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SIP 2002
FOZ DO IGUASSU - BRAZIL

Society For Invertebrate Pathology

VIII International Colloquium on
Invertebrate Pathology and Microbial Control (ICIPMC)

XXXV Annual Meeting of the SIP

VI International Conference on Bacillus thuringiensis (ICBt)

ogram and Abstracts

August 18 to 23, 2002

Embrapa

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Documentos 183

VIII International Colloquium on Invertebrate Pathology and Microbial Control

VI International Conference on *Bacillus thuringiensis*

35th Annual Meeting of the SIP

Foz do Iguassu, Brazil
18 - 23 August 2002

PROGRAM AND ABSTRACTS

Society for Invertebrate Pathology

SIP

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GENERAL PROGRAM GRID

SUNDAY, AUG. 18	MONDAY, AUG. 19	TUESDAY, AUG. 20
COUNCIL MEETING Room Araucaria **8:30 - 17:00** **REGISTRATION** Bourbon Convention Center **10:00 - 17:00** **HOTEL CHECK-IN** Hotel Front Desk	**REGISTRATION** **OPENING CEREMONY** Room: Cataratas Welcome: SIP 2002 Chairman SIP President **FOUNDERS' LECTURE** Honoree: Dr. Huguette de Barjac Introduction: Dr. Dudley Pinnock Founders' Lecturer: Dr. Peter Luethy **8:30 - 10:00**	**CONTRIBUTED PAPERS** Microbial Control 1 (Iguaçu I) Bacteria 2 (Cataratas I) **SYMPOSIA** Fungi 2 (Iguaçu II): Microecology of Entomopathogenic Fungi Virus 2 (Cataratas II): Prospects for the Use of Viral Pesticides **8:00 - 10:00**
	COFFEE BREAK 10:00 - 10:30	COFFEE BREAK 10:00 - 10:30
	PLENARY LECTURES Room: Cataratas Baculoviruses and the Bonus of Biotechnology 1. Baculovirus genetics and gene regulationD. A. Theilmann 2. Improvements in insect cell culture for recombinant protein production. R. R. Granados 3. Baculovirus molecular pathology. L. E. Volkman 4. Engineered baculovirus insecticides. V. Romanowski **10:30 - 12:30**	**CONTRIBUTED PAPERS** Microsporidia 1 (Iguaçu I) Bacteria 3 (Cataratas I) **SYMPOSIUM** Nematodes 1 (Iguaçu II): Entomopathogenic Nematodes: Current Status **WORKSHOP** The Future of Scientific Publications (Cataratas II) **10:30 - 12:30**
	LUNCH 12:30 - 14:00	LUNCH 12:30 - 14:00
	CONTRIBUTED PAPERS Bacteria 1 (Cataratas I) Nematodes 1 (Iguaçu I) **SYMPOSIA** Fungi 1 (Iguaçu II): Toward the Integration of Fungal Entomopathogens with Other Biological Control Agents Viruses 1 (Cataratas II): Arthropod-borne Virus **14:00 - 16:00**	**POSTER SESSION II** **14:00 - 16:00** **WORKSHOP** **Poster Format - Poster Session II** Microsporidia: Techniques in Microsporidia Research **14:00 - 16:00**
	COFFEE BREAK 16:00 - 16:30	COFFEE BREAK 16:00 - 16:30
	POSTER SESSION I **16:30 - 18:30**	**CONTRIBUTED PAPERS** Fungi 1 (Iguaçu I) Virus 1 (Iguaçu II) **SYMPOSIA** Bacteria 1 (Cataratas I): Bacterial Insecticidal Proteins: Specificity, Improvement and Novel Toxins Fungi 3 (Cataratas II): Genetic Structure of Fungal Populations **16:30 - 18:30**
	DINNER 18:30 - 20:00	DINNER 19:00 - 20:00
MIXER **18:30 - 20:30**	**DIV. BUSINESS MEETING** Fungi (Iguaçu I) Virus (Cataratas I) Bacteria (Cataratas II)) Nematodes (Araucária) Microsporidia (Ipê) **20:00 - 22:00**	**DIV. BUSINESS MEETING** Microbial Control (Cataratas II) **20:00 - 22:00**

