticulum until the final stages of the baculovirus replication cycle i.e cell lysis. We have produced recombinant baculoviruses with the KDEL retention signal deleted, (AcΔKDEL) under the control of the very late polyhedrin promoter. *In vitro* assays in *Spodoptera frugiperda* cells shows that chitinase is actively secreted from the infected cell. Chitinase has been shown to be present in the culture medium of AcΔKDEL infected cells, whereas chitinase has not been detected in the medium of wild-type virus infected cells. Both wild-type and the mutant virus showed chitinase to be present in cell pellet. Confocal Laser Scanning Microscopy and Transmission Electron Microscopy have also been utilised to elucidate the localisation of chitinase in infected cells. Preliminary larval assays in *Trichoplusia ni* larvae have indicated that premature liquefaction may be induced in AcΔKDEL infected larvae. Subsequently recombinant baculoviruses have been generated with the ΔKDEL chitinase gene, under the control of its natural chitinase promoter. These recombinant viruses will be tested both *in vivo* and *in vitro*, to see if the secretion of chitinase from the ER of infected cells may facilitate premature liquefaction and increase the 'speed of kill' of the virus for pest control.

WORKSHOP I - Tuesday, 16:30 (Viruses)

An introduction to the ICTV and invertebrate RNA viruses

Anette Schneemann and John E. Johnson

Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

The International Committee on Taxonomy of Viruses (ICTV) was established in 1966 at the International Congress of Microbiology in Moscow. It operates under the auspices of the Virology Division of the International Union of Microbiological Societies. The first objective of this presentation is to provide a general introduction to the ICTV, its structure and mandate. This will include examples of currently ongoing debates such as how the names of viruses should be constructed and represented in the literature. The second objective is to review the position of invertebrate viruses within the ICTV classification scheme and to illustrate how high resolution X-ray structures of intact viruses can assist in invertebrate virus classification. Examples of invertebrate viruses for which high resolution structures are available include members of the family *nodaviridae*, *tetraviridae* and *picornaviridae*. Nodaviruses and tetraviruses are both icosahedral viruses but the number of coat protein subunits in the viral capsids differs. While nodaviral capsids contain 180 subunits (T=3 symmetry), tetraviruses contain 240 subunits (T=4 symmetry). Despite this difference, X-ray analysis has revealed significant structural analogies between the two particles including (1) the highly conserved fold of two domains in the coat protein subunits, (2) a conserved cleavage event in the same part of the molecule and (3) a conserved pentameric helical bundle at the fivefold axes of the virion. These observations suggest that the tetraviruses have evolved from the nodaviruses. Another case in which structural analysis provides evidence for evolutionary relationships is that of cricket paralysis virus. This virus was classified as a picornavirus despite its dicistronic genome organization. It is clear that from a taxonomic viewpoint the dicistronic insect viruses, which also include *Drosophila* C virus and *Plautia stali* intestine virus, represent a distinct lineage compared to the true picornaviruses. From a structural standpoint, however, both of these distinct groups are related indicating that different regions of the picornavirus genomes may represent exchangeable modules. These modules may have recombined in different ways and then evolved significantly from the time of genetic recombination. Similar observations have been made for picorna-like plant viruses that have similar genetic modules physically separated in bipartite genomes, but still form particles readily recognizable as picorna-like structures. Thus, taken together, structures can provide a means of correlating virus relationships that gene organization and sequences alone may miss.

SYMPOSIUM III - Tuesday, 17:30 (Bacteria)

Visualization of the pore formed by the insecticidal *Bacillus thuringiensis* Cry1Aa toxin in lipid membranes

Jean-Louis Schwartz[1,2], Nicole Van Mau[3], Véronique Vié[4], Christian Le Grimellec[4], Frédéric Heitz[3], Raynald Laprade[2], Cécile Rang[5], Roger Frutos[5] and Luke Masson[1]

[1]BRI, National Research Council of Canada, Montreal (Qc), Canada, H4P 2R2, [2]GRTM, Université de Montréal, Montreal, H3C 2J7, [3]CRBM, CNRS UPR 1086, 34293 Montpellier Cedex 5, France, [4]CBS, 34090 Montpellier Cedex, France, [5]CIRAD, 34032 Montpellier Cedex 1, France

Bacillus thuringiensis (*Bt*) insecticidal toxins permeabilize cells, midgut brush border membrane vesicles and liposomes, and form ion channels in planar lipid bilayers (PLBs). One of the most challenging aspects of the molecular mode of action of *Bt* toxins relates to the elucidation of how these water-soluble proteins become membrane proteins, and of the final architecture of the lesion they make in the target cell membrane.

The three-dimensional structure of *Bt* activated Cry proteins shows three structural domains. Domain 1 (D_1) is made of seven "-helices, whereas D_2 and D_3 are made of β-sheets. D_1 appears to be responsible for pore formation in PLBs and cell membranes. Based on PLB data from disulfide bond engineering and substituted cysteine accessibility studies, we have proposed a model in which D_1 swings away from D_2 and D_3, and the most hydrophobic α_4/α_5 hairpin inserts transversally into the bilayer, while the other helices remain parallel to the membrane surface. Based on

steric considerations, the functional Cry1Aa ion channel would then assume a tetrameric structure in its minimal configuration. The membrane-inserted α_4/α_5 hairpins of four toxin molecules are thought to aggregate so that the lumen of the channel is lined by the hydrophilic faces of the α_4 helices while the "$_5$ helices anchor the molecules in the lipid membrane.

Conformational changes of the *Bt* Cry1Aa toxin structure during association and insertion into lipid monolayers and bilayers and the morphology of the formed membrane pores were investigated using Fourier transform infrared absorption spectroscopy (FTIR) and atomic force microscopy (AFM). FTIR revealed that, in the presence of lipids, Cry1Aa undergoes a major conformational change in which the ß-sheets are lost to a disorganized structure while the α-helical content remains unaltered. Integration into lipid monolayers was facilitated in neutral, but inhibited in acidic lipids and when the acyl chains were in an ordered rather than a disordered state. AFM imaging showed that the putative pores are organized in aggregates. Each 5-nm unit possessed four 1.4-nm subunits protruding 0.5-0.6 nm above the membrane surface, with a central depression measuring 1.5 nm in diameter. Our data describe a tetrameric structure in which most of the toxin (i.e. presumably D_2 and D_3) is present in a disorganized state. This is the first physical description of the nature of the lipid-toxin interactions and the assembled pores formed by *Bt* toxins in lipid membranes.

POSTER BP22 - Tuesday (Bacteria)

Binding analysis of *Bacillus thuringiensis* Cry11Bb toxin to *Aedes aegypti* brush border membrane vesicles

Cesar Segura[1], Sergio Orduz[1] and Mike Adang[2]

Biotechnology and Biological Control Unit. Corporación para Investigaciones Biológicas. Apartado Aéreo 7378 Medellin, Colombia[1]. Entomology Department, University of Georgia Athens, GA 30602-2605., USA[2].

The Cry11 proteins produced by *Bacillus thuringiensis* are potent insecticides against mosquito larvae. It is assumed that the mode of action of these toxins is similar to that of Cry1 toxins. Interaction of Cry11Bb toxin produced by *B. thuringiensis* subsp. *medellin* with *Aedes aegypti* brush border membrane vesicles (BBMV) was studied. The Cry11Bb protoxin is a 94 kDa protein that is proccesed into 30/35 protein through an intermediate of 68 kDa, thus the 68 kDa toxin and the activated-94 kDa toxin that results in a polypeptide of 30/35 kDa were radiolabeled and incubated with *Ae. aegypti* brush border membrane vescicles (BBMV). Labeled toxin (^{125}I-68 kDa) showed specific interaction with the BBMV, experiments of saturation with the protein in the presence of increasing amounts of BBMV indicated that ^{125}I-68 kDa-BBMV interaction was saturable in the range of 10-80 µg of BBMV. Trypsinized 94 kDa (30/35 kDa) was not properly labeled. Binding of 68 kDa protein was susceptible to mosquito proteases. An evidence of toxin aggregation, determined by dynamic light scattering, was obtained for the 30/35 kDa toxin. Both 68 kDa and 30/35 kDa proteins were toxic to mosquito larvae. Our results suggest that 68 kDa protein interacts specifically with the mosquito midgut, while 30/35 kDa, due to its toxicity and aggregation might play a role in events post-binding.

POSTER BP24 - Tuesday (Bacteria)

Gene organization of large plasmids of *Bacillus thuringiensis* subsp. *israelensis* and its related strains

Kikuo Sen[1], Hideyuki Katagiri[1], Shin-ichi Kurosawa[1, 2], Hiroshiro Shibai[1], So Takebe[3] and Tohru Komano[3]

[1]Faculty of Agriculture, Shinshu University, Nagano 399-4598, JAPAN;

[2]Akita Prefectural University, Akita 010-0195, JAPAN; [3]Department of Genetic Engineering, Kinki University, Wakayama 649-6493, JAPAN

Bacillus thuringiensis subsp. *israelensis* (*Bti*) produces a parasporal inclusion body called crystal that is composed of several proteins toxic to diptera such as mosquitoes. *Bti* has at least seven plasmids ranging 3.6 - 82 Md. Interestingly the several genes of *Bti* mosquitocidal proteins localize on its 70-Md plasmid that can transfer between *Bacillus thuringiensis* (*Bt*) cells. Moreover, the *p20* gene of chaperone-like protein, and ISs (insertion sequences) and transposon structure are also found in the same plasmid. Elucidation of structure of the *Bti* 70-Md plasmid is very important to know evolution of *Bt*. Therefore, in this study, we analyzed gene organization of large plasmids from *Bti* HD522 strain, its plasmid-cured mutant strain O-4 that was obtained by drug- and heat-treatment, and a *Bti*-related strain TK-1F9 that was newly isolated from Wakayama prefecture, Japan.

Total plasmids of *Bti* HD522 and fragments cloned from the 70-Md plasmid were analyzed by the Southern hybridization, DNA sequencing and PCR, and thereby about 55 kb of the plasmid (corresponding to about 50%) was mapped. Two gene clusters in about 20-kb and about 35-kb fragments were found; the former had the *cry4A*, *cry10A*, IS*231*W and IS*240*A and the latter had the *cyt1A*, *cyt2B*, *cry4B*, *cry11A*, *p20* and IS*231*W. Ben-Dov *et al.* reported that the *cry4B* exists upstream from the *cry10A* in the case of *Bti* 4Q5 strain (*Plasmid*, **42**(3),186 - 191, 1999), but the *cry4A* existed in our case of HD522. TK-1F9 harbored as many as 12 plasmids, and had the almost same gene contents on one plasmid as *Bti* described above. However, the position and orientation of genes from TK-1F9 were different from those from the both *Bti* strains of HD522 and 4Q5. These results show polymorphism of the large plasmids encoding insecticidal proteins among the *Bti* and *Bti*-related strains.

In PCR analysis using template DNA of plasmids from the mutant O-4, several insecticidal protein genes have not been detected except the *cry4A* and *cry10A*. This result suggests that some DNA rearrangement occurred during the plasmid-curing in laboratory. It is very possible that a similar event has occurred in nature during the evolutional process of *Bti* strains.

POSTER FP9 - Tuesday (Fungi)

Effects of constant and fluctuating temperatures on sporulation and infection by *Erynia neoaphidis*

Paresh A. Shah*, Markus Aebi and Urs Tuor

Mikrobiologisches Institut, ETH-Zentrum, CH-8092, Zürich, Switzerland
*IACR-Rothamsted, Harpenden, AL5 2JQ, England

Studies were performed with an isolate of *E. neoaphidis* from *Nasonovia ribisnigri*, the currant-lettuce aphid. Discharge of primary conidia from mycelium formulated as alginate granules and unformulated mycelial mats was studied, as well as infection of the potato aphid, *Macrosiphum euphorbiae*, inoculated using Petri dish culture plugs. Experiments at constant temperatures indicated the optimum temperature range for sporulation was 10-20 °C for both mycelial preparations and that there was no or very little sporulation at 30 ºC. In two experiments, infection of young adult aphids kept at 15 ºC was 39 and 55%, infections of 77 and 81% were recorded at 20 ºC, while 10 and 40% of aphids were infected at 25 ºC. Under fluctuating temperature cycles, numbers of discharged conidia did not differ when mycelial preparations were maintained at 18-25 °C compared with 18-20 ºC, but fewer were produced when preparations were exposed continuously to a cycle varying between 18-30 °C. In one experiment, infections of 41 and 33% were found from inoculated aphids kept at 18-20 ºC and 18-25 ºC, respectively. In another experiment, 18% infection was obtained for aphids kept at 18-20 ºC, while only 7% infection was found for aphids exposed to an 18-30 ºC cycle.

The variability in infections from bioassays were probably due to the age and quality of different batches of aphids rather than the doses used, which varied between 29-38 conidia mm^{-2} for all experiments.

The results suggest that low infections obtained in a previous greenhouse trial, with the same host-pathogen combination, could partly be explained by ambient temperatures of 15-25 ºC during the trial.

SYMPOSIUM III - Tuesday, 18:10 (Bacteria)

Studies 0n The Mode of Action of *B.Thuringiensis* d-Endotoxin Suggest an "Umbrella-Like" Model for its Folding and Insertion Into Membranes.

Yechiel Shai.

The Department of Biological Chemistry. The Weizmann Institute of Science, Rehovot, 76100 Israel.

Bacillus thuringiensis, a gram-positive bacterium, produces parasporal crystals. Within these crystals lie the δ-endotoxins, which are specific insect toxins. The δ-endotoxins are digested by the insects in the midgut and are enzymatically modified into their active form, after which, they bind to target cells through a two-stage process to form channels/pores. The first step involves reversible binding to a receptor [1], followed by an irreversible step, in which the pore-forming domain inserts into membrane, a process which is not yet clearly understood [2]. We have synthesized and characterized the seven helices which compose the pore forming domain of Cry3A [3], as well as helices from the Cry1Ac including the hairpin domain, α4-loop-α5, its α4 and α5 helices, as well as mutant α4 peptides, with mutations known to alter toxin toxicity [4]. Spectrofluorimetric and structural studies combined with functional studies revealed that the α4-loop-α5 hairpin is the part of the toxin which inserts into the target membrane, while the other helices lie on the membrane surface like ribs of an umbrella [3,5]. Strikingly, addition of the active α4 mutant peptide completely inhibits pore formation by α4-loop-α5. Altogether, our data suggest also that α4 and α5 line the lumen of the channel as suggested previously [5,6,7], and that α5 also participates in the oligomerization of the toxin [8].

1. Knight, P. J., Crickmore, N., and Ellar, D. J. (1994) *Mol. Microbiol.* 11, 429-436.
2. Rajamohan, F., Lee, M. K., and Dean, D. H. (1998) *Prog. Nucleic Acid Res. Mol.Biol.* 60, 1-27
3. Li, J. D., Carroll, J., and EllarD. J. (1991) *Nature* 353, 815-821.
4. Manoj Kumar, A. S., and Aronson, A. I. (1999) *J. Bacteriol.* 181, 6103-6107.
5. Gazit, E., La Rocca, P., Sansom, M. S., and Shai, Y. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 12289-12294.
6. Schwartz, J. L., Juteau, M., Grochulski, P., Cygler, M., Prefontaine, G., Brousseau, R., & Masson, L. (1997) FEBS Lett. 410, 397-402: Masson, L., Tabashnik, B. E., Liu, Y. B., Brousseau, R., and Schwartz, J. L. (1999) *J. Biol. Chem.*274, 31996-32000.
7. Uawithya, P., Tuntitippawan, T., Katzenmeier, G., Panyim, S., and Angsuthanasombat, C. (1998) *Biochem. Mol. Biol. Int.* 44, 825-832.
8. Gerber, D. & Shai Y (2000). *J. Biol. Chem* . in press.