GENERAL PROGRAM GRID

WEDNESDAY, AUG. 21	THURSDAY, AUG. 22	FRIDAY, AUG. 23
CONTRIBUTED PAPERS Fungi 2 (Iguaçu I) Virus 2 (Iguaçu II) 8:00 - 10:00 **SYMPOSIUM** Cross-Division 1 (Cataratas I): Bacteria /Insect Interactions: Virulence Aspects **8:00 - 11:00** (NO COFFEE BREAK) **WORKSHOP** Bioinsecticide Production Issues, with a Focus on Latin America (Cataratas II) **8:00 - 10:00**	**CONTRIBUTED PAPERS** Fungi 3 (Iguaçu I) Virus 3 (Iguaçu II) **SYMPOSIA** Bacteria 2 (Cataratas I): Bt Transgenic Plants and Insect Resistance to Bt Toxins Microbial Control 1 (Cataratas II): Solar Irradiation of Fungal Pathogens: Deleterious Effects, and Mitigation through Genetics and Formulation **8:00 - 10:00**	**CONTRIBUTED PAPERS** Fungi 4 (Iguaçu I) Microsporidia 1 (Iguaçu II) **SYMPOSIA** Bacteria 3 (Cataratas I): Bti and Bsh Mosquitocidal Strains: Use and Necessities **9:00 - 11:00**
COFFEE BREAK 10:00 - 10:30	COFFEE BREAK 10:00 - 10:30	
SYMPOSIA Cross-Division 2 (Iguaçu I): Microbial Germplasm Repositories: The Legacy, the Problem, the Future Nematodes 2 (Iguaçu II): Entomopathogenic Nematodes: Research Trends **10:30 - 12:30**	**GENERAL BUSINESS MEETING** **10:30 - 12:30**	HAVE A GOOD TRIP BACK! (BOA VIAGEM!) SEE YOU IN VERMONT, USA
LUNCH 12:30 - 14:00	LUNCH 12:30 - 14:00	
EXCURSION **14:00 - 18:00**	**SYMPOSIA** Cross-Division 3 (Iguaçu II): Microsporidia within Entomophthorales **14:00 - 16:00** Microbial Control 2 (Cataratas II): Microbial Control of Insect Pests of Potato - from Tierra del Fuego to the Great White North **14:00 - 17:00** **WORKSHOP** Ethics, legal and regulatory concerns of transgenic plants (Cataratas I) **14:00 - 16:00**	
	COFFEE BREAK 16:00 - 16:30	
	CONTRIBUTED PAPERS Virus 4 (Iguaçu II) Bacteria 4 (Cataratas I) **16:30 - 18:30** **WORKSHOPS** Preservation of Entompathogenic Fungi (Iguaçu I) Microbiol Control of the Coffee Berry Borer by Entomopathogens Fungi (Cataratas II) **16:30 - 18:30**	
BARBECUE **18:30 - 21:30**	**BANQUET** **19:30**	

Society for Invertebrate Pathology

VIII International Colloquium on Insect Pathology and Microbial Control (ICIPMC)

VI International Conference on *Bacillus thuringiensis* (ICBt)

SIP 35th Annual Meeting

Foz do Iguassu, Brazil - 18-23 August 2002

PROGRAM

SUNDAY, AUGUST 18

SIP COUNCIL MEETING, Room Araucaria
8:30 - 17:00

REGISTRATION, Bourbon Convention Center
10:00 - 17:00

HOTEL CHECK-IN, Hotel Bourbon Front Desk
All day

MIXER
19:00 - 21:00

MONDAY, AUGUST 19

REGISTRATION, Bourbon Convention Center
10:00 - 17:00

OPENING CEREMONY
8:30 - 10:00
Room: Cataratas

Welcome: Flávio Moscardi, SIP 2002 Chairman
James Harper, SIP President
Embrapa Representative

Founders' Lecture
Honoree: Huguette de Barjac
Introduction: Dudley Pinnock
Founders' Lecturer: Peter Lüthy

Coffee Break
10:00 - 10:30

Monday, 10:30 - 12:30
Room: Cataratas
PLENARY SESSION
Baculoviruses and the Bonus of Biotechnology
Chair: J. M. Vlak

10:30 Baculovirus genetics and gene regulation. D. A. Theilmann. Pacific Agri-Food Research Centre, Agriculture and Agri-Food, Summerland, BC V0H 1ZO, Canada.

11:00 Improvements in insect cell culture for recombinant protein production. R.R. Granados. Boyce Thompson Institute for Plant Research, Cornell University, Tower Road, Ithaca, NY, 14853-1801, USA.

11:30 Baculovirus molecular pathology. L.E. Volkman. Department of Plant and Microbiology, University of California, 251 Koshland Hall, Berkeley, CA 94720, USA.

12:00 Engineered baculovirus insecticides. V. Romanowski. Instituto de Bioquímica y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Lunch
12:30 - 14:00

Monday, 14:00 - 16:00
Cataratas I
CONTRIBUTED PAPERS - Bacteria 1
Chair: A. Bravo **pgs 33 - 35**

14:00 Hydropathic complementarity determines interaction of epitope ^{869}HITDTNNK^{876} in *Manduca sexta* Bt-R_1 receptor with loop 2 of domain II of *Bacillus thuringiensis* Cry1A toxins. I. Gómez, J. Miranda-Rios, E. Rudiño-Piñera, D.I. Oltean, S.S. Gill, A. Bravo, and M. Soberón. Instituto de Biotecnología, Departamento de Microbiología Molecular, Departamento de Reconocimiento Molecular y Bioestructura. UNAM. Apdo postal 510-3, Cuernavaca, Morelos 62250, México. Department of Cell Biology and Neuroscience, University of California, Riverside, CA 92521, USA.

14:15 A cell–cell signaling peptide activates the PlcR virulence regulon in bacteria of the *Bacillus cereus* group L. Slamti and D. Lereclus. Unité de Biochimie Microbienne, CNRS (URA2172), Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris cedex, France. Unité de Lutte Biologique, INRA, La Minière, 78285 Guyancourt cedex, France.

14:30 The importance of N-terminal activation. A. Bravo, J. Sánchez, T. Kouskoura and N. Crickmore. Instituto de Biotecnología, Universidad Nacional Autonoma de Mexico, Morelos, México and School of Biological Sciences, University of Sussex, Brighton, UK.

14:45 Expression of cadherin-like receptors for Cry1Aa from silkworm in cultured mammalian cells. Y. Tsuda, K. Hashimoto, F. Nakatani, T. Fukada, K. Sugimoto and M. Himeno*. Department of Applied Biochemistry, College of Agriculture, Osaka Prefecture University, 1-1 Gakuen-cyo, Sakai, Osaka 599-8531, Japan (*present address: Faculty of Home Economics, Kobe Women's University, Higashisuma, Suma, Kobe 654-8585, Japan).

15:00 Glycosyltransferases mediate Bt toxin action in *Caenorhabditis legans*. J.S. Griffitts, L.D. Marroquin and R.V. Aroian. Division of Biology, University of California, San Diego, USA.

15:15 Charged residues in Helix-4 of the *Bacillus thuringiensis* Cry4B Toxin are involved in ion channel conductivity: Site directed mutagenesis and molecular dynamics simulations studies. I. Sramala, W. Fischer, M. Sansom, and S. Panyim, C. Angsuthanasombat. Laboratory of Molecular Biophysics, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom, 73170 Thailand Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, UK Laboratory of Molecular Biophysics, University of Oxford, Oxford, OX1 3QU, UK.

15:30 Early transcription of Bacillus thuringiensis cry genes in Lepidoptera-active strains. M. Porcar, C. Martínez, J.E. Ibarra, and V. Juaréz-Pérez. Laboratoire des Bactéries et Champignons Entomopathogènes. Institut Pasteur, Paris, France and Departamento de Biotecnología y Bioquímica, CINVESTAV-I.P.N., Irapuato, Gto. México.

15:45 Addition of the *Bacillus sphaericus* binary toxin to *B. thuringiensis* subsp. *morrisoni* (PG-14) and *B. thuringiensis* subsp. *jegathesan* markedly improves their toxicity. Hyun-Woo Park[1], D.K. Bideshi[1] and B.A. Federici[1,2]. [1]Department of Entomology and [2]Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, Riverside, Riverside, CA 92521, USA.

16:00 *Bacillus sphaericus* resistance: in vitro binding of the binary toxin to *Culex quinquefasciatus* larvae selected with the strain IAB59. M.H. Silva-Filha[1], C.M.F. Oliveira[1], C. Nielsen-LeRoux[2] and L. Regis[1]. [1]Centro de Pesquisas Aggeu Magalhães- FIOCRUZ, Recife, Brazil and [2]Unité de Biochimie Microbienne, Institut Pasteur, Paris, France.