SYMPOSIUM I - Monday, 15:40 (Bacteria)

Development and management of resistance to Bt toxins in the diamondback moth

M. Shelton

Department of Entomology
Cornell University/NYSAES
Geneva, New York 14456

Field control failures to sprays of products containing *Bacillus thuringiensis* subsp. *kurstaki* began to be observed in populations of the diamondback moth, *Plutella xylostella*, in the late 1980s and through the 1990s in important crucifer growing regions of the world. Studies on some of these populations have been undertaken to understand what specific Bt toxins the populations are resistant to as well as the genetic and mechanistic basis of such resistance. Far less emphasis has been placed on why this resistance came about and what, if anything, can be done to manage this resistance. Resistance to Bt toxins appears to have arisen primarily in intensively managed crucifer production areas where resistance had already developed to organophosphate, carbamate and pyrethroid insecticides. In other words, generally a crisis had arisen before Bt was used intensively against diamondback moth populations. Growers who could no longer rely on older insecticides began to rely extensively and intensively on the use of products containing *B. thuringiensis* subsp. *kurstaki* and later on products containing *B. thuringiensis* subsp. *aizawai.* How a grower has managed these Bt products has influenced the resistance situation in his own area as well as sometimes in other areas. Resistance management for Bt depends on using multiple control strategies which include cultural and biological controls, as well as insecticides with different modes of action. With the advent of some new insecticides with novel modes of action, growers now have other tools which, if used wisely, can be incorporated into an area-wide management program that will help preserve the effectiveness of Bt toxins.

CONTRIBUTED PAPER - Monday, 12:15 (Viruses IV)

Expression of phenoloxidase genes from a parasitoid wasp in recombinant baculoviruses

Masamitsu Shikata, Ian Smith and Neil Parkinson

Central Science Laboratory, Sand Hutton, York, United Kingdom

Biochemical characterization of fractionated venom from the pupal endoparasitoid wasp *Pimpla hypochondriaca* indicated that this fluid, which is injected into the haemocoel of the host during oviposition, contains a number of biologically active components including cytotoxic and paralytic factors and phenoloxidase (PO). Genes encoding three related but distinct POs have been isolated from a cDNA library made from adult female *Pimpla* venom gland tissue. While related to other arthropod POs, the *Pimpla* POs are distinctive both in possessing signal sequences for secretion and in lacking a proteolytic cleavage site which occurs in the primary translation products (prophenoloxidases) of other species. In the present work, we have constructed three recombinant genotypes of *Autographa californica* nucleopolyhedrovirus (AcNPV), each of which contains one of the *Pimpla* PO cDNAs inserted adjacent to the polyhedrin promoter. In each case, PO was expressed at high levels in infected Sf9 insect cells. However, despite the presence of a signal peptide, the POs invariably remained cell-bound and were not secreted into the extracellular medium. To assess whether the insecticidal activity of these PO-expressing recombinant AcNPVs was any different to that of wild-type AcNPV, virus particles were injected into the haemocoel of fourth-instar *Heliothis virescens* larvae. While the levels of PO activity in the haemolymph were somewhat higher in larvae infected with each recombinant than in larvae infected with wild-type AcNPV, there were no differences among the survival times of insects infected with any of the recombinants or with wild-type AcNPV.

POSTER VP7 - Tuesday (Viruses)

Hyphantria cunea* nucleopolyhedrovirus (NPV) interferes with *Bombyx mori* NPV replication in a cell line from *B. mori

Noriko Shirata[1], Motoko Ikeda[1], Katsumi Kamiya[2], Satoshi Kawamura[2] and Michihiro Kobayashi[1]

[1]Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan; [2]Gifu Prefectural Institute of Bio-industrial Technology, Minokamo, Gifu, 505-0004, Japan

BmN-4 cells from the silkworm, *Bombyx mori*, are permissive for *B. mori* NPV (BmNPV) replication, while infection of BmN-4 cells with *Hyphantria cunea* NPV (HcNPV) results in an abortive infection at the point prior to the expression of viral late genes (Shirata et al., *Appl. Entomol. Zool.* 34, 507-516, 1999). In the present study, we examined interactions between BmNPV and HcNPV and found that HcNPV coinfection drastically restricted BmNPV replication in BmN-4 cells.

Light microscopy showed that in BmN-4 cells coinfected with BmNPV and HcNPV, CPE characteristic of NPV infection was clearly observed but few infected cells produced polyhedra. Analysis of polyhedrin gene expression indicated that restricted production of polyhedra was due to transcriptional restriction of polyhedrin gene expression. Examination of budded virions (BVs) and viral structural polypeptides including GP64 protein revealed that BmNPV BV yield in coinfected BmN-4 cells was less than one-twentieth of that in cells infected with BmNPV only, and accumulation of BmNPV GP64 was abrogated in coinfected BmN-4 cells. Slot-blot analysis further showed that accumulation of BmNPV genomic DNA in coinfected BmN-4 cells was approx. 20-fold lower than that in BmNPV-infected BmN-4 cells, indicating that BmNPV replication in BmN-4 cells coinfected with HcNPV was restricted at the point prior to viral DNA replication.

To further characterize the observed restriction of BmNPV replication in BmN-4 cells coinfected with HcNPV, superinfection experiments were conducted, in which BmN-4 cells were primarily infected with BmNPV and then superinfected with HcNPV at various times post BmNPV infection. The results showed that production of BmNPV structural polypeptides and polyhedrin was restricted substantially when HcNPV

superinfection occurred by 3 h post BmNPV infection but their production increased gradually as the times at HcNPV superinfection were delayed.

These results indicate that HcNPV interferes with BmNPV replication in BmN-4 cells and imply that a product(s) from HcNPV gene(s) is involved in the observed restriction of BmNPV replication in coinfected BmN-4 cells.

CONTRIBUTED PAPER - Thursday, 16:30 (Microbial Control I)

Dimethylsulfoxide as an enhancer of bacterial formulations

Margarita V. Shternshis[1] and Vladimir V. Gouli[2]

Department of Plant Protection,[1] Novosibirsk State Agrarian University, Russia 630039 and Entomology Research Laboratory[2] University of Vermont, Burlington, Vermont 05401-3404 USA

Bacterial formulations based on *Bacillus thuringiensis (Bt*) are the most popular in microbial insect control. However, these formulations require the permanent improvement. Based on the knowledge of the mechanism of Bt's action on insects we selected an optical brightener or enhancer for testing. Domain I of *Bt* Cry protein is known to be responsible for pore formation in cell membranes and nontoxic dimethylsulfoxide (DMSO) is known to help the transfer of biological molecules by increasing the size of membrane pores. Therefore, we use DMSO as an enhancer of two commercial formulations based on *Bt subsp. galleriae* (entobacterin) and *Bt subsp. dendrolimus = sotto* (dendrobacillin). We studed the influence of entobacterin on the beet webworm larvae, *Loxostege sticticalis* L., and dendrobacillin on the larvae of cabbage white butterfly, *Pieris brassicae* L. under the laboratory conditions. Larvae of instars 2-3 were placed on natural diet and treated with a mixture of bacterial suspension and DMSO in concentration 0 –2 %. The LC_{50} of entobacterin was reduced 7 times for *L.sticticalis* larva when DMSO was added to the bacterial suspension. Larva mortality of *P. brassicae* when treated with 0.2% suspension of *Bt* was equal to that caused by a 0.1% bacterial suspension combined with DMSO. The increasing mortality of insects under the treatment of bacterial formulation with DMSO was confirmed in the field on cabbage. Entobacterin was applied with the addition of DMSO on cabbage plots infested by diamondback moth larvae, *Plutella xylostella* L., and cabbage moth larvae, *Mamestra brassicae* L. The addition of DMSO to bacterial formulations caused the enhancement of insect mortality of both species. In 10 days after treatment, we observed a 30 and 50% mortality increase for *P.xylostella* and *M.brassicae*, respectively. Thus, addition of DMSO to the *Bt* formulations increase the larval mortality and the result did not depend on the investigated insect species or *Bt* subspecies. DMSO is known to be the plant growth regulator as well, and therefore, could be an advantageous additive to plant protection formulations.

SYMPOSIUM II - Tuesday, 8:35 (Bacteria)

Ecotoxicology *of Bacillus thuringiensis* and *Bacillus sphaericus*, with an emphasis on mammalian safety

Joel P. Siegel

United States Department of Agriculture, Agricultural Research Service,
2021 South Peach Avenue, Fresno, California, 93727

Entomopathogenic spore forming bacteria, most notably *Bacillus thuringiensis* (Bt), are the most widely used microbial pest control agents (MPCA). As part of the registration process, a series of studies must be conducted that assess the toxicity and infectivity of the candidate organism to a designated group of vertebrate nontarget organisms (NTOs). The emphasis of these studies has traditionally been direct effects, typically assessed in one-month laboratory studies. Initially, one of the main issues raised about the safety of *Bt* was its close relationship to *Bacillus anthracis*. A recent example of this issue surfacing in Canada will be discussed. Questions have also been raised focusing on the relationship between *B. cereus* and *Bt* because *B. cereus* has been recognized as the causal agent of an increasing number of cases of food poisoning and as a source of ocular infections. There are three commonly cited reports associating human infection with *Bt* dating back to the early 1980's. Questions arising the most recent case, involving infection following injury from a land mine blast will be explored. Researchers have reported that this isolate, *Bt* subspecies konkukian, was pathogenic to both immune suppressed and immune intact mice and these findings will be discussed. Data on the pathogenicity of *Bt* as well as other bacilli following invasive exposure will be presented and an attempt will be made to place the data on *Bt* subsp. *konkukian* in perspective. Finally, issues addressing the impact of *Bt* on NTO in the wild will be discussed.

SYMPOSIUM IV - Friday, 8:15 (Bacteria)

Entomopathogenic bacteria for the control of mosquitoes and black-flies in Brazil

Maria Helena Neves Lobo Silva-Filha and Lêda Regis

Department of Entomology, Centro de Pesquisas Aggeu Magalhães-Fundaçã Oswaldo
Cruze, Av. Moraes Rêgo s/n, Cidade Universitária, Recife-PE 7472 Brazil 50670-420

The bacteria *Bacillus thuringiensis* ssp *israelensis* (Bti) and *B. sphaericus* (Bs) show toxicity against some species of Culicidae. In addition, Bti is also a pathogen of black fly larvae. The utilizaion of these agents is expected to increase due to the wide-spread resistance in vectors to chemical insecticides and the environmental concerns due to the use of some of these. Brazil is one of the largest countries suffering from the recrudescence and emergence of vector-born diseases. The main goal of this presentation is to discuss the current status of Bti and Bs utilization in vector control in this country.

Two official programs using Bti have been pioneered in Brazil. These programs have been carried out since the 80's for controlling the black fly *Simulium pertinax* in large areas of Rio Grande do Sul State (south) and in the north coast of São Paulo State (southeast). In both cases, *S. pertinax* does not act as a vector but it is a major source of nuisance. Dengue threat in many cities is also necessitating the control of the vector, *Aedes aegypti*, using Bti, which will be introduced in many areas of Rio de Janeiro State, as part of a program attempting *A. aegypti* "eradication" in Brazil. Bti has been used in small field trials to control *A. aegypti* and *C. quinquefasciatus* in urban areas and cities of Brasília (center) and Londrina (south). In all cases mentioned above, Bti introduction was due to insect resistance to organophosphate insecticides. On the other hand, no loss of susceptibility of the insect populations subjected to Bti treatments is reported to the present time.

Large scale field trials using Bs have been successfully carried out by research groups in order to control *C. quinquefasciatus*, vector of filariasis and source of enormous nuisance, in urban areas of Brazil. Since the beginning of the 90's, trials using Bs have been done in districts of Recife city (northeast) showing high levels of filariasis and dramatic mosquitogenic conditions. Results obtained to date show that Bs is an effective agent and could be adopted at the operational level, under the local conditions. Data obtained in those trials also indicated that rotation of Bs and Bti during extensive treatment period is highly recommended to avoid selection of Bs resistance among *C. quinquefasciatus* larvae. The recent elaboration of a national program for eliminating filariasis from endemic areas might open perspectives for official programs using Bs in long-lasting control programs. Brazil has a large potential for the use of entomopathogenic bacteria and there is a need for the development of a variety of Bti and Bs based products for utilization in diverse control programs.

POSTER FP8 - Tuesday (Fungi)

Compatibility of Selected Fungicides with Fungal Pathogens of *Bemisia* Whiteflies

N. A. Silva Uribe, T. J. Poprawski[1], K. Arévalo-Niño and L. J. Galán-Wong

Universidad Autonoma de Nuevo Léon, Facultad de Ciencias Biológicas, Monterrey, Mexico; [1]USDA-ARS, Beneficial Insects Research Unit, Weslaco, TX 78596

A laboratory spray chamber, calibrated to deliver 282 liters/ha using 3 TXVS-6 nozzles (2 on drops) at 2.2 kg/cm^2, and 4.8 km/h, was used to

apply fungicides to whitefly-infested melon leaves, either 2 days before [2DB], on the same day (2-3 h before the fungi) [SD], or 2 days after [2DA] spray application of aerial conidia suspensions of *B. bassiana* and *P. fumosoroseus*. On SD, each leaf was sprayed with one 2-ml aliquot of spore suspension using a Potter spray tower. Treated and control (0.01% aqueous Tween 80) leaves were then isolated individually in vented plastic Petri dishes, and incubated for 24 h at 25ºC and 100% RH under a photophase of 16:8 (L:D) h. Thereafter, leaves were maintained under similar temperature and light regimes, but at 50-55% RH. The dishes of each treatment and control were incubated in isolation for the reason given above. Whiteflies were scored for mycosis 7 days after spore application. The angular values of proportion mycosis were analyzed by one-way ANOVA. Means were separated using the Tukey HSD test. Untransformed means are presented. In the *P. fumosoroseus* series, control mycosis (95.4%) was higher but not significantly different from mycosis in the 2DB, SD and 2DA Top Cop ($P = 0.598$), the 3 Quadris ($P = 0.077$), and the 3 Tilt ($P = 0.057$) treatments. Mycosis rate was 71.7, 49.1, and 65.8% in the 2DB, SD, and 2DA Bravo treatments, respectively [$P = 0.007$; Tukey test: control (a), 2DB (ab), SD (b), 2DA (b)]). In the *B. bassiana* series, control mycosis (81.2%) was significantly higher than mycosis in any of the 3 Bravo [$P < 0.001$; Tukey test: control (a), 2DB (26.7%b), SD (18.3%b), 2DA (45.0%b)] and any of the 3 Tilt [$P < 0.001$; Tukey test: control (a), 2DB (57.5%b), SD (41.7%b), 2DA (40.8%b)] treatments. Significant differences also were found in the Quadris treatments [$P = 0.001$; Tukey test: control (a), 2DB (65.8%ab), SD (63.3%bc), 2DA (45.8%c)] and in the Top Cop treatments [$P = 0.005$; Tukey test: control (a), 2DB (38.3%b), SD (48.3%b), 2DA (60.8%ab)]. At all 3 times of application, the fungicides were generally more compatible *in vivo* with *P. fumosoroseus* than with *B. bassiana*.