Monday, 14:00 - 15:15
Iguaçu I
CONTRIBUTED PAPERS - Nematodes 1
Chair: P. Grewal **pgs 35 - 37**

14:00 Entomopathogenic nematodes and conservation biological control. C. Hoy, J. Lawrence and P. Grewal. Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University. Wooster, Ohio 44691, USA.

14:15 Adaptation of entomopathogenic nematodes to insect food plant. M.E. Barbercheck, J. Wang and C. Brownie. Dept. of Entomology, and Dept. of Statistics, North Carolina State University, Raleigh, North Carolina 27695, USA.

14:30 Endotoxin activity of lipopolysaccharide produced by *Moraxella osloensis* against the Grey Garden Slug *Deroceras reticulatum*. L. Tan and P.S. Grewal. Department of Entomology, Ohio State University, Wooster, Ohio 44691, USA.

14:45 The Australian clinical isolates of *Photorhabdus* constitute a new taxon. R. Akhurst, C. Beard, P. Jansen and N. Boemare. CSIRO Entomology, Canberra, Australia, University of Melbourne, Melbourne, Australia; INRA, Montpellier, France.

15:00 Endemic entomopathogenic nematodes in diverse vegetable landscapes. J. Lawrence, C. Hoy and P. Grewal. Department of Entomology. Ohio Agricultural Research and Development Center. The Ohio State University. Wooster, Ohio 44691. USA. **(STUDENT PAPER)**

Monday,14:00 - 16:00
Iguaçu II
SYMPOSIUM – Fungi 1: Toward the Integration of Fungal Entomopathogens with Other Biological Control Agents
Convenor: S. P. Wraight

14:00 Interactions between fungi and insect predators. J.K. Pell and H.E. Roy. Department of Entomology and

Nematology, IACR - Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK, and Department of Life Sciences, Anglia Polytechnic University, East Road, Cambridge CB1 1PT, UK.

14:25 Interactions between fungi and insect parasitoids. L.A. Lacey and A.L. Mesquita. Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Road, Wapato, Washington 98951, USA, and EMBRAPA-CNPAT, Rua Dra. Sara Mesquita, N 2270, Bairro Pici, P.O. Box 3761, Fortaleza 60511-110, Ceará, Brazil.

14:50 Interactions between fungi and other entomopathogens. T.R. Glare and T.A. Jackson. AgResearch, P.O. Box 60, Lincoln, New Zealand.

15:15 Interactions between fungi and chemical insecticides. P.M.O.J. Neves, S.B. Alves, J. E.M.de Almeida and A. Moino Jr.. Depto. de Agronomia, Universidade Estadual de Londrina, P.O. Box 6001, Londrina 86051-970, Paraná, Brazil, Depto. de Entomologia, Fitopatologia e Zoologia Agrícola, USP/ESALQ, P.O. Box. 9, Piracicaba 13418-900, São Paulo, Brazil, Instituto Biológico de São Paulo, P.O. Box 70, Campinas 13001-970, São Paulo, Brazil, and Depto. de Entomologia, UFLA, P.O. Box 37, Lavras 37200-000, Minas Gerais, Brazil.

15:40 Discussion

Monday, 14:00 - 16:00
Cataratas II
SYMPOSIUM (Viruses 1) - Arthropod-borne Virus
Convenor: J. Becnel

14:00 Contributions of Invertebrate Pathology to Vector Control. J. J. Becnel, USDA, ARS, CMAVE, 1600 S.W. 23rd Drive, P.O. Box 14565, Gainesville, FL 32604, USA.

14:30 Yellow fever in South America. P.F.C. Vasconcelos, Instituto Evandro Chagas, Av. Almirante Barroso, 492, 66090-000, Belém, PA, Brazil.

15:00 Dengue transmission and *Aedes aegypti* control in Brazil. P.T.R. Vilarinhos, Coordenação de Vigilância de Fatores de Riscos Biológicos, ASDCE/CENEPI, Fundação Nacional de Saúde, Setor de Autarquias Sul lote 04, Bloco N sala 730, 70 058-902, Brasília, DF, Brazil.

15:30 West Nile virus: an exotic emerging pathogen in North America. T.G. Andreadis, The Connecticut Agricultural Experiment Station, 123 Huntington Street, PO Box 1106, New Haven, CT 06504, USA.

15:40 Discussion

16:00 - 16:30 Coffee break

Monday, 16:30 - 18:30
POSTER SESSION I **pgs 37 - 62**

Fungi

FP1 Transformation of *Beauveria bassiana* mediated by *Agrobacterium tumefaciens* using herbicide resistance gene as a selectable marker. W. Fang, Y. Zhang, X. Yang and Y. Pei. Biotechnology Research Center Southwest Agricultural University Beibei Chongqing 400716 China. **(STUDENT POSTER)**

FP2 Cloning and characterization of cuticle degrading enzyme CDEP-1 from *Beauveria bassiana*. W. Fang, Y. Zhang, X. Yang and P. Yan. Biotechnology Research Center Southwest Agricultural University Beibei, Chongqing 400716 China. **(STUDENT POSTER)**

FP3 Kinetics and mechanism of the amyloid-like interfacial self assembly of the hydrophobin Sc3. P.A. Stroud, J.S. Goodwin, C.L. McCormick, G.C. Cannon and P. Butko. University of Southern Mississippi, Hattiesburg, MS 39406, USA.

FP4 Entomopathogenic fungi for white grub control in south of Chile. M. Rodríguez, M.G. Gerding, A. France and M. Gerding. Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Quilamapu, Chillán, Chile.

FP5 Susceptibility of chilean target pests to native entomopathogenic nematodes. A. France, S. Espinoza, and M. Gerding. Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Quilamapu, Chillán, Chile.

FP6 Selection of *Beauveria bassiana* (Bals.) Vuill. colonies resistant to pesticides. R.S. Cavalcanti, A. Moino Jr., G.C. Souza and A.S.M. Duarte. Federal University of Lavras, Department of Entomology, Lavras, MG, Brazil.

FP7 Characterization and mass production of *Paecilomyces tenuipes*, entomopathogenic fungus collected in Korea. S-H. Nam[1], S. Lee[2], I-Y. Jung[1], S.-D. Ji[1] and S.-Y. Cho[1]. [1]Department of Sericulture and Entomology, NIAST, RDA, Suwon 441-400, Korea.

[2]Department of Plant Pathology, University of Stellenbosch P. Bag X1, Matieland 7602, South Africa.

FP8 Effects of temperature on the survival of propagules of the entomopathogenic Hyphomycete *Paecilomyces fumosoroseus* (Wize) Brown and Smith. A.L. Carmona, A. Asaff, O. Gómez, and M. de la Torre. Centro de Investigación y de Estudios Avanzados del IPN, Departamento de Biotecnología y Bioingeniería, México D.F., México. **(STUDENT POSTER)**

FP9 Interactions between two species of mitosporic fungi, larvae of *Musca domestica* and *Stomoxys calcitrans*, and the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae). C. Nielsen[1,2], T. Steenberg[1] and H. Skovgaard[1]. [1] The Danish Pest Infestation Laboratory, Denmark and [2]Department of Terrestrial Ecology, Zoological Institute, University of Copenhagen, Denmark. **(STUDENT POSTER)**

FP10 Mass cultivation of *Nomuraea rileyi* in bioreactors. B. Vertens[1], U. Tuor[2], M. Hassani[2] and U. Baier[3]. [1]Hochschule Anhalt, Germany, [2]Swiss Federal Institute of Technology, ETH, Microbiology, Zuerich, Switzerland and [3]HSW University of Applied Sciences, Waedenswil, Switzerland.

FP11 Effects of aphid-induced plant volatiles on intraguild interactions at the third trophic level. J. Baverstock[1], J.K. Pell[1] and P.G. Alderson[2]. [1]Plant and Invertebrate Ecology Division, IACR-Rothamsted, [2]Division of Agricultural Sciences, University of Nottingham, UK.

FP12 Molecular, morphological, and functional characterization of a Peruvian isolate of *Metarhizium anisopliae* var. *acridum*. B.P. Magalhães[1], M.S. Tigano[1], I. Martins[1], H. Frazão[1] and H.G. Ramirez[2]. [1]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil and [2]SENASA, Lima, Peru.