POSTER NP1 - Tuesday (Nematodes)

Entomopathogenic nematodes for the control of turfgrass insect pests in Quebec

Louis Simard[1], Guy Bélair[2] & Jacques Brodeur[1]

[1]Département de Phytologie, Université Laval, Ste-Foy (Quebec) Canada
[2]Agriculture & Agri-Food Canada, St-Jean-sur-Richelieu (Quebec) Canada,

The activity of entomopathogenic nematodes against the European chafer (*Rhizotrogus majalis*) (Coleoptera:Scarabaeidae) and the sod webworm (*Chrysoteuchia topiaria*) (Lepidoptera:Pyralidae) was evaluated under laboratory and field conditions. In the laboratory, bioassays with *R. majalis* were performed in 25-ml plastic containers filled with a pasteurized sandy soil and inoculated with infective juveniles (IJ) of the following nematodes species: *Heterorhabditis bacteriophora*, *Steinernema glaseri*, *S. feltiae* and *S. carpocapsae*. Mortality rates of *R. majalis* were extremely low for all tested nematodes species, except *S. glaseri* which caused 93% mortality of 3rd instar larvae at a concentration of 2000 IJ per larva. Laboratory tests have also shown that mortality rates caused by *S. glaseri* were significantly reduced when changing soil type from sandy to sandy loam, loamy clay and organic soil. Based on these results, no application was performed to assess the efficiency under field conditions. In the laboratory, *C. topiaria* 3rd and 4th instar larvae were exposed to eight concentrations of each species in petri dishes at 24 C for a 5-day period. *Heterorhabditis megidis* was the most virulent species with a LD_{50} of 6 IJ/larva. *S. glaseri*, *S. carpocapsae*, and *S. feltiae* revealed LD_{50} values of 34, 68, and 126 IJ, respectively. The effect of nematode exposure time on *C. topiaria* larval mortality was evaluated with *H. megidis* and *S. carpocapsae*. With a concentration of 1000 IJ/larva, a 24-hour exposure time or more was required to reach >80% mortality rates. Preliminary field tests against sod webworm larvae were conducted with *H. megidis*, *S. carpocapsae*, and *S. feltiae* on several residential lawns located in Quebec city and Montreal. In some lawns, sod webworm larval populations were significantly reduced by nematode treatments when compared to the untreated control plots. The efficiency of nematode applications was similar to the Diazinon insecticide treatment on most sites. An effective monitoring of this pest would allow to make targeted nematode applications, and thus reduce the use of insecticide in urban areas.

CONTRIBUTED PAPER - Thursday, 12:30 (Bacteria I)

Hybrid *Bacillus thuringiensis* δ-endotoxins provide enhanced spectrum of activity on Lepidopteran pests

Sakuntala Sivasupramaniam[1], Victor T. Kabuye[1], Thomas Malvar[1], James A. Baum[1], Amy Jelen Gilmer[2], Angela Peters[2], Dolores Coyle[2], K. S. Mohan[3], Farah Deeba[3] and K. C. Ravi[3]

[1]Monsanto Co. 700 Chesterfield Parkway North, St. Louis, MO 63198.
[2]Ecogen Inc. 2005 Cabot Boulevard West, Langhorne, PA 19047.
[3]Monsanto Co. Bangalore, India

Bacillus thuringiensis (Bt) δ-endotoxins comprise a diverse set of proteins that are toxic to a variety of lepidopteran, coleopteran, and dipteran larvae. Sequence comparisons between δ-endotoxins suggest that recombination between related δ-endotoxin genes contributes to their diversity. One such example is Cry1Ab which appears to have resulted from a recombination event between *cry1Ac* and *cry1Aa*. In these studies we describe the insecticidal and biochemical properties of hybrid proteins between Cry1Ac and Cry1F that were generated by established molecular genetic techniques. Hybrid Cry1Ac/Cry1F were expressed in Bt and assessed for the desired traits of crystal formation, protein stability, and insecticidal activity. Our results showed that certain hybrid toxins expressed by EG11063, EG11074, EG11751, and EG11768 exhibited the insecticidal characteristics of the parent δ-endotoxins, thereby enhancing the spectrum of Lepidopteram activity. These same hybrid proteins were also efficiently expressed in Bt cultures and were similar to the parental toxins in their resistance to proteolytic degradation. Our results also showed that by varying the exchange site between Cry1Ac and Cry1F, activity was improved against particular insects or had severe effects on insecticidal activity or protein stability without affecting efficient crystal formation in Bt.

The increase in host range specificity obtained with these novel δ-endotoxins such as those described in this study, can provide useful genes for *in-planta* applications and can serve as important tools for addressing concerns about insect resistance to Bt.

CONTRIBUTED PAPER - Tuesday, 10:45 (Viruses III)

EGT activity in granulovirus-infected insect larvae

Ian Smith[1], Robert Weaver[1] and Frances Hunter[2]

[1]Central Science Laboratory, Sand Hutton, York, United Kingdom
[2]School of Animal and Microbial Sciences, University of Reading, United Kingdom

Many, but not all, baculovirus genomes encode ecdysteroid UDP-glucosyltransferase (EGT), an enzyme which is secreted from infected cells and which conjugates the sugar moieties of UDP-galactose and/or UDP-glucose to ecdysone. As a result of this activity, ecdysone-mediated insect development may be disrupted, the feeding period and life-span of an infected larva prolonged, and the yield of progeny virus increased. We have previously identified and characterized the *egt* gene in a granulovirus of the tomato moth, *Lacanobia oleracea*, and we have now detected EGT activity in the haemolymph of infected *L. oleracea* larvae using high performance liquid chromatography. The same methodology was used to investigate haemolymph from *Spodoptera littoralis* (Egyptian cotton leafworm) larvae that had been infected with either of two distinct granuloviruses. Preliminary examination of the genomes of these two viruses by Southern hybridization suggested that one possessed a homologue of the *L. oleracea* granulovirus *egt* gene, whereas the other did not. However, no detectable EGT activity was found in larvae infected with either of the *S. littoralis* granuloviruses at various times post-inoculation in insects which subsequently succumbed to viral infection. Our results will be discussed in the context of the pathobiology of these viruses in their infected hosts.

SYMPOSIUM III - Tuesday, 17:50 (Bacteria)

Evidences for inter-molecular interaction as a necessary step for pore-formation activity and toxicity of *Bacillus thuringiensis* Cry1Ab toxin.

Mario Soberon, Rigoberto V. Perez, Maria E. Nuñez-Valdez, Isabel Gomez, Jorge Sanchez, and Alejandra Bravo.

Instituto de Biotecnologia, U.N.A.M., Apdo Postal 510-3 , Cuernavaca, Morelos 62271. Mexico.

Based on the observation of large conductance states formed by Bacillus thuringiensis Cry toxins in synthetic planar lipid bilayers and the estimation of a pore size between 10-20 um, it has been proposed that the pore could be formed by an oligomer containing four to six toxin monomers. However, there is a lack of information regarding the insertion of Cry toxins into the membrane and oligomer formation. Here we provide direct evidences showing that the intermolecular interaction between Cry1Ab toxin monomers is a necessary step for pore formation and toxicity. Two Cry1Ab mutant proteins affected in different steps of their mode of action (F371A in receptor binding and H168F in pore formation) were affected in toxicity against *Manduca sexta* larvae. Binding analysis showed that F371A protein bound more efficiently to *Manduca sexta* brush border membrane vesicles when mixed with H168F in a one to one ratio. These mutant proteins also recovered pore-formation activity and toxicity against *M. sexta* larvae when mixed, showing that monomers affected in different steps of their mode of action can form functional hetero-oligomers.

CONTRIBUTED PAPER - Tuesday, 16:30 (Protozoa I)

Development of *Vairimorpha* sp. in the fat body tissues of *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae and effects on host food utilizaton values

Leellen F. Solter[1] and Michael W. Henn[2]

[1] Illinois Natural History Survey, 607 East Peabody, Champaign, IL 61820, USA [2]Institute of Applied Zoology, Technical University of Munich, Am Hochanger 13, 85354 Freising, Germany

Infection of the gypsy moth, *Lymantria dispar*, with the microsporidium *Vairimorpha* sp. strongly influences the development of the host in ways typical of many species of terrestrial entomopathogenic microsporidia; growth is reduced while development time is extended in infected insects. The appearance of the different stages of the pathogen in the host relative to the elapsed time after oral infection and the influence of pathogen proliferation on food utilization of the host were examined. At 72 hours post infection, primary spores and germinated spores were present in midgut epithelial cells and muscle cells and the fat body tissues contained meronts, sporonts, and primary spores. Many more fat body cells contained vegetative stages and primary spores at 96 and 120 hr post infection, and both diplokaryotic spores and immature octospores were present. After this time period, the fat body cells continued to be invaded, with primary spores present at all time periods to 240 hours. Diplokaryotic spores matured first (beginning 96 hr post infection, octospores begin to mature at 145 hr post infection. While primary spores have been shown to germinate within the midgut cells of the host, and are presumed to be a means by which the pathogen invades the target tissues for production of mature environmental spores, they have not been previously reported to be important in the spread of the pathogen within the target tissues. Approximate digestibility of infected larvae increased during this time period, whereas the conversion of ingested and digested food to body substance decreased. The relative growth rate of infected and uninfected groups did not differ significantly between 96 and 120 hr post infection, although the relative consumption rate in infected *L. dispar* larvae was higher. Between 192 and 240 hr post infection, the relative growth rate of uninfected larvae increased. The infected group did not demonstrate this increase at a time period characterized by maturation of diplokaryotic spores and octospores in larval fat body tissues. Total body weight of uninfected larvae remained higher than that of infected larvae after 192 hr post infection. Calculated utilization values of infected larvae were significantly lower than those of the uninfected group; the lack of relative growth of infected larvae between 192 and 240 hrs post infection may be caused by lower food utilization or other factors currently under investigation.

STUDENT POSTER VP17 - Tuesday (Viruses)

Cydia pomonella granulovirus: Horizontal transmission of the virus is closely related to its distribution

Susanne B. Steineke and Johannes A. Jehle

State Education and Research Centre Neustadt/Weinstr., Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany

Cydia pomonella granulovirus (CpGV) can effectively control the codling moth in apple orchards. Due to the biology of the host, horizontal transmission generally is not considered an important aspect in the population dynamics of this virus. The low density of codling moth make virus transmission from one larva to another unlikely. Yet it is generally difficult for pathogens to solely rely on vertical transmission. In order to fully understand the population dynamics of CpGV, there remains a need to study the horizontal transmission. A *C. pomonella* larva infected as neonate and killed as second instar can produce up to 10^8 virus particles. Most larvae this age have just started tunnelling and die a few millimetres beneath the apple's surface. A mathematical model shows that if these particles are spread somewhat from their point of origin, the chance of infection is increased greatly. According to the model the chance of infection after two days increases from 0% to about 16% if the virus released by a second instar cadaver is spread across an area of only 3 cm^2. We will discuss to what extent data can support this model with the results from an experiment using detached apples.

CONTRIBUTED PAPER - Monday, 15:30 (Fungi II)

Testing a liquid medium for industrial production of submerged spores of *Metarhizium anisopliae var. acridum*

Dietrich Stephan[1], Dirk Mager[1], Helmut Junge[2], Hermann Strasser[3] and Gisbert Zimmermann[1]

Federal Biological Research Centre for Agriculture and Forestry[1], Institute for Biological Control , Heinrichstrasse 243, D-64285 Darmstadt, Germany, FZB Biotechnik GmbH[2], Glienicker Weg 185, D-12489 Berlin, Gemany, Leopold-Franzens-University[3], Institute for Microbiology, Technikerstrasse 25, A-6020 Innsbruck, Austria

GREEN MUSCLE® is a mycoinsecticide based on the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* (IMI 330189), which contains solid state fermented conidia. As this fungus can also easily be produced in liquid culture, we focussed on the development of a liquid medium for industrial production of submerged spores. In this presentation, the effect of two standardized media on various fungal parameters are discussed.

First experiments in Erlemeyer flasks demonstrated that the spore yield and the germination rate did not differ between the two media. Furthermore, the effect on spore formation and spore size, the production of enzymes, the utilisation of nutrients and the production of toxins were investigated, and the results will be presented. When the virulence of submerged spores grown in the two different media was compared by superficial application or by injection of larvae of *Locusta migratoria*, slight differences were obtained after application but significant differences after injection.

In additional experiments, the medium was tested in a 4 l laboratory fermenter. Growth, spore formation, pH-value and O_2-concentration were measured over a fermentation period of four days. In a further experiment, the medium was used for production of submerged spores in fermenters with a capacity of up to 3000 l. The economic feasibility of the liquid medium and the production system will be discussed.

The investigations were carried out within the framework of the GTZ-Project "Production and marketing of a biological preparation for control of locusts and grasshoppers"

SYMPOSIUM - Tuesday, 8:45 (Nematodes)

From Steiner to present: Recapitulations and considerations of entomopathogenic nematode systematics in the 21st century

S. Patricia Stock

Department of Nematology. University of California Davis
One Shields Ave. Davis, CA 95616-8668

Since the isolation of the first described entomopathogenic nematode, *Steinernema kraussei*, a significant amount of progress has been made in our understanding of the biology and genetics of these nematodes and their symbionts. Of all research areas involved in the study of entomopathogenic nematodes (EPN), 'systematics' has been the most debatable discipline. From traditional morphological descriptions to the implementation of molecular methods and the application of phylogenetic approaches in the interpretation of species concepts, EPN systematics has undergone to a significant amount of change. In this presentation, the current state of affairs in the taxonomy of the Steinernematidae and Heterorhabditidae is reviewed based on a combined re-examination of morphological attributes and interpretation of their recovered phylogenetic relationships. Hypotheses of the evolution of key diagnostic characters are examined and discussed. Considerations for future identification/description of EPN taxa are proposed.

POSTER BP49 - Thursday (Bacteria)

Persistence of the *B.t.* CryIA(c) Protein from Cotton in the Soil

D. A. Streett

USDA, ARS, Southern Insect Management Research Unit
Stoneville, MS, USA 38776

Since 1996, when commercial *B.t.*-transgenic cotton varieties were first introduced, the planted acreage has steadily increased each year. In 1998, approximately 55% of the cotton planted in Mississippi was *B.t.*-transgenic varieties. The recent report on persistence of the CryIA(b) protein from transgenic corn tissue in the soil led to our interest in the fate of CryIA(c) protein from transgenic cotton tissue in the soil. Larval growth bioassays were conducted using neonate *Heliothis virescens* on artificial diet incorporating lyophilized transgenic cotton powder or MVP II (Mycogen Corporation) as the source of CryIA(c) protein. Larval growth bioassays using neonate *H. virescens* could detect 300ng of a MVP II + soil mixture per ml diet. The enzyme-linked immunosorbent assay (ELISA) could only be used as a qualitative assay for the presence of CryIA(c) protein. Soil samples from field locations planted with *B.t.* and non-*B.t.* cotton varieties were monitored for CryIA(c) bioactivity for six months post harvest in 1999-2000. CryIA(c) bioactivity was not detected at any of the field locations in this study.

CONTRIBUTED PAPER - Thursday, 11:30 (Viruses IV)

Laboratory and field evaluation of genetically modified *Helicoverpa armigera* single-nucleocapsid nucleopolyhedroviruses (HaSNPV) in cotton

Xiulian Sun[1,2], Xinwen Chen[1,2] Zhongxin Zhang[1] Felix J.J.A. Bianchi[2], Hualin Wang[1], Huiying Peng[1,2], Just M. Vlak[2] and Zhihong Hu[1]

Joint-Laboratory of Invertebrate Virology[1], Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P R China, and Laboratory of Virology[2], Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus (HaSNPV) has been developed as a commercial biopesticide to control the cotton boll worm, *H. armigera*, in China (Zhang, 1989). The major limitation to a wider application has been the relative long time to incapacitate the target insect. Two HearNPV recombinants with improved insecticidal properties, engineered previously (Chen *et al*., 2000) were field-tested in China for the first time. One recombinant (HaCXW1) lacked the ecdysteroid UDP-glucosyltransferase (*egt*) gene. In another recombinant (HaCXW2) the *egt* gene was replaced by an insect-selective scorpion toxin (*AaIT*) gene. Both recombinants carried a Green Fluorescent Protein gene as a marker. HaCXW1 and HaCXW2 reduced the median survival time (ST_{50}) of second instar *H. armigera* larvae with 27% and 32%, respectively, in comparison to wild-type HaSNPV (HaSNPV–wt). The recombinants and wild type HaSNPV can be discriminated by a PCR analysis. Their biological activity (LD_{50}) values were similar to the wild type HaSNPV.

In the field, the number of surviving larvae in plots treated with these recombinants was significantly lower than in plots treated with HearNPV-wt or in control plots when sampled over time after spraying. Second instar *H. armigera* larvae were collected from the treated plots and investigated in the laboratory. Treatments with HaCXW1 and HaCXW2 resulted in 15.3% and 26.3% reduction in ST_{50} values as compared to HaSNPV–wt. This is in line with the results obtained in laboratory bioasays. Feeding reduction by larvae infected with HaCXW1 (egt-minus) and HaCXW2 (*egt*-minus; AaIT-plus) approximated 50% and 63%, respectively, within 4 days after treatment as compared to HaSNPV–wt. These results indicated that in a cotton field situation the recombinants were a more effective control agents of the cotton boll worm than wild type HaSNPV and behaved here in a similar way as predicted from the laboratory assays.

CONTRIBUTED PAPER - Friday, 9:15 (Microbial Control II)

Shelf-life of *Anagrapha falcifera* nuclear polyhedrosis virus (*Af*MNPV) microcapsular lignin-based formulations

Patricia Tamez-Guerra*[1], Michael R. McGuire[2], and Robert W. Behle[2]

[1]Dep. de Microbiología e Inmunología, Fac. de Ciencias Biológicas, UANL, San Nicolás de los Garza, N. L., México. AP 46-F. Fax (8-352-4212). e-mail: patamez@ccr.dsi.uanl.mx
[2]Bioactive Agents Research Unit, NCAUR-USDA-ARS. 1815 N. University St., Peoria, IL USA. 61604. Fax (309-671-6693), e-mail: mcguirmr@mail.ncaur.usda.gov.

The *Anagrapha falcifera* nucleopolyhedrosis virus (*Af*MNPV) is a natural insecticide with commercial potential for control of lepidopteran pests of crops. Development of viruses as bioinsecticides has been limited because activity is rapidly lost during storage and after application in the field. Lignin-based spray dried formulations (SDF) of *Af*MNPV have demonstrated improved residual activity after field application when compared with unformulated *Af*MNPV (NFV) and a commercial formulation (CF). We evaluated lignin-based formulations for the loss of activity due to spray drying and after storage at room (30°C) and cold (~4°C) temperatures every month for 6 months. Eight lignin-based formulations were prepared using 3 production lots of *Af*MNPV (24 SDF total). A droplet-feeding assay was used to determine the dose response and LC_{50} to neonate *Trichoplusia ni.* A similar dose-response assay using treated cotton-leaf tissue was conducted at 0, 1, 4 and 6 months. Lignin-based formulations were compared with unformulated *Af*MNPV that was stored frozen (-20°C). Dose response assays showed that spray drying reduces the activity of *Af*MNPV by about 10%. During 6 months storage at 4°C, activity of the formulations was not significantly less than the frozen *Af*MNPV. At 30°C, most SDF (22/24) maintained insecticidal activity for four months, but lost significant activity afterwards (21/24). CF samples from two of the three lots also lost activity after 6 months of storage at 30°C. SDF and CF, which were stored at cold temperature for 7 months, were compared with freshly-prepared SDF for residual activity after field application. Treatments were applied to field-grown cabbage at a rate of 1.0×10^{12} pibs/A. Each virus lot was used as a replicate. Insecticidal activity against neonate *T. ni* was determined for leaf samples collected at 3, 7, 27 and 51 h after application. SDFs had significantly higher insecticidal activity (~70% mortality) than that of NFV (30% mortality) three hours after application. At 7 h and 27 h after application, SDFs had significantly higher residual activity (65% and 50% mortality, respectively) compared with the CF (42% and 23% mortality, respectively). No differences in activity were observed between samples of SDFs stored for 7 months at 4°C and freshly prepared. These experiments demonstrated that *Af*MNPV in lignin-based SDF has a shelf-life of up to 4 months at room temperature and at least 7 months in cold temperature, with longer residual activity in field compared with NFV of CF.

STUDENT PAPER - Tuesday, 17:15 (Protozoa I)

Phylogenetic analysis of the Protist *Helicosporidium sp.*

A. Tartar, D. Boucias, J. Becnel*, and B. Adams

Department of Entomology and Nematology
University of Florida, Gainesville, Florida, 32611 USA
*Center for Medical, Agricultural and Veterinary Entomology
USDA, ARS, Gainesville, Florida 32604, USA

At present very little is known about the protist *Helicosporidium sp*., a pathogen of insects, mites, cladocerans and trematodes. Historically, this pathogen was first detected in a certopogonid and described by Keilin in the early 1900s. Kudo (1931) placed it in a separate order Helicosporidia within Cnidiospora. In the 1960s, Weiser, examining both type material and a new isolate from a hepialid larva, proposed that this organism should be transferred to the Ascomycetes. Later studies by Lindegren and Hoffman (1976) proposed that the developmental stages of this organism were closer to the Protozoa than fungi. An *Helicosporidium* sp. isolated from the blackfly *Simulium jonesi* Stone & Snoddy (Diptera: Simuliidae) has been amplified under both *in vitro* and *in vivo* conditions. In order to evaluate the phylogenetic position of this organism, the 18S, 26S, 5.8S regions of the *Helicosporidium* DNA, as well as some partial sequences of the actin and tubulin genes, were amplified by PCR and sequenced. BLAST analyses were performed and surprisingly, the most significant "hits" suggested similarities with unicellular green algae (Chlorophytae) for all five sequences. These sequences were then aligned with similar sequences from representative eukaryotes and analyzed using maximum-parsimony. Such analyses led to the construction of two phylogenetic trees that evaluate the position of *Helicosporidium sp*. within the phylogeny of eukaryotes, in regard to its rDNA genes and its nuclear genes (actin and tubulin), respectively. Both trees failed to associate *Helicosporidium* with any of the sampled eukaryotic taxa. This suggests that *Helicosporidium sp*. may represent a unique lineage that diverged very early from other major eukaryote clades.

STUDENT POSTER PP9 - Thursday (Protozoa)

Does pebrine exist in Brazilian silkworm farms?

Rie Teramoto[1], Mihoko Hashiguchi[1], Chisa Yasunaga-Aoki[1], Takeshi Kawarabata[1], and Hidehiro Tomimaru[2]

[1] Institute of Biological Control, Faculty of Agriculture, Kyushu University,
Fukuoka 812-8581, Japan
[2] Fiacao De Seda Bratac S/A, Caixa Postal 39-Cep, 17690-000 Bastos-Est,
Sao Paulo, Brazil

Several microsporidians were isolated from Brazilian silkworm farms in 1995. Biological characteristics of the new isolate designated TB-3 were studied in vivo and in vitro.

TB-3 spores showing an ovocylindical shape with the size of 4.1 µm x 2.3 µm were similar to *Nosema* sp. NIS M11 spores. When studied by the latex adhesion test using latex particles sensitized with monoclonal antibodies against *Nosema bom- byci*s NIS 001, *Nosema* sp. NIS M11, and *Vairimorpha* sp. NIS M12 spores, it revealed that spores of TB-3 did not react with any latex particles.

Partially purified spores of TB-3 were perorally inoculated into the silkworms, *Bombyx mori*. Pathogenicity of this microsporidium against the silkworms was lower and the number of spores produced in the infected larvae was less than the case of *N. bombycis*.

The percentages of germinated TB-3 spores were evaluated when prime with KOH or EDTA solutions. KOH-primed spores were also inoculated into *Antheraea eucalypti* cells suspended in a Rinaldini's solution. Under those various conditions, the rates of spore germination were ranged from 10% to 20%.

WORKSHOP I - Tuesday, 16:50 (Viruses)

The Baculoviridae, current taxonomic issues

David A. Theilmann

Pacific Agri-Food Research Centre
Agriculture and Agri-Food Canada
Summerland, B. C. Canada

The taxonomic classification of baculovirus has evolved significantly over the last three decades. The current taxonomy statement has tried to accurately reflect the currently known and data for physical, biological, and genetic aspects of baculoviruses. In addition, significant changes have occurred to adhere to the ICTV rules that are attempting to bring uniformity to virus taxonomy in general.

In this discussion I will overview the current taxonomy statement, highlighting areas that might evolve in the future. It is a very exciting time for baculovirus research at several baculovirus genomes have been completely sequenced and several more are in progress. This database of baculovirus genomes is fast becoming a treasure trove of information concerning the genetic and evolutionary relatedness between baculovirus species.

STUDENT POSTER BP50 - Thursday (Bacteria)

Effect of the á-helix 4 mutation, N135Q, on the properties of the Cry1Ac1 and Cry1Ab5 toxins.

Natalie J. Tigue, Juliette Jacoby & David J. Ellar

Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge, CB2 1GA

Mutations in domains I, II and III in the Cry I A toxins have revealed important residues involved in the functioning of the individual domains. Mutation of residues in á -helices 4 and 5 of domain I, for example, have revealed a critical role for this helical hairpin in pore formation (Kumar and Aronson (1999) *J. Bacteriol.* **181(19)**: 6103-6107; Masson *et al*. (1999) *J. Biol. Chem.* **274(45):** 31996-32000). From such studies, it has been suggested that residues in á -helix 5 are involved in oligomerisation, whereas á -helix 4 lines the lumen of the pore and is therefore involved in the function of the ion channel.

In a previous study (Cooper *et al.* (1998) *Biochem J.* **333**: 677-683) the CrylAc1 domain I mutant, N135Q, was shown to have lost toxicity to *Manduca sexta* . Also, this á -helix 4 mutant was unable to form pares in *M. sexta* brush border membrane vesicles (BBMV) as measured by light scattering experiments. In SPR experiments, however, the mutant was able to bind to arninopeptidase N, isolated from the *M sexta* midgut. The results of further studies with this mutant and the equivalent mutant of Cry1Ab5 (also N135Q) will be reported.

POSTER NP4 - Tuesday (Nematodes)

Influence of abiotic factors on the parasitism of *Steinernema feltiae* (Rhabditida: Steinernematidae) on larvae of *Anastrepha obliqua* (Diptera: Tephritidae).

Jorge Toledo[1], Concepción Pérez[1], Pablo Liedo[1], Trevor Williams[1] and Jorge E. Ibarra[2].

[1]ECOSUR, A.P. 36, Tapachula 30700 , Chiapas, Mexico;
[2]CINVESTAV-IPN, A.P. 629, Irapuato 36500, Guanajato, Mexico.

The West Indian fruit fly, *Anastrepha obliqua*, is a widely distributed species, ranging from southern United States to Brazil. This is an important pest in mango orchards and control methods involve the use of chemical insecticides, either in baits, for the control of adults, or as soil applications, for control of the pre-pupal and pupal stages that have left the fruit. The potential of entomopathogenic nematodes to control fruit flies depends on their ability to parasitize larvae and pre-pupae, during the period between egression from the fruit and pupation. Parasitism of *Steinernema feltiae* in a wide range of insect hosts varies due to differences in host susceptibility and parasite virulence. Abiotic factors may also play an important role in parasitism. This report deals with the effect of temperature, soil depth and soil texture on the parasitism of *S. feltiae* in larvae of *A. obliqua*. Virulence levels were established by estimating LC_{50}s in sandy soil, on 6 and 8-day old larvae at three different soil depths (2, 5 and 8 cm). The 8-day old larvae were between 1.5 and 2 times less susceptible to nematode parasitism than 6-day old larvae. Soil depth increased LC_{50} values from 2 to 10 times. When parasitism was tested in three different soil textures (sand, clay and loam), significant

differences in parasitism were observed. Interestingly, the highest parasitism was observed in clay, followed by that observed in sand and loam, respectively. Because these bioassays were performed using the previously estimated LC_{50}s at three different soil depths and two larval ages, no significant differences were observed between soil depths except in those bioassays carried out in clay where parasitism was more prevalent in the deepest containers. As clay proved to be a better environment for parasitism, temperature tests were performed using clay soil. Three temperatures (19, 25 and 30°C) were tested at three different soil depths and on two larval ages. Mortality counts indicated that the lowest parasitism occurred at the lowest temperature, and the highest parasitism occurred at 25 and 30ºC. Correlation analyses indicated that the optimum temperature for parasitism lay between 26 and 27°C. These bioassays also corroborated that high levels of parasitism occurred in the deepest containers, in a clay soil environment.

POSTER BP14 - Tuesday (Bacteria)

Effect of *Bacillus thuringiensis* b-exotoxin on three species of *Anastrepha* (Diptera: Tephritidae)

Jorge Toledo[1], Pablo Liedo[1], Trevor Williams[1] and Jorge E. Ibarra[2]

[1]ECOSUR, Apdo .Postal 36, 30700Tapachula, Chis., Mexico;
[2]CINVESTAV-IPN, Apdo. Postal 629, 36500 Irapuato, Gto., Mexico

Fruit flies of the genus *Anastrepha* (Schiner) (Diptera: Tephritidae) represent a major problem for fruit and vegetable production from southern United States to the North of Argentina. *Anastrepha* larvae leave the fruit and are in contact with the soil for approximately 24 h prior to pupation. Soil applications of insecticides such as diazinon have been considered as a useful contribution to fly control in systems based on integrated pest management. The β-exotoxin of *B. thuringiensis* has shown high toxicity to a number of insect species from diverse orders. Dipteran larvae appear to be particularly susceptible and deleterious effects have been observed following topical application, ingestion or injection of the exotoxin. This report describes toxic effects of the *B. thuringiensis* β-exotoxin towards third instar larvae of three fruit fly species: *Anastrepha ludens, A. obliqua* and *A. serpentina.* Bioassays were performed in 9 cm diameter Petri dishes containing a filter paper disk impregnated with each toxin solution. Third instar *Anastrepha* larvae were randomly selected and placed in groups of 30 in each Petri dish. After 24 h exposure, larvae were transferred to vermiculite where they were left to pupate for 12 days. Adult emergence was recorded. The β-exotoxin was highly toxic to all three species tested, with LC_{50} values estimated at 0.641, 0.512 and 0.408 µg/cm^2 of filter paper, for *A. ludens, A. obliqua* and *A. serpentina*, respectively. Only *A. ludens* was statistically less susceptible than *A. serpentina*. Exposure to β-exotoxin was associated with an increase in the incidence of deformed pupae. The adult survivors from β-exotoxin treatments showed no negative effects in terms of their longevity, fecundity or egg eclosion (fertility). We conclude that the β-exotoxin may have potential as a control agent for fruit fly pests.

SYMPOSIUM I - Monday, 14:30 (Viruses)

The role of hemocytes in AcMNPV pathogenesis in *Helicoverpa zea* and *Heliothis virescens*.

Dominique Trudeau, Jan O. Washburn and Loy E. Volkman.

Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA.

AcMNPV is the most widely investigated and best characterized insect virus to date. Even so, our understanding of AcMNPV pathogenesis and the roles of specific host components in this process is incomplete. Hemocytes have been implicated in the resistance of *Helicoverpa zea* to fatal infection by AcMNPV. In order to investigate more fully the role of hemocytes in *H. zea* 's resistance, we compared AcMNPV infection in *H. zea*, a semi-permissive host, and *Heliothis virescens*, a closely-related, permissive host. Specifically, we characterized AcMNPV infection in the hemocyte populations of both species in time course studies both *in vivo* and *in vitro*. We found that hemocytes of *H. zea* take up AcMNPV and transport the nucleocapsids to the nucleus, but unlike the hemocytes of *H. virescens*, do not support viral replication. In *H. zea* hemocytes, infection is blocked prior to early gene expression. Hence, hemocytes of *H. zea* not only fail to amplify virus but also contribute to its removal from the hemolymph. Hemocytes of *H. zea* also participate in the encapsulation of melanized, viral-infected tracheal elements. We suggest that melanization and encapsulation are triggered by AcMNPV-induced pathology in the host tracheal epithelia and do not represent a specific antiviral response. The failure of *H. zea* hemocytes to amplify virus after having removed it from the hemolymph, together with the host melanization and encapsulation responses, result in the elimination of viral foci, reduced hemolymph viral titers and subsequent attenuation of disease progression.