FP13 Attenuation of fungal infection in thermoregulating locusts, *Locusta migratoria*, is accompanied by changes in hemolymphal proteins. R.M. Ouedraogo[1], A. Kamp[2], M.S. Goettel[3], J. Brodeur[1] and M.J. Bidochka[2]. [1]Département de Phytologie, Université Laval, QC, Canada, G1K 7P4, [2]Department of Biological Sciences, Brock University, St. Catharines, ON Canada L2S 3A1, [3]Agriculture and Agri-Food Canada Research Centre, P.O. Box 3000, Lethbridge, AB, Canada T1J 4B1.

FP14 Field testing of new biocontrol strategies to decrease the population density of *Melolontha hippocastani*, an important scarab species in Germany. K. Jung[1], J. Gonschorrek[2], J. Ruther[3] and G. Zimmermann[1]. [1]Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany; [2]Hessen-Forst, Prof.-Oelkers-Str. 6, D-34346 Hann. Münden, Germany; [3]University of Berlin, Haderslebener Str. 9, D-12163 Berlin, Germany.

FP15 *Metarhizium anisopliae* and *Trichoderma viride* efficiently control colonies of *Atta cephalotes* in the field. E.L. Lopez and S. Orduz. Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas (C.I.B.) A.A. 7378, Medellín, Colombia.

FP16 Advanced studies about biological control of Chagas disease vectors with entomopathogenic fungi in Central Brazil. C. Luz[1], L.F.N. Rocha[1], G.V. Nery[1], R.O. Silva[1], M. Unterseher[1/2] and N.R. Silva[1]. [1]Institute of Tropical Pathology and Public Health – Federal University of Goiás, 74001-970 Goiânia-GO, Brazil. [2]Tübingen University, Germany.

FP17 Characterization of *Nomuraea* sp. isolated from long-horned grasshopper (Tettigoniidae). Ö. Kalkar[1], G.R. Carner[1] and D.G. Boucias[2]. [1]Department of Entomology, Clemson University, Clemson, South Carolina 29634 USA, [2]Department of Entomology and Nematology, University of Florida, Gainesville, Florida, 32611 USA. **(STUDENT POSTER)**

FP18 Soil as an environment for winter survival of aphid-pathogenic Entomophthorales. C. Nielsen [1], A.E. Hajek[2], R.A. Humber [3], J. Bresciani [1] and J. Eilenberg[1]. [1]The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, 1871 Frederiksberg C., Denmark., [2]Cornell University, Department of Entomology, Ithaca, NY 14853, USA, [3]USDA-ARS Plant Protection Research Unit, US Plant Soil & Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA.

Viruses

VP1 Polyhedral envelope protein mutants of *Rachiplusia ou* multi-nucleocapsid nucleopolyhedrovirus. H. Jin, R.L. Harrison, D. Schroeder, A.J. Boughton and B.C. Bonning. Department of Entomology and Interdepartmental Program in Genetics,

Iowa State University, Ames, IA 50011, USA.

VP2 Insecticidal activity and risk assessment of a recombinant baculovirus expressing a basement membrane-degrading protease. R.L. Harrison[1], A.J. Boughton, J.J. Obrycki and B.C. Bonning[1]. Department of Entomology and [1]Interdepartmental Genetics Program, Iowa State University, Ames, Iowa 50011, USA.

VP3 Bioassays of balsam fir sawfly nucleopolyhedrovirus against its natural host and other insects. K. Barber[1], S. Holmes[1], J. Dedes[1], K. van Frankenhuyzen[1], E.G. Kettela[2], D.B. Levin[3] and C.J. Lucarotti[2]. [1]Canadian Forest Service, Sault Ste. Marie, Ontario, [2]Canadian Forest Service, Fredericton, New Brunswick, and [3]Biology Department, University of Victoria, Victoria, British Columbia, Canada.

VP4 Characterisation of *pif* (*per os* infectivity factor) from *Spodoptera littoralis* nucleopolyhedrovirus. I. Kikhno, S. Gutiérrez, L. Croizier, G. Croizier and M.L. Ferber. Laboratoire de Pathologie Comparée, UMR 5087, 30380 Saint Christol-les-Alès, France.