POSTER FP10 - Tuesday (Fungi)

Effect of the growth media composition in the biopesticide activity of *Beauveria bassiana* strains against second-third instar larvae of *Spodoptera frugiperda*

Uribe D, Aponte L and J Cerón

Instituto de Biotecnología, Universidad Nacional de Colombia, A.A. 14-490, Santafe de Bogotá, Colombia

Spodoptera frugiperda is one of the most important polyphagous insect pest in our country, which affect a huge variety of agricultural crops like cotton, rice, corn and sorghum. The biological control strategy appears as a good alternative to make the management of this specie in order to diminish the selection's pressure of the chemical insecticides to develop pesticide resistant insect. In this work, it is shown the results of a screening of native isolates of *Beauveria bassiana* and *Metarhizium anisopliae* strains against insect larvae of *S frugiperda*. At the end of the screening it was possible to select some isolates with very good biopesticide activity, which may be considered as a good alternative for the control of this insect in field conditions.

As it is well known, in the production of entomogenous fungi some characteristics like the culture medium composition may affect the biopesticide activity and it will happen in a different way depending of the entomogenous fungi strain. In order to know the effect of the nutrients in the biopesticide activity, four entomogenous fungi strains were tested in two different media Sabouroud Dextrose Agar (SAD) and Wheat agar (WA). Three of the entomogenous strains had good biopesticide activity (60% to 90% of mortality at $1x10^8$ conidia/ml) against second third instar larvae of *S frugiperda* and one had no good biopesticide activity (11% at $1x10^8$ conidia/ml). The results suggest that when the strains were grown at WA presented the better performance that when they were grown at the SDA media. Although it is worth to mention that for the best two strains that differences were not statistically significant. As there is a huge interest in our country in the entomogenous fungi production as a biocontrol agents, it is shown the results found with the better entomogenous isolate, when it was evaluated in four different growth media for conidia production. It was included sterilized rice grains which is one of the most widely employed media in our country to produce entomogenous fungi in the agricultural industry in Colombia.

STUDENT POSTER BP25 - Tuesday (Bacteria)

Development of a bioassay methodology for the evaluation of the biopesticide activity of *Bacillus thuringiensis* native strains against first instar larvae of *Tecia solanivora*.

Uribe D, Castelblanco A, Grosso V, Martinez W and J Cerón.

Instituto de Biotecnologia Universidad Nacional de Colombia A.A. 14-490, Santafe de Bogotá, Colombia

Colombia is the main potato producer at the Latin- América región, with more than 160.000 Ha sown and a yield per Ha of 17.5 ton. At the moment around 90.000 families live of working in this crop in our country, it makes the potato agricultural industry one of the most important crops of our agricultural economy nowadays.

The high yields of this crop are supported in the management of nutrition and phytosanitary problems with chemically derived products. However, instead of this management, some pest insect like the "Guatemalan potato moth" *Tecia solanivora*, produce huge economic losses to the growers. During 1997, in the last outbreak of the insect pest,

it was obtained a profit decay in 40% of the annual yield of the crop in Colombia. The explanation of the low capacity for the appropriate management of the pest may reside in that there is not a good commercial product of chemical or biological source with good performance against the insect. It may happen because of the low research investment in the control of this insect in the region of the insect distribution, which is from Central America to Ecuador.

As a response for the situation described above, last year it was designed an Insecticidal Pest Management against *T solanivora* in our country. In this program the biological control had a remarkable place in the research priorities of the institutions interested in the problem. *Bacillus thuringiensis* was identified as an important entomogenous microorganism in this strategy, because it is one of the options for the biological control of the pest in storage conditions. In this work, it is shown the methodology designed to make a good insect breeding of *T solanivora* in the laboratory. In addition to that it is shown a method of bioassay which use small pieces of potato tuber to evaluate the biopesticide activity of *B thuringiensis* native strains against first instar larvae of *T solanivora*. Finally it is worth to remark the activity of at least three Bt native strains which have around 60% more activity against the insect larvae than the control strain HD1 kurstaky, which is used in some commercial products for the biological control of the pest in storage conditions.

POSTER BP51 - Thursday (Bacteria)

Bacillus thuringiensis and _Beauveria bassiana_ based formulation as an alternative for the control of _Spodoptera frugierda_

Uribe D, Aponte L, Martinez W, Grosso V and J Cerón

Instituto de Biotecnología, Universidad Nacional de Colombia, A.A. 14-490, Santafe de Bogotá, Colombia

Spodoptera frugiperda is one of the most important polyphagous insect pest in our country. It is widely distributed in most of American countries, affecting a variety of agricultural crops like cotton, rice, corn and sorghum. The biological control strategy is a good alternative to manage this specie in order to diminish the selection's pressure of the chemical insecticides that develop pesticide resistant insect. Bt native strains have been testing against first instar larvae of *S frugiperda* with good results in both laboratory and field conditions. On the other hand some entomogenous fungi species have shown relatively good activity against second-third instar larvae of *S frugiperda*. In this context both entomogenous microorganisms are complementary strategies for the control of this pest insect in field conditions. In this work it is shown the results in terms of percentage of mortality of three different prototypes of formulation which contain 1) a Bt native strain; 2) *Beauveria bassiana* native strain and 3) a mix of both of these microorganism in the same formulation. The results suggest that this kind of strategies may be a good alternative to improve the pesticide performance of the biological control of *S frugiperda* in field conditions.

CONTRIBUTED PAPER - Monday, 18:00 (Bacteria II)

Role of α-helix 3 charged residues in pore formation by the _Bacillus thuringiensis_ insecticidal toxin Cry1Aa

Vincent Vachon[1], Florence Coux [1,2], Gabrielle Préfontaine[3], Cécile Rang[2], Lucie Marceau[1], Luke Masson[3], Roger Frutos[2], Jean-Louis Schwartz[1,3], Roland Brousseau[3] and Raynald Laprade[1]

Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec, Canada[1], CIRAD, Montpellier, France[2], and Biotechnology Research Institute, National Research Council, Montreal, Quebec, Canada[3]

Each of the charged residues of α-helix 3 of the *Bacillus thuringiensis* Cry1Aa toxin, except those involved in an intramolecular salt bridge, was mutated individually to either a neutral or to an oppositely charged amino acid. Most of the resulting mutants were considerably less toxic to *Manduca sexta* larvae than Cry1Aa. The ability of these mutants to permeabilize brush border membrane vesicles isolated from *M. sexta* larvae to KCl, sucrose, raffinose, N-methyl-D-glucamine-HCl and potassium gluconate was analyzed, at pH 7.5 and 10.5, with an osmotic swelling assay based on light-scattering measurements. Mutations at position 99 (R99C, R99E and R99Y) resulted in an almost complete loss of pore-forming ability. Replacing either Glu101, Glu116, Glu118 or Asp120 by cysteine, glutamine or lysine residues, however, had only minor effects on the properties of the pores formed by the toxin after an hour of preincubation with the vesicles. Mutants E101C, E116Q, E116K and E118C were nevertheless slightly less active at pH 10.5 than at pH 7.5. In addition, half of the mutants (E101C, E101Q, E101K, E116K, E118C and D120K) had a significantly slower rate of pore formation when compared with Cry1Aa. Both pH and rate of pore formation therefore appear to be important modulating factors of toxin potency in the larval midgut. The above results strongly suggest that α-helix 3 plays an important role in the mechanism of pore formation, but probably does not line the lumen of the pore.

CONTRIBUTED PAPER - Monday, 17:45 (Bacteria II)

Analysis of the pores formed by α-helix 4 mutants of the _Bacillus thuringiensis_ insecticidal toxins Cry1Aa

Vincent Vachon[1], Gabrielle Préfontaine[2], Cécile Rang[3], Florence Coux[1,3], Marc Juteau[1], Jean-Louis Schwartz[1,2], Roland Brousseau[2], Roger Frutos[3], Raynald Laprade[1] and Luke Masson[2]

Groupe de recherche en transport membranaire, Université de Montréal, Montreal, Quebec, Canada[1], Biotechnology Research Institute, National Research Council, Montreal, Quebec, Canada[2] and CIRAD, Montpellier, France[3]

The role played by α-helix 4 of the *Bacillus thuringiensis* toxin Cry1Aa in functional pore formation was investigated by individually replacing, by site-directed mutagenesis, each of its charged residues by either a neutral or an oppositely charged amino acid. Like α-helix 3 mutants, most of the resulting mutants were considerably less toxic to *Manduca sexta* larvae than Cry1Aa. In contrast with most α-helix 3 mutants, however, most α-helix 4 mutants, with the notable exception of those at amino acid position 127 (R127N and R127E), located near the N-terminal end of the helix, also had a strongly reduced ability to form pores, at pH 7.5 and 10.5, in midgut brush border membrane vesicles isolated from this insect. This reduced activity was accompanied by a reduced rate of pore formation. In addition, the pores formed by most mutants that retained a partial but detectable pore-forming ability (E128C, R131D, R131Q, R131E, R131H, D136N, D136C and D136Y) were either reduced in size or modified in their ionic selectivity. Introducing a negatively charged amino acid near the C-terminal end of the helix (Thr142Asp and Thr143Asp), a region normally devoid of charged residues, caused a total loss of activity. These results provide further support for a model in which α-helix 4 lines the lumen of the pore formed by Cry1Aa.

CONTRIBUTED PAPER - Thursday, 12:00 (Bacteria IV)

Insecticidal activity of walnut plants transformed to contain Insecticidal Crystal Protein Fragments of _Bacillus thuringiensis_

P. V. Vail[1], A. Dandekar[2], J. S. Tebbets[1], S. L. Uratsu[2], G. H. McGranahan[2] and C. A. Leslie[2]

[1]USDA-ARS, Horticultural Crops Research Laboratory, 2021 S. Peach Ave., Fresno, CA 93727

[2]University of California, Department of Pomology, 1045 Wickson Hall, Davis, CA 95616

In 1989 we began studies of the toxicity of two Insecticidal Crystal Protein Fragments (ICPF) *cry*IA(b) and *cry*IA(c) to two lepidopterous pests important to walnut production, codling moth, *Cydia pomonella* (L.) and the navel orangeworm, *Amyelois transitella* (Walker). We also tested susceptibility of the Indianmeal moth, *Plodia interpunctella* (Hübner), a major postharvest storage pest. These studies demonstrated that all three species were susceptible to the two ICPFs tested. Indianmeal moth larvae were the most susceptible and navel orangeworm the least susceptible of the three species.

These results prompted us to determine the possibility of engineering walnuts to express ICPFs at sufficient levels to control both production and storage pests. Walnut somatic embryos containing transfer DNA responsible for the production of ICPF, *cry*IA(c), were successfully

transformed. Presumptive transformation was based on marker genes for kanamycin resistance, GUS, and southern blot analysis with later constructs. However, expression of the ICPF was low using early gene constructs and provided little or no insecticidal activity in plant tissue. Later studies conducted with two new gene constructs resulted in higher levels of expression of the ICPFs. We assayed 61 putative transformed embryos and categorized them based on the level of insecticidal activity (high, moderate and low) and/or their effect on larval development. Twenty-one lines expressed ICPFs at levels sufficient to kill most or all of the test insects and stop development. Twelve had moderate expression and 28 had low expression levels. Plantlets from high expression embryos also showed high levels of expression.

Plants derived from the somatic embryos and plantlets demonstrating high levels of expression were planted outside in the spring of 1996 at Parlier, California. However, none of these transformed plants survived through the second year of growth. Plant mortality was attributed to the poor vigor of the trees derived from a relatively old somatic culture. In the Spring of 1998, approximately 70 transformed "third" generation transformed plants derived from embryos/plantlets were planted at Parlier, CA and have survived to the third growing season (2000). We are now preparing to evaluate vegetative and reproductive tissues from these plants to determine their insecticidal activity. Damage will be assessed in the field; levels of insecticidal activity in the transformed plants will be determined in the laboratory.

CONTRIBUTED PAPER - Tuesday, 8:30 (Fungi III)

Differential susceptibility of *Bt*-resistant and *Bt*-susceptible Colorado potato beetle, *Leptinotarsa decemlineata*, to *Beauveria bassiana*

John D. Vandenberg,[1] Michael H. Griggs,[1] Stephen P. Wraight[1] and Leah S. Bauer[2]

[1] USDA Agricultural Research Service, U.S. Plant Soil & Nutrition Lab,
Tower Road, Ithaca, NY 14853 and [2] USDA Forest Service, Center for Integrated Plant Systems, East Lansing, MI 48824

The Colorado potato beetle, *Leptinotarsa decemlineata*, is a perennial pest of potatoes and other solanaceous crops throughout much of the world. *Beauveria bassiana* occurs naturally in this host and can be an effective control agent applied augmentatively or inundatively. One of us (L.S.B.) observed *B. bassiana* infections within a population of *L. decemlineata* resistant to the Cry3a toxin of *Bacillus thuringiensis*. Such infections were rarely observed in the *Bt*-susceptible colony. We wished to test the hypothesis that beetles from this *Bt*-resistant population were more susceptible to infection by *B. bassiana*. We conducted laboratory bioassays using 2 isolates of *B. bassiana*: 1) ARSEF 5813, isolated from *L. decemlineata* from the *Bt*-resistant colony, and 2) Mycotech GHA, a commercially available strain against *L. decemlineata* from 3 sources: 1) a *Bt*-resistant colony, 2) its *Bt*-susceptible wild-type parent colony, and 3) a commercially available colony. We found that *Bt*-resistant beetles were significantly more susceptible to infection by *B. bassiana* 5813 and slightly more susceptible to infection by *B. bassiana* GHA. Isolate 5813 had smaller spores and germinated more slowly than GHA and was less virulent against all 3 test populations. *Bt*-resistant beetles produced smaller egg masses and larvae grew more slowly than wild-type beetles. These findings suggest that the greater susceptibility of the *Bt*-resistant beetles is due to their slower growth rate. These results demonstrate the potential of using *B. bassiana* as an alternative management tool for populations of this pest expressing *Bt* resistance.

Beetles were reared on that had been selected for resistance to the Cry3a toxin expressed in transgenic potato plants through XXX generations.

Strain 5813 was significantly more virulent against Bt-resistant beetles

Strain 5813 has smaller spores and slower germination (at 16 h)

Strain	Assay	Res. LC50	Sus.LC50	Intercept diff?
	Slope diff?			
5813 2	38	230	yes	no
3	35	241	yes	no
4	7	90	yes	no
5	57	316	yes	no
GHA 4	4	14	no	no
5	13	34	yes	no

STUDENT PAPER - Tuesday, 9:30 (Viruses II)

Three major structural proteins of White Spot Syndrome Virus have evolved by gene duplication

Marielle C.W. van Hulten, Fokko Zandbergen, Marcel Westenberg, Stephen D. Goodall and Just M. Vlak

Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, the Netherlands

White spot syndrome is a worldwide disease of penaeid shrimp. The disease agent, white spot syndrome virus (WSSV) is unclassified taxonomically, but resembles baculoviruses based on its morphology. The virion is a bacilliform to ovoid enveloped particle with a rod-shaped nucleocapsid. To study the taxonomic position of the virus the structural proteins were analyzed and compared to those of baculoviruses. Two major structural proteins of 28 kDa (VP28) and 19 kDa (VP19) were identified in the envelope and two proteins of 26 kDa (VP26) and 24 kDa (VP24) in the nucleocapsid. The N-terminal amino acids of these proteins were obtained by protein sequencing and used to locate the genes for these proteins on the WSSV genome. The open reading frames of the three largest proteins (VP28, VP26 and VP24) were all about the same size coding for proteins with theoretical sizes of around 22 kDa. To confirm that these open reading frames are coding for the major structural proteins, they were expressed in insect cells using baculovirus vectors and analyzed by Western analysis. The expression products of these putative structural proteins were the same size as their counterparts in the WSSV virion. They also reacted with a polyclonal WSSV antibody, confirming their virion origin. No serological relationship was found between baculovirus and WSSV structural proteins and also the sequence showed no homology to other proteins available in the GenBank. Surprisingly, statistically significant similarity was found between these three structural protein sequences. Further research showed that VP28, VP26 and VP24 have most probably evolved by gene duplication. The most surprising observation is that these proteins have diverged to give proteins with different functions in the WSSV virion, i.e. envelope and nucleocapsid. This unique feature supports our proposition that WSSV might be a representative of a new virus genus (Whispovirus) or perhaps a new family (Whispoviridae).

POSTER VP18 - Tuesday (Viruses)

Effect of sublethal dosages of *Granulovirus* (Baculoviridae) on *Spodoptera littoralis* (Lepidoptera, Noctuidae)

E. Vargas-Osuna and A. Fernández-Vilchez

Entomología Agrícola y Forestal. Departamento de Ciencias y Recursos Agrícolas y Forestales. E.T.S.I.A.M. Universidad de Córdoba. Apartado 3048. 14080 Córdoba. Spain

The *Granulovirus* (GV) was isolated from *Spodoptera littoralis* infected larvae field-collected in Egypt and obtained from Natural Resources Institute (Kent, UK) as a purified suspension of occlusion bodies (OBs). A dosage determined to provide 50% mortality was used to inoculate fifth-instar *S. littoralis* larvae by the leaf-disc bioassay method. Lethally infected larvae died in the last instar (L6) and showed an extended developmental time, ranging 10 to 30 days after GV treatment.

Both male and female survivors of the larval inoculation exhibited a significantly longer developmental time in the pupal phase compared with control larvae. No significant differences were found in fecundity and egg viability between pairs in which male or female came from GV treated larvae and control pairs. Adult longevity was not significantly affected by the GV treatment. A low level of GV mortality was recorded in the progeny of the adults which had developed from treated larvae

The slow larval infection caused by the GV isolate in *S. littoralis* larvae was associated to the mechanisms that aid to the long-term reduction of the *S. littoralis* populations.

CONTRIBUTED PAPER - Monday, 15:00 (Fungi II)

Steam-exploded agricultural wastes as a novel source of nutrients for production of *Metarhizium anisopliae.*

Larry Vaughan[1] and Herman Warren[2]

Office of International Research and Development[1] and Department of Plant Pathology, Physiology, and Weed Science[2]. Virginia Tech, Blacksburg, VA 24061 USA

The steam-explosion of cellulose wastes results in simple sugars that can be used by microorganisms for growth and reproduction. However, other by-products such as acetic acid, dehydrated pentoses, and furfural can inhibit microbes. Peanut shells, corn cobs, and rice hulls each underwent steam explosion treatment at three different severities. Higher severity (higher steam/temperature combination) typically corresponds to greater breakdown of the cellulose and hemicellulose into sugars, but is accompanied by greater production of inhibitory organic acids. Growth and germination of *Metarhizium anisopliae* (IMI 330189) and *Aspergillus niger* was inhibited by native steam-exploded material. However, pH-adjusted extracts did not inhibit growth and conidiation of *M. anisopliae* and common saprophytes in paper disk assays. *M. anisopliae* was found to grow well and conidiate on steam-exploded peanut shells and rice hulls, but not corn cobs. Steam explosion may provide a means for economically transforming commonly available waste products from food processing into media for fermentation of entomopathogenic fungi.

POSTER FP24 - Thursday (Fungi)

Characterisation of native entomophthoralean fungi associated with *Plutella xylostella* (Lepidoptera: Plutellidae) in the Bajio region, Guanajuato, Mexico.

José Luis Velasco-Silva[1], Raquel Alatorre-Rosas[1], Judith K. Pell[2] and Ariel Guzman Franco[1]

[1]Instituto de Fitosanidad, Colegio de Postgraduados, Km 36.5 Carretera Mexico-Texcoco, CP56230 Montecillo, Edo. México.
[2]Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK.

Crucifers, particularly broccoli and cauliflower, are the most important crops in the Bajio region of Guanajuato, Mexico and are grown on a large scale for both the local and the international market. The diamond back moth, *Plutella xylostella*, is the most serious insect pest on these crops. In addition to causing direct feeding damage, significant economic losses are incurred if they contaminate the high quality frozen commercial products destined for export. Various strategies are under development for *P. xylostella* control, including the use of biological control. Entomopathogenic fungi have great potential to regulate this pest, particularly species from the order Entomophthorales (Zygomycotina) that have been recorded causing epizootics in *P. xylostella* populations in other countries. In 1997, entomophthoralean fungi were recorded, for the first time, from *P. xylostella* in the northern region of the state of Guanajuato. A sampling programme was established to define the natural incidence of these fungi in Guanajuato state and to identify, isolate and characterise the isolates. Isolates were characterised with respect to their growth and sporulation at temperatures between 5° and 37°C (90% RH, 12:12 light:dark regime) and selected isolates were bioassayed against 3rd instar larvae of *P. xylostella* to compare their virulence. From the *P. xylostella* populations sampled in Guanajuato, 25 strains belonging to *Pandora blunckii*, *Zoophthora radicans* and *Conidiobolus* sp. were isolated. Measurements of growth at different temperatures demonstrated that these isolates grew well between 18° and 25°C but were unable to grow at 37°C. There were some small differences between the isolates with respect to virulence against *P. xylostella*; treated larvae began dying on the second day after inoculation and the lethal concentration (LC50) corresponded to approximately 3 conidia mm^{-2} leaf surface for most isolates. Resting spores were produced in some larvae. Further tests demonstrated that these isolates attacked all larval instars, pupae and adults of *P. xylostella*. We conclude that *P. blunckii*, *Z. radicans* and *Conidiobolus* sp. are present in the Bajio region of Guanajuato, have great potential for control of *P. xylostella* and could be developed as a biological alternative for use in integrated pest management of *P. xylostella*.

POSTER BP57 – Thursday (Bacteria)

Serratia spp and other Enterobacteriaceae active against *Phyllophaga* spp (Coleoptera: Melolonthidae) larvae in Mexico.

Villalobos, F.J.[1], Ramírez-Gama, R.M.[2], Calderón. M.A.[2], Hernández, L.[2], Tenango, J.L.[2] Nuñez-Valdez, M.E.[3]

Facultad de Ciencias Agropecuarias, UAEM[1], Facultad de Química, UNAM[2], Instituto de Biotecnología, UNAM[3].

Root-ingesting soil-dwelling larvae of scarab beetles are important cause of damage to several crops in Mexico. Most strategies used to control these insects have lead to negative environmental consequences. Efforts to manage scarab beetles in Mexico within the framework of a sustainable agriculture are currently being made and entomopathogens have been detected as a crucial component of this approach. Strains of *Serratia entomophila* cause amber disease in *Costelytra zealandica* (White) and have been developed as a successful commercial biological control agent (INVADE™ Monsanto) in New Zealand pastureland. In Mexico, larvae of *Phyllophaga trichodes* from Tamaulipas, *P.anodentata* from Jalisco and *P.blanchardi* from Morelos, have been observed showing symptoms similar to those reported for amber disease. In this work, the isolation and identification of Enterobacteriaceae strains that induce pathogenic symptoms (mortality, amber color and anti-feeding responses) in larvae of *Phyllophaga* from Mexico are presented. The Koch´s postulates have been demonstrated by bioassays using pieces of carrot root coated with these isolates to experimentally infect healthy third instars of *Phyllophaga*. We have found strains of *Serratia* spp to be the causal agent of mortality and anti-feeding effect in *P.trichodes* and *P.blanchardi.* However, amber color has expressed differently in both species of *Phyllophaga* in comparison with the amber color observed in *C.zelandica.* Moreover, a longer post-infection period was required in both species of *Phyllophaga* than is normally observed in *C.zealandica*. Both mortality and anti-feeding responses induced in the insects by *Serratia entomophila* (strain UC9) and *S.proteamaculans* (strain AgR142) infection were significantly higher ($P<0.05$) in comparison with the control treatment in third instars of *P.trichodes*. The *Serratia* strain MOR4.1 isolated from amber diseased third instars of *P.blanchardi* collected in Morelos has also shown pathogenic activity against third instars of *P.trichodes* from Tamaulipas. Strains of *Enterobacter* spp (AIL3) isolated from dead third instars of *P.trichodes* have also shown a significantly ($P<0.05$) higher mortality, amber color and anti-feeding effect in healthy third instars of *P. trichodes* than in the control treatment. It is concluded that more strains of Enterobacteriacea than previously suspected may be active against scarab larvae.

WORKSHOP I - Tuesday, 17:10 (Viruses)

On the taxonomy of white spot syndrome virus (whispovirus): a case study

Just M. Vlak and Marielle C.W. van Hulten

Laboratory of Virology, Binnenhaven 11 6709 PD, Wageningen University, The Netherlands.

White spot syndrome virus (WSSV) cause a major zoonotic disease in penaeid shrimp. WSSV is infectious for a wide range of marine and sweet water crustaceans, including crab and crayfish. The virus predominantly infects tissues of ecto- and mesodermal origin and is most likely transmitted *per os.* The virions have a bacilliform shape with a flagella-like terminal extension at one end. The nucleocapsid is rod-shaped and contains a circular, double stranded DNA of approximately 300 kilobase pairs. Hence, WSSV is among the largest DNA viruses in animals to date. The virus replicates in the cell nucleus, where the virions are also assembled. Due to these characteristics, in particular the rod-shaped morphology, the virus has been previously classified as a non-occluded baculovirus (Francki *et al*., 1991). In the 1995 revision of the Baculovirus family the non-occluded rod-shaped viruses including WSSV were orphaned (Murphy *et al.*, 1995) and this situation pertains to exist in the latest revision (Van Regenmortel *et al.*, 1999).

The taxonomy of baculoviruses is based on the presence of common morphological (rod-shaped virions and occlusion bodies), pathological (disease symptoms) and genetic (DNA genome structure and gene homology) characteristics. Some of these characteristics are shared with WSSV, such as the nuclear replication, the rod-shaped form of the

nucleocapsid and some structural features of the viral DNA (circular, homologous regions). However, others in particular the lack of occlusion bodies and unique genetic characteristics underscore the distinct position of WSSV relative to baculoviruses. WSSV has an estimate of less than 5% of gene homologues with baculovirus genes, whereas members of the baculovirus family share up to half of their genes. Phylogenetic analysis of genes WSSV and baculoviruses have in common, such as those encoding ribonucleotide reductases and protein kinases, show that baculoviruses and WSSV are located in different clades suggesting they have no most recent common ancestor. Finally, the major structural proteins of WSSV share no homology at all with those of baculovirus occlusion body- or budded virus-derived proteins. In this contribution we will present the collective evidence including molecular genetic data to suggest that WSSV is a representative of a new genus or perhaps a new virus family. For this genus we coined the name 'whispovirus'. The position of WSSV relative to the baculovirus family will be discussed from a taxonomic perspective.

POSTER VP11 - TUESDAY (Viruses)

Biodiversity of *Spodoptera litura* NPVs from apparently healthy *Spodoptera litura* larvae collected in Indonesia from 1995 to 1997

Era Wahyuni[1,3,4], Liliane Croizier[3], André Pollet[1,2], Soeprapto Mangoendihardjo[1], Miguel López-Ferber[3] and Guy Croizier[3]

1- Research Center for Biological Control. University of Gadjah Mada (UGM). Yogyakarta. Indonesia. 2- Institut de Recherche pour le Développement (IRD). Paris. France. 3- UMR5087 INRA-CNRS-Université MontpellierII. Saint Christol-lès-Alès. France. 4- RILET Malang. Indonesia

To study the *Spodoptera litura* NPVs (SpltMNPV) present in *Spodoptera litura* larvae in Indonesia and then to appreciate the degree of homogeneity of the viruses in the wild, a survey was undertaken in the main islands of the archipelago namely: Sumatra, Kalimantan, Bali, Lombok, Sulawesi, Iran Jaya, Flores, Maluku and Java. From about 3000 individually-collected larvae, 103 were tentatively classified as positive (infected with NPVs) using Loeffler's staining for the presence of inclusion bodies (IBs). Dot blotting and Southern of the total larval DNA extracted from the positive larvae using type A and type C SpltMNPV DNAs as probes, reduced the number of positive larvae under 20 and indicated the predominance of type C over type A. Oligonucleotide primers were designed to amplify a 627 nt DNA fragment internal to the polyhedrin gene (*polh*). These fragments were sequenced and the sequences found were distributed in three groups named A, B, C corresponding respectively to group IV, II and III of Maeda (Maeda *et al.*,1990). *Polh* from an isolate of SpltMNPV-C from Wuhan China was sequenced as a reference (this work). The 627nt-polyhedrin sequence from 6 larvae present three silent mutations in comparison with the homologous sequence of SpltMNPV polyhedrin gene of the Chinese isolate. The 627nt-polyhedrin sequence of 4 larvae were identical to the homologous sequence of SpliMNPV type B (Croizier & Croizier, 1994). Then 4 larvae were naturally infected with NPV of the group A. This NPV was very closed from SpltMNPV type A. Polyhedrin genes of SpltMNPV type C (Chinese or Indonesian isolates) and SpltMNPV type B (Indonesian isolates) code for identical proteins. On the other hand the DNA restriction profiles of type B and type C SpltMNPV and/or SpliMNPV were quite different from each other. In the DNA sequence analysis of the 627 nt fragments as many as 39 silent mutations differentiated SpltMNPV-C type from -B type. Phylogenetic analysis of SpltMNPV *polh* of the three virus types present in the wild in Indonesia shown that *polh* of the B and C types are very close (polyhedrin tree subgroup II-B) whereas *polh* from SpltMNPV-A is a member of the subgroup II-A polyhedrin tree containing *Spodoptera exigua*-, *Spodoptera frugiperda*- and *Mamestra brassicae*-nucleopolyhedrovirus polyhedrins (Bulach *et al.*, 1999).

CONTRIBUTED PAPER - Thursday, 17:00 (Bacteria V)

The ecology distribution of *Bacillus thuringiensis* and *cry* gene diversity in China

Jin-hong Wang Wei-hui Wu Yue-hua Chen Gai-xin Ren

Department of microbiology, College of Life sciences, NanKai University, Tianjin, P.R.China 300071

1080 samples were collected from pest habitats of dust, soil, dead insect and plant leaves from 27 provinces, municipalities and 4 directed cities all over China. From 406 samples of the collection 965 *Bacillus thuringiensis* (*Bt*) strains were isolated, especially it is the first time to obtain *Bt* isolates from dust and soil samples of Tibet. We found that the ratio of *Bt* isolates in dead insect samples is the highest, then the dust, plant leaves and soil sample respectively. The ecology distribution of *Bt* in dust and soil samples is different between east-half and west-half part of the country. In east-half part of China where most are plain areas, the number of *Bt* isolates from south is lower than north in soil samples, but higher in dust samples. It is interested that the isolation results of west-half part where most are altiplano areas are opposite to that of the east-half part. There are eight mainly parasporal crystal shapes with various sizes can be visualized by microscopic observation, bipyramid shape is the most abundant, the others were spherical, cubical, rectangular, elliptical, pointed, embedded and amorphic irregular shapes in order. Most of the isolates contained at least 2 to 3 crystal shapes, only few strains contain one crystal shape. 221 *Bt* isolates were identified by the PCR method with general primers of *cry*I, *cry*II, *cry*III, *cry*IV and *cry*V genes. It is found that *cry*I gene is the most abundant gene, then the *cry*II, *cry*V and *cry*III genes, they were occupied in 75.6%, 67.9%, 58.4% and 14.5% of the strains respectively, no *cry*IV gene was found, this results is quite different from the former reports of other counties. There are 10 *cry* gene combination types were concluded, the most abundant type is the strains with *cry*I, *cry*II and *cry*V genes; we also found several good combination types which both contain Lepidopteran-toxic *cry*I and/or *cry*V genes and Coleopteran-toxic *cry*III genes in one isolate. The *Bt* strains that contained *cry*I genes were further characterized by additional PCR detection with specific primers of the *cry*IAc, *cry*IC and *cry*IE genes. Among them 20 *Bt* isolates with *cry*IAc, *cry*IC, *cry*II and *cry*V genes were found, the bioassay results showed that one strain *Bt*-15A3 was high toxic to *Heliothis armigera, Spodoptera exigua* and *Plutella xylostella* and has high developing value in future applying.