VP5 Differences in the peritrophic membrane of susceptible and resistant *Anticarsia gemmatalis* larvae to the insect nucleopolyhedrovirus (AgMNPV). S.M. Levy[1], A.M.F. Falleiros[2], F. Moscardi[3] and E.A. Gregório[1]. [1]Centro de Microscopia Eletrônica, IB, UNESP, Botucatu-SP-Brazil; [2]Depto de Histologia, CCB, UEL, Londrina-PR-Brazil; [3]Centro Nacional de Pesquisa de Soja, Embrapa Soja, Londrina-PR-Brazil. **(STUDENT POSTER)**

VP6 Establishment, growth kinetics, and susceptibility to AcNPV of heat tolerant lepidopteran cell-lines. Guo-xun Li[1,2], Y. Hashimoto[1] and R.R. Granados[1]. [1]Boyce Thompson Institute for Plant Research at Cornell University, Ithaca, New York, USA [2]Laiyang Agricultural University, Shandong, P.R. China.

VP7 The effects of an entomopoxvirus on the development of a pupal parasitoid, *Brachymeria lasus*, in its host *Homona magnanima* (Lepidoptera: Tortricidae). M. Hoshino, M. Nakai, J. Takatsuka, S. Okuno and Y. Kunimi. Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan.

VP8 Comparative analysis of the genome and host range of *Chilo* iridescent virus and Cricket iridovirus isolate. N.J. Jakob[1], R.G. Kleespies[2], C.A. Tidona[1], K. Müller[1], H.R. Gelderblom[3] and G. Darai[1]. [1]Institute for Medical Virology, University of Heidelberg, Germany, [2]Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany, and [3]Robert Koch-Institute, Berlin, Germany.

VP9 Biology, ecology and host-virus interactions of invertebrate iridescent viruses (*Iridoviridae*) in Diptera and Lepidoptera: recent advances. C.F. Marina[1,2], M. López[1], A. Gómez[1], M.Constantino[1], A. Reyes[1], G. Martínez[1], A. Hernández[1] and T. Williams[1,3]. [1]ECOSUR, AP 36, Tapachula 30700, Chiapas, Mexico, [2]Centro de Investigación de Paludismo-INSP, Tapachula 30700, Chiapas, Mexico, [3]Depto. Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain (current address).

VP10 A baculovirus mutant with a host-specific defect in occlusion body formation in insect cells. B.J. Kelly[1,2], S. Chapple[1], L.A. King[2] and R.D. Possee[1]. [1]Centre for Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR. [2]School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK. **(STUDENT POSTER)**

VP11 Effects of an optical brightener on the development of resistance to SfMNPV, the severity of sublethal effects and growth of *Spodoptera frugiperda*. A.M. Martínez, T. Williams and P. Caballero. Depto. Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain.

VP12 High prevalence of *pif* deficient genotypes in a wild-type nucleopolyhedrovirus of *Spodoptera frugiperda*. O. Simón[1], D. Muñoz[1], M. López-Ferber[2] and P. Caballero[1]. [1]Depto. Producción Agraria, Universidad Pública de Navarra, 31006, Pamplona, Spain, and [2]Station de Recherches de Pathologie Comparée, INRA, St. Christol-les-Ales, 30380, France.

VP13 Effects of Tinopal LPW on the infectivity and productivity of the *Spodoptera exigua* nucleopolyhedrovirus. R. Murillo[1], R. Lasa[1], D. Goulson[2], T. Williams[1], D. Muñoz[1] and P. Caballero[1]. [1]Depto. Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain, and [2]Biological Sciences, University of Southampton, Southampton, SO16 7PX, UK.

VP14 A novel vaccine delivery system using recombinant baculovirus occlusion bodies. Y. H. Je[1], I. Nobiron[1], J.A. Olszewski[1] and D.R. O'Reilly[1,2]. [1]Department of Biological Sciences. Imperial College of Science, Technology and Medicine, London, U.K. [2]Syngenta, Jealotts Hill Research Station, Bracknell, UK. **(STUDENT POSTER)**

VP15 Suppressive effects of *Xestia c-nigrum* granulovirus on nucleopolyhedrovirus infection in *Mamestra brassicae* and *Helicoverpa armigera*. C. Goto. National Agricultural Research Center, Tsukuba, 305-8666, Japan.

VP16 ORF94 of HaSNPV encodes a novel major ODV envelope protein ODV-E43. M. Fang, H. Wang, X. Chen and Z. Hu. Joint-laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P. R.China.