POSTER VP26 - Thursday (Viruses)

Stably transformed cell lines from *Trichoplusia ni* with baculovirus p35 or SV40 T antigen genes produce high levels of AcMNPV and recombinant proteins

P. Wang[1], G. Li[1], J. Garcia[1], Helge Zieler[2], and R. R. Granados[1]

[1]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853, and [2]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

Two established cell lines from the cabbage looper, *Trichoplusia ni,* BTI TN 5B1-4 (High Five Cells), and BTI MG 1 cells were stably transformed with the baculovirus p35 and the simian virus 40 (SV 40) T antigen (T Ag) genes. P35 inhibits programmed cell death by inhibiting cellular capsases and the T Ag has been used to alter cell differentiation. The *T. ni* cells transformed with p35 were selected and cloned following actinomycin D screening, while the T Ag transformed clones which were cotransfected with a neomycin resistant gene were selected by neomycin resistance. Screening of over 100 selected cell clones demonstrated that cells transformed with p35 were more tolerant to culture stresses and had greater cell viability. These engineered cells containing inserted transgenes showed differential response to wild-type AcMNPV infection and some were high producers of viral occlusion bodies. Expression of a secreted glycoprotein (Secreted Alkaline Phosphatase) and an intracellular protein (Beta Galactosidase) with recombinant baculoviruses demonstrated that some transformed cell lines were superior in protein production in comparison to nontransformed control cells.

SYMPOSIUM I - Monday, 14:00 (Viruses)

Establishment of systemic infection in *Heliothis virescens* by HzSNPV and AcMNPV: A comparison of 'S' versus 'M' strategies.

Jan O. Washburn[1], James E. Wong[2] and Loy E. Volkman[1]

[1] Department of Plant and Microbial Biology, University of California Berkeley, CA 94720-3102

[2] DuPont Agricultural Products, Stine-Haskell Research Center, P.O. Box 30 Newark, DE 19714

Species in the genus *Nucleopolyhedrovirus* (Baculoviridae) traditionally have been divided into the SNPVs and the MNPVs. The S and M designations refer to the number of nucleocapsids within the occlusion-derived virus (ODV) that initiate infection of the insect host; in the SNPVs, the ODV contain a single nucleocapsid, whereas in the MNPVs, the ODV contain from one to many nucleocapsids. We compared infection strategies of the S and MNPVs *in vivo* using recombinants of *Helicoverpa zea* SNPV (HzSNPV) and *Autographa californica* MNPV (AcMNPV) containing *lacZ* driven by the *Drosophila hsp70* promoter. We orally inoculated newly-molted fourth instar larvae of the permissive host, *Heliothis virescens*, with dosages yielding ~85% mortality (HzSNPV = 15 OBs; AcMNPV = 20 OBs) and subsequently monitored the course of primary and secondary infection by elucidation of the reporter gene signal. For both viruses, rates of primary infection of midgut columnar cells (% lacZ positive larvae vs time) were linear and nearly identical during the first 24 hours post inoculation (hpi), but HzSNPV lacZ signals within the midgut were first observed at 4 hpi compared to 10 hpi for AcMNPV. While final mortalities were similar, HzSNPV generated twice as many primary foci, suggesting that midgut cells infected by HzSNPV were less efficient at transmitting BV into the hemocoel and establishing systemic infection. Secondary (= systemic) infection of tracheal epidermal cells servicing the midgut was observed at 10 and 12 hpi, respectively, for HzSNPV and AcMNPV, and at 12 hpi, the proportions of larvae with secondary infections were identical. Rates of secondary infection (% of foci with lacZ in the tracheal epidermis vs time) also were characterized by linear equations having identical slopes and intercepts. Thus, despite the 6 h difference in the onset of primary infection, ODV of both viruses established systemic infection at the same time and rate. At 14 and 16 hpi, respectively, we found evidence that hosts were sloughing midgut cells infected by AcMNPV and HzSNPV. Our results support the hypothesis that the infection strategies of AcMNPV and HzSNPV have been selected for rapid establishment of secondary infection of the tracheal epidermis to counter sloughing of infected midgut cells.

STUDENT PAPER - Monday, 14:15 (Fungi II)

Variability and adaptability in mitosporic fungi selected for biocontrol of insect pests

E Watson, N E Jenkins & M B Thomas

Leverhulme Unit for Population Biology and Biological Control, NERC Centre for Population Biology and CABI Bioscience, Imperial College at Silwood Park, Ascot, Berks, SL5 7PY. Tel: 01344 872 999 Fax. 01491 829 123 E-mail: e.watson@cabi.org

Any commercially produced pesticide, whether chemical or biological, has to satisfy strict criteria prior to registration to ensure its efficacy, safety and regulation. Unlike agrochemicals however, biological control organisms are able to respond to their environment as a result of both their intrinsic phenotypic plasticity and their ability to evolve. This potential population-level response, which is largely unpredictable in the field, can have considerable consequences for the success of a biological control agent, particularly longer-term, as well as for the ability to identify it in the field. This poster provides an introduction to the project which aims to investigate the variability, plasticity and potential for adaptation present in a widely used fungal entomopathogen, *Metarhizium anisopliae*. Possible mechanisms for genetic change, and methods to investigate their significance in pathogens used in biological control such as this species, are considered.

SYMPOSIUM I - Monday, 15:30 (Viruses)

Polydnavirus-mediated alteration of insect physiology

Bruce A. Webb

Department of Entomology
University of Kentucky, Lexington KY 40546-0091

The role of polydnaviruses in their mutualistic associations with parasitic wasps is to suppress the immune system and disrupt other physiological systems of the parasite's lepidopteran host. Polydnaviruses inhibit insect growth and alter development by expressing a limited number of viral genes after parasitization in the absence of virus replication. This 'expressed' gene set is characterized by the presence of gene families whose members are expressed to varying degrees. Our laboratory has pursued functional analyses of the *Campoletis sonorensis* polydnavirus genes by developing bioassays for use in protein purification and by sequencing this genome. Some secreted, virally-encoded proteins, the cys-motif proteins, interact with hemocytes and are thought to alter their morphology and function. Other viral gene families lack signal peptides and are thought to have intracellular, at this point undefined, cellular targets. Protein purification has led to identification and partial purification of a protein inhibiting host protein synthesis at a post-transcriptional level. This protein, host translation inhibiting factor (HTIF), inhibits synthesis of several proteins involved in growth and immunity (e.g. arylphorin, juvenile hormone esterase, lysozyme). Translation of other 'constitutive' host proteins (actin, transferrin) are not affected by HTIF. HTIF may redirect nutritional resources toward the developing parasite and clearly exemplifies a means through which the virus inhibits multiple physiological systems. Genome sequence analysis and homology searches recently identified a third CsIV gene family, the viral innexins or vinexins. The discovery of a viral innexin homolog suggests that polydnaviruses alter gap junctions in infected insects. In general, characterization of polydnavirus function in bioassays and elucidation of structure-function relationships within these genomes indicate that a limited number of genes and mechanisms produce polydnavirus pathologies.

CONTRIBUTED PAPER - Friday, 11:00 (Microsporidia II)

Prevalence and incidence of microsporidia in *Ips typographus* (Col., Scolytidae)

Rudolf Wegensteiner*[1] and Jaroslav Weiser*[2]

*[1] Institute of Forest Entomology, Forest Pathology and Forest Protection, University of Natural Resources-BOKU-Vienna, Austria; *[2] Insect Pathology, Institute of Entomology, Academy of Sciences, Ceske Budejovice, Czech Republic.

Ips typographus is the most serious insect pest in spruce forests of central and northern Europe. In spite of the importance of this bark beetle species there is still a great lack of knowledge about pathogen prevalence, pathogen incidence through a year and about impact of different pathogen species on bark beetle populations.

Adult *I. typographus* were collected from standing, attacked trees or from trap logs. They were collected by hand and dissected in the lab. In addition, log sections were brought to the lab and emerging beetles were dissected after removal. Pheromone traps were used in one locality. In most cases living beetles were dissected, dead beetles were examined in some few cases only.

Special focus of the present study was on observation of prevalence of microsporidia in beetles collected at a given date. In order to gain data on the incidence of pathogens during a year, beetles were examined collected from pheromone traps with regard to time of their approach to the traps. Furthermore, beetles were examined from log sections with regard to time of their emergence from the logs (in lab). Frequencies of microsporidia were compared between beetles from managed forests and natural reserves as well as from a forest conservation area. In addition, infection rates were examined concerning age of beetles.

The current state of knowledge on the phenology of microsporidia in *I. typographus* is presented along with information on their transmission, mode of action and persistence in populations.

CROSS-DIVISION SYMPOSIUM - Friday, 9:10

Surface proteins on stagings of non-insect microsporidians

Earl Weidner

Department of Biology, Louisiana State University. Baton Rouge, Louisiana 70803

Dynactin, a protein which mediates vesicle motility via a dynein-driven motor, is expressed within microsporidian sporoplasms of *Spraguea lophii*. Dynactin is bound to the sporoplasm surface and may

be the means by which the sporoplasms motor up axons to the neuronal cell bodies within the brain of host animals. Desmoplakin protein is expressed within sporophorous vesicles of *Thelphania* sps. and surrounds the spores In the form of desmosomal jackets. Keratin intermediate filaments bind the desosomal elements in the sporophorous vesicles of *Thelohania*. The spore stage of *Spraguea lophii* has clathrin and a kinase within pits near the surface. These proteins and others have been characterized by Western blot analyses and their distribution has been partially worked out. Experiments are currently underway to further define some of the functional adaptations of these proteins for the specific stages of the microsporidian parasites.

SYMPOSIUM II - Monday, 18:10 (Viruses)

The secret life of invertebrate iridescent viruses

Trevor Williams

ECOSUR, A.P. 36, Tapachula 30700, Chiapas, Mexico

Invertebrate iridescent viruses (IIV's) are icosahedral particles with a dsDNA circularly permuted genome. They typically infect invertebrates, especially insects, in damp or aquatic habitats. Their potential as biocontrol agents is considered virtually nil, due to the apparent rarity of IIV-diseased individuals in nature.

There is now increasing evidence, however, that these viruses may show marked differences in virulence. Certain isolates, such as those from blackflies (*Simulium* spp.) have been observed to cause obvious lethal infections in a tiny fraction of the host population and at the same time covert non-lethal infections in a substantial proportion of apparently healthy hosts. It is possible to detect covert infections by PCR and highly sensitive insect bioassay techniques. Recent studies have demonstrated that mosquitoes covertly infected by IIV type 6 showed extended larval development times, smaller adult body size, reduced longevity and fecundity compared to uninfected conspecifics. Other isolates, such as those from *Helicoverpa zea* or *Spodoptera frugiperda*, show only the highly virulent patent form of infection.

I present an overview of recent advances in the study of these viruses focussing on changes in IIV taxonomy, sublethal effects of covert infection, the toxic nature of IIV proteins, genetic variability of IIV populations, stability of IIVs in the environment, host specificity, and the possible routes of transmission of IIVs.

STUDENT POSTER VP34 - Thursday (Viruses)

Molecular characterization of *Adoxophyes orana* granulovirus

Sally Wormleaton and Doreen Winstanley

Department of Entomological Sciences, Horticulture Research International, Wellesborne, Warwick, CV35 9 EF

Adoxophyes orana (Lep. Tortricidae) is an important pest of apples and pears in Europe and Asia. A granulovirus isolated from diseased *A. orana* larvae has potential to control this pest. This English isolate has similar restriction endonuclease fragment profiles to isolates from Japan, Switzerland and Italy. *A. orana* granulovirus (AoGV) is a slow killing GV with a median survival time of 37 days for first instar larvae. A physical map of the AoGV genome has been constructed and hybridizations to the genomes of a fast killing GV (*Cydia pomonella* GV) and another slow killing GV (*Trichoplusia ni* GV) have been carried out. The GV appears to be more similar to *Cydia pomonella* GV that has a genome of similar size. The genome organization of 6 kbp within the granulin region is presented and compared to the equivalent area in other GVs. Within this region are homologues of granulin, protein kinase, *me*53 and *egt*. Phylogenetic studies to determine the relatedness of AoGV to other members of the Baculoviridae family will be presented based on these ORFs.

CONTRIBUTED PAPER - Tuesday, 9:00 (Fungi III)

Persistence of *Beauveria bassiana* conidia applied to dorsal versus ventral surfaces of potato foliage

Stephen P. Wraight and Mark E. Ramos

USDA, ARS Plant Protection Research Unit; US Plant, Soil and Nutrition Laboratory, Ithaca, NY 14853

Conidia of *B. bassiana* GHA were applied to potato foliage in research plots near Ithaca NY during the 1997–99 field seasons and monitored for persistence. Unformulated conidia (applied 1998) and conidia formulated as a clay-based wettable powder (applied 1997) and oil-based emulsifiable suspension (applied 1999) were applied by hand-held atomizing sprayer to the point of runoff on the dorsal or ventral surfaces of potato leaves (sprays deposited a mean of 15,000 conidia/mm^2). Following application, sample leaves were collected at 24-hr intervals for 3–4 d and thereafter at 2–3 d intervals. In some tests, leaves were also collected 4 h postapplication. Disks were cut from the leaves and washed in a solution of Silwet L-77 (0.01–0.04%) on a mechanical shaker. Removal of spores was confirmed by fluorescence staining and microscopic examination. Wash samples were serially diluted and plated on Sabouraud dextrose agar with 10 g yeast extract and 100 mg gentamicin sulfate per liter (SDAY+G) for CFU counts. Small droplets of undiluted suspension were applied to SDAY+G plates, incubated for 16 hr at 25 C and examined at 400x to determine viability of conidia. Finally, the wash samples were stained with acid fuchsin (0.1mg/ml), and conidia were counted by hemacytometer at 400x. As expected, viability of conidia recovered from leaf dorsal surfaces declined more rapidly than viability of conidia from ventral surfaces. For example, during the initial 3 d postapplication, viability of conidia applied in 1997 to the dorsal versus ventral surfaces declined at the rate of 54% vs only 6% per day, respectively. Respective rates from the 1998 test were 67 vs 17% per day. Despite the greater survival of inoculum on the ventral surfaces, hemacytometer evaluations indicated high rates of spore loss from both leaf surfaces. Assessments for 1997-98 revealed losses of total viable conidia from dorsal surfaces at a mean daily rate of 71%. Nearly all of this loss (86%) was attributable to loss (physical removal) of conidia. On the ventral surfaces, viable conidia were lost at a daily rate of 46%, with 89% attributable to removal. Loss rates were high during even the initial 4 h postapplication and during stable weather conditions. Interestingly, tests in 1999 indicated that conidia applied via oil-in-water emulsions were also rapidly lost from foliage.

POSTER BP27 - Tuesday (Bacteria)

Investigations of Cry4A toxin structure and function

Masashi Yamagiwa[1], Tohru Komano[2], and Hiroshi Sakai[1]

[1]Department of Bioscience and Biotechnology, Okayama University, Okayama 700-8530, Japan

[2]Department of Genetic Engineering, Faculty of Biology-oriented Science and Technology, Kinki University, Wakayama 649-64, Japan

Cry4A toxin is a dipteran-specific insecticidal protein produced by *Bacillus thuringiensis* subsp. *israelensis*. The mode of action of dipteran-specific δ-endotoxins is poorly elucidated compared with lepidopteran-specific ones. Structure-function interpretation of Cry4A was investigated. We previously reported the active form of Cry4A is a heterodimer of the 20- and 45-kDa fragments. The 20-kDa fragment of activated Cry4A consists of α1, α2, α3, α4, and α5 helices of domain I, and the 45-kDa fragment contains α6 and α7 helices of domain I, domain II, and domain III according to the amino acids sequences alignment of Cry1Aa, Cry3A, and Cry4A. Several deletion mutants were constructed to investigate the functions of these two subuints of activated Cry4A. *In vitro* coprecipitation experiments revealed that the N-terminal 74 amino acids including α6 and α7 helices of domain I was important for the association of the 20- and 45-kDa subunits of Cry4A. It also suggested that α4 and α5 helices were not involved in the association of the two fragments. The mutant that lacked α4 and α5 helices had no insecticidal activity against *C. pipiens* larvae. The binding property of activated Cry4A to the membrane of midgut epithelial cells of mosquitoes was also investigated by both *in vitro* and *in vivo* binding experiments. Cry4A bound specifically to the apical microvilli of larval midgut epithelial cells in the gastric caecae and in the posterior midgut of mosquito by immunohistochemical staining. The data of binding assay using the brush border membrane vesicles purified from larval midgut of mosquitoes will be also discussed.

POSTER VP6 - Tuesday (Viruses)

Establishment of a new cellline having high phagocytic ability from hemocytes of the beet armyworm, *Spodoptera exigua,*

Chisa Yasunaga-Aoki[1], Takeshi Kawarabata[1], Kazuhiro Iiyama[1], and Shigeo Imanishi[2]

[1] Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
[2] National Institute of Sericultural and Entomological Science, Tsukuba 305-8634, Japan

Lepidopteran cell lines have been derived from a variety of tissues including ovaries, embryos, hemocytes, and fat bodies. For the purpose of obtaining a highly phagocytic cell line, we selected a hemolymph of the beet armyworm larvae and a new hemocyte cell line, *Spodoptera exigua* SeH920-1a, could be established by supplementing the culture medium with a reduced form of glutathione to avoid the activation of prophenoloxidase cascade.

To evaluate a phagocytic ability of the *S. exigua* SeH920-1a cells, two different sizes of polystyrene microspheres (6.14 ± 0.45 µm and 2.84 ± 0.14 µm in diameter each) and inactivated spores of entomopathogenic microsporidium, *Vairimorpha* sp. NIS M12, (5.10 ± 0.21 µm x 2.00 ± 0.11 µm) were respectively mixed with the cell culture. The *S. exigua* SeH920-1a cells maintained higher phagocytic ability against foreign particles than the other lepidopteran cell lines which were not derived from the hemocytes. When microsporidian spores were inoculated, 27% of phagocytotic cells in a *S. exigua* SeH920-1a cell line were observed to take up 1.7 spores per cell on the average.

SYMPOSIUM II - Tuesday, 8:15 (Bacteria)

***Bacillus sphaericus* in the environment**

Allan A. Yousten

Microbiology Section, Biology Department, Virginia Tech, Blacksburg, VA 24061

B. sphaericus is a heterogeneous species of bacteria containing both mosquito pathogens and non-pathogens. These bacteria form round spores in a terminally swollen sporangium. At least five, and likely more, DNA similarity groups are present, but all of the pathogens that have been examined belong in a single, closely related group designated IIA. The members of this group are identified by pathogenicity, probe or PCR and will likely be assigned a new species name. Pathogenic strains have been isolated from soil, water, and dead mosquito larvae. This strict aerobe has rather limited catabolic capabilities and fails to metabolize glucose, several other common sugars, or starch. Deamination of amino acids results in a final alkaline pH under most growth conditions. Pathogenicity is due to the production of any or all of three types of protein toxins. Both pathogens and non-pathogens have been serotyped, but serotyping is generally not predictive of the level of toxicity. The gene for the binary toxin is located on the chromosome rather than on a plasmid. The formation of the binary toxin only by strains of similarity group IIA suggests that horizontal gene transfer may be restricted to closely related strains within this species. The formation of multiple, unrelated insecticidal proteins by this species is a remarkable feature shared with *B. thuringiensis*. Although spores of the pathogenic strains germinate and recycle in dead mosquito larvae, spores fail to germinate and are eliminated from the guts of non-target aquatic insects. There are no reports of epizootics caused by B. sphaericus under naturally occurring conditions.

SYMPOSIUM II - Tuesday, 10:30 (Bacteria)

Biodiversity of entomopathogenic sporeforming bacteria

Allan A. Yousten

Microbiology Section, Biology Department, Virginia Tech, Blacksburg, VA 24061

Entomopathogens are found among four different genera of sporeforming bacteria. Some of these bacteria are pathogenic because of their invasiveness and others because of the production of one or more toxins. Among the toxin-producers, the best known is *Bacillus thuringiensis*. This bacterium produces four well-characterized, unrelated toxins (delta endotoxin, beta exotoxin, CYT toxin, VIP toxin) affecting various insects by different mechanisms. This species has been isolated from a wide variety of ecological niches worldwide. *Clostridium bifermentans*, the only anaerobic sporeforming species recognized as an insect pathogen, produces a toxin related to the delta endotoxins of *B. thuringiensis*. It has been isolated only from a single geographic area. *Bacillus sphaericus* is a heterogeneous species containing both pathogenic and non-pathogenic strains. The pathogens may produce up to three distinct toxins (binary toxin, Mtx, Mtx2/3) which affect only mosquito larvae. The pathogens have been isolated from dead larvae and also from aquatic sites and even from soil. The commercialized strain (2362) was isolated from an adult black fly although *B. sphaericus* is not pathogenic for that insect. *Brevibacterium laterosporus* produces an uncharacterized toxin that is active against mosquitoes and black flies. The invasive sporeforming entomopathogens are all in the genus *Paenibacillus*. *P. popilliae* and *P. lentimorbus* are pathogens producing milky disease in various scarab larvae, and *P. larvae* subsp. larvae is the causative agent of American foulbrood of honeybees. *P. popilliae* and most isolates of *P. lentimorbus* produce parasporal bodies containing a protein related to the delta endotoxins of *B. thuringiensis.* However, there is as yet no direct evidence that this protein is related to the pathogenicity of these bacteria. The scarab pathogens have only been found to sporulate within the diseased larvae, and isolations have been made almost exclusively from dying or dead larvae. The presence of delta endotoxin-like proteins in three different genera suggests considerable horizontal gene transfer among these bacteria.

CONTRIBUTED PAPER - Monday, 12:00 (Bacteria I)

A molecular chaperone from *Bacillus thuringiensis* triggering the expression of cryptic ICP-genes

Jianxiu Yu and Yi Pang

State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou, 510275, People's Republic of China.

Molecular chaperones control protein structure, function, localization and transportation. A novel chaperone-like *p21zb* gene was cloned and characterized from a new strain of *Bacillus thuringiensis*. As expected, *p21zb* enhanced the net yield of insecticidal crystal proteins (ICPs), but it was found that this gene had a striking characteristic of triggering the expression of cryptic (or "silent") ICP-genes and helping the formation of crystals when it was transformed into three acrystalliferous strains of *B. thuringiensis.* Bioassay results showed that the trigger-expressed ICPs produced by *p21zb*-transformed *B. thuringiensis* strains were toxic to insects and/or wider insect-killing range than their parental strains. PCR and reverse transcription (RT)-PCR demonstrated that P21zb activated the mRNA transcription of cryptic ICP-genes. These findings suggest that it may open up a new opportunity for learning more about the functions of molecular chaperones, and that employing this kind of molecular chaperone genes we can construct a range of broad-spectrum toxic and over-producing engineered strains of *B. thuringiensis* as the novel insecticides.

POSTER BP53 - Thursday (Bacteria)

Cloning and Subcloning of the *cyt* Gene of *Bacillus thuringensis subsp.israelsis TN-189*

Li Yubing, Chen Yuehua, Ren Gaixin

Department of Microbiology, Nankai University, Tianjin, 300071, P.R.China

Bacillus thuringensis subsp. israelensis (Bti) TN-189 is one of the isolates in our lab, which has high toxicity to the dipteran insect such as *Cudex pipiens, Aedex albopictus* and *Scrara sp*.. Primers were designed according to the sequences of the cyt1A gene in *Bti.* Using PCR technique a 982bp fragment of the *cyt* gene was obtained in 189, the size of which is identical as that in *Bti*. The PCR product was inserted into PbluescriptSk (+) and transformed into *E. coli* JM109. Then the *cyt* gene was subcloned into the shuttle vector PHT315 and transformed into *E.coli* JM109. The positive recombinant was gained by selecting the white

clone. Through the hemolytic assay and detecting O.D value variation of the broth during the growth of recombinant the results showed that *cyt* gene was expressed by IPTG inducing, however, the level of expression is low. The recombinant has no apparent toxicity to the mosquito C. *pipiens*.

Key words: *Bacillus thuringensis subsp. israelensis TN-189, cyt* gene, clone, expression

POSTER VP35 - Thursday (Viruses)

Euprosterna elaeasa virus: a member of a new group of insect RNA viruses

Jean-Louis Zeddam(1), Fiona Pringle(2), Karl Gordon(3), Vernon Ward(2) and Terry Hanzlik(3)

1: IRD, 213, rue La Fayette, 75483 Paris Cedex 10 (France)
2: Department of Microbiology, Otago University, P. O. Box 56, Dunedin (New Zealand)
3: CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601 (Australia)

In Peru, massive mortalities periodically reported in the wild populations of the oil-palm pest *Euprosterna elaeasa* (Lepidoptera; Limacodidae) were found to be caused by a small isometric virus. Its efficacy suggested that the virus could be used as a biological control agent in pest management programs to greatly reduce the need for chemical pesticides which are overused in industrial plantations. From this perspective, a detailed characterization of the new virus (named EeV) was undertaken.

Electron microscopy observations showed that the viral particles were icosahedral with a diameter of about 40 nm; many of them were exhibiting a central white core. Two major proteins (58.8 and 6.8 kDa, respectively) were found using denaturing polyacrylamide gel electrophoresis. The viral genome was found to be a non-polyadenylated single-stranded RNA of positive polarity whose complete sequence was determined. The 5698 nucleotides-long RNA (52.1% GC content) contains two overlapping ORFs. The larger one (ORF1), located at the 5' end, encodes a protein of 1257 amino-acids with a calculated molecular weight of 140.5 kDa which is the viral replicase (or RNA-dependent RNA polymerase, RdRP). The second gene (ORF2) is in the +1 reading frame relative to the first one, starts at nucleotide 3348 and produces the coat protein precursor. Comparison of the ORF2 sequence with the capsid gene of *Thosea asigna* virus, a presently unassigned member of the family *Tetraviridae* (Pringle *et al* (1999) J. gen. Virol. 80:1855-63), shows very strong homology (78% at the amino-acid level). Although an antigenic relationship has been detected between EeV and *Nudaurelia* β virus (NβV, the type virus of the genus *Betatetravirus*, family *Tetraviridae*), there is only limited amino-acid sequence homology (34%) between the NβV and EeV capsid proteins. Moreover, EeV's ORF1 appears to be quite different to the corresponding region in NβV and has no significant homology to previously sequenced tetraviral RdRps. It also completely lacks a helicase domain.

In conclusion, although the bio-physical and serological properties of EeV as well as the overall genome organization appeared very similar to those reported for the betatetraviruses, the sequencing of its genome established that, in fact, this virus should be considered as a member of a new group of insect viruses. Furthermore, as suggested by Pringle *et al. (1999)*, these results should lead to a re-examination of the current status of the viruses assigned to the genus *Betatetravirus* because there are no sequence data presently available for the majority of them. Finally, genome comparison could also help in clarifying the taxonomic relationships existing between these two groups of RNA viruses.

POSTER BP54 - Thursday (Bacteria)

Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C

J. Z. Zhao[1] H. L. Collins[1] J. D. Tang[1] J. Cao[2] E. D. Earle[2] R. T. Roush[3] S. Herrero[4] B. Escriche[4] J. Ferré[4] A. M. Shelton[1]

[1]Dept. of Entomology, Cornell University, NYSAES. Geneva, NY 14456;
[2]Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853;
[3]Dept. of Crop Protection, Waite Institute, South Australia 5064, Australia;
[4]Dep. Genética, Universitat de Valencia, 46100-Burjassot (Valencia), Spain

Although several insect species have developed resistance to *Bacillus thuringiensis* (Bt) formulations or toxins, there are only three reported species for which resistant strains can survive on Bt transgenic insecticidal plants. In the resistant strains of the three insect species that can survive from the neonate to adult stage on Bt plants, resistance did not develop directly from selection on Bt crops. We selected a field-collected colony of the diamondback moth (DBM), *Plutella xylostella,* which had 31-fold resistance to Cry1C, using Cry1C protoxin and transgenic broccoli expressing a Cry1C protein. After 26 generations of selection the resistance ratio of this strain to Cry1C protoxin was 12,391- and 63,124-fold, respectively, for the neonates and second instars using a leaf dip assay. The neonates of the resistant strain could complete their entire life cycle on transgenic broccoli expressing high levels of Cry1C. The resistance remained stable until G34 under continuous selection, but decreased to 2,760-fold at G34 when selection ceased at G28. The Cry1C resistance in this strain was inherited as an autosomal and incompletely recessive factor when evaluated using a leaf dip assay, and as a recessive factor when using Cry1C transgenic broccoli. More than one locus is probably involved in the Cry1C resistance in this strain.

Saturable binding of ^{125}I-Cry1C was found with brush border membrane vesicles (BBMV) from both susceptible and Cry1C resistant strains. Significant differences in Cry1C binding to BBMV from the two strains were detected. BBMV from the resistant strain had about 7-fold lower affinity for Cry1C and 3-fold higher binding site concentration than BBMV from the susceptible strain. The overall Cry1C binding affinity was just 2.5-fold higher for BBMV from the susceptible strain than from the resistant strain. These results suggest that reduced binding is not the major mechanism of resistance to Cry1C. More studies are needed for examining other potential mechanisms. We are also testing the cross-resistance pattern for the Cry1C resistance.

STUDENT POSTER BP55 - Thursday (Bacteria)

Protein-Protein Interactions May Reveal New Roles for a Novel Insect Protein

Meibao Zhuang[1], Hyun-Ku Lee[2], Sarjeet S. Gill[3]

Environmental Toxicology Graduate Program[1,3] & Department of Cell Biology and Neuroscience[3], University of California, Riverside, CA 92521
The Scripps Research Institute[2], 10550 N. Torrey Pines Road, La Jolla, CA 92037

p85 is a novel protein found in *Heliothis virescens*, *Drosophila melanogaster* and human. The *H. virescens* p85 (Hvp85) has high affinity to the Cry1Ac toxin from *Bacillus thuringiensis*. Although both *Drosophila* p85 (Dmp85) and human p85 (Hp85) have been identified as components of the SWI/SNF-like transcriptional activation complexes, their actual cellular functions are yet to be elucidated. Interestingly, Hvp85 binds four-Way Junction (4-WJ) DNA, while Cry1Ac prevents its DNA-binding ability. Using the yeast two-hybrid and the co-immunoprecipitation assays, we demonstrated that Hvp85 and Dmp85 interact with *Drosophila* Deep Orange (Dor), a protein that plays an important role in lysosomal transport. The C-terminus of Dor is crucial for its function, while the C-terminus of Dor binds p85 stronger than the full-length Dor. By using histochemical and mutational studies, we will attempt to reveal the possible roles of p85 in protein transport and its roles in Cry1Ac toxicity.

INDEX OF AUTHORS

H

I

J

K

L

M

N

O