VP17 Characterization of a J domain gene of *Spodoptera litura* multicapsid nucleopolyhedrovirus. L. Wang, J. Yu, C. Yin, Z. Li, X. Hu and Y. Pang. State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P.R.China. **(STUDENT POSTER)**

VP18 Identification of a novel protein associated with envelope of occlusion-derived virus in *Spodoptera litura* multicapsid nucleopolyhedrovirus. C. Yin, J. Yu, L. Wang, Z. Li, P. Zhang and Y. Pang. State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P.R. China. **(STUDENT POSTER)**

VP19 A comparison of ascoviruses isolates from Indonesia and the United States. Y.M. Kusumah[1] and G.R. Carner[1]. [1]Department of Entomology, Clemson University, Clemson, South Carolina, USA. **(STUDENT POSTER)**

VP20 Activity of Selected Nucleopolyhedroviruses against Larvae of the Beet Armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). Pudjianto[1], B.M. Shepard[1], G.R. Carner[1], and M. Shapiro[2]. [1]Department of Entomology, Clemson University, Clemson, SC, USA. [2]Insect Biocontrol Laboratory, USDA-ARS, Belstville, MD, USA. **(STUDENT POSTER)**

VP21 Identification and characterization of Hz-2V structural protein genes. W. Kim[1]and J.P. Burand[1,2]. Dept. of Entomology[1] & Microbiology[2] Univ. of Massachusetts-Amherst, Amherst, Massachusetts 01003, USA.

Bacteria

BP1 Quantification of K^+-dependant transmembrane potentials in *Manduca sexta* midgut brush border membranes by diS-$C_3(5)$ assay induced by alkaline pH and Cry1Ab toxin. C.M. Garay, J. Sánchez, R. Miranda, A. Darszon and A. Bravo. Instituto de Biotecnología. Universidad Nacional Autónoma de México. Apdo postal 510-3, Cuernavaca, Morelos 62250. México.

BP2 Characterization of *Bacillus thuringiensis aizawai* UNI498 and histopathology analysis of the toxic effect on midgut of the *Anticarsia gemmatalis*. V.L. Bobrowski[1,4], R. Scheneumann[2], G. Pasquali[3], M.H. Bodanese-Zanettini[4] and L.M. Fiuza[2]. [1]DZG, Instituto de Biologia, UFPel; [2]Lab. de Microbiologia, Centro de Saúde, UNISINOS; [3]Depto. de Biotecnologia, IB, UFRGS; [4]Depto de Genética, IB, UFRGS. RS - Brazil. **(STUDENT POSTER)**

BP3 Mutagenic analysis of conserved residues in β17 within domain III of the *Bacillus thuringiensis* Cry4B toxin. P. Chayaratanasin, G. Katzenmeier, S. Panyim and C. Angsuthanasombat. Laboratory of Molecular Biophysics, Institute of Molecular Biology and Genetics, Salaya Campus, Mahidol University, Nakornpathom, Thailand 73170. **(STUDENT POSTER)**

BP4 Mutations within the α4-α5 loop region of Cry4B affect membrane pore-forming properties Y. Kanintronkul and C. Angsuthanasombat. Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Bhudamonthol, Nakornpathom, Thailand, 73170. **(STUDENT POSTER)**

BP5 Factors affecting crystallization of the Bt toxins. Z. Walters, C. Roquain and N. Crickmore. School of Biological Sciences, University of Sussex, Brighton, UK.

BP6 Investigation of parasporal inclusions from a mosquitocidal *Bacillus thuringiensis* serovar *sotto* strain. A. Ohgushi[1], N. Wasano[2], M. Maeda[3] and M. Ohba[1]. [1]Graduate School of Agriculture, Kyushu University, Fukuoka, Japan; [2]Biotechnology & Food Research Institute, Kurume, Fukuoka, Japan;[3] Kyushu Medical Co., Ltd., Kitakyushu, Japan. **(STUDENT POSTER)**

BP7 Biological fitness of a *Culex quinquefasciatus* population its resistance to *Bacillus sphaericus*. C.M.F. Oliveira, F.C. Filho, J.F. Beltràn, M.H. Silva Filha, and L. Regis. Centro de Pesquisa Aggeu Magalhães, Recife, PE, Brazil.

BP8 Occurrence of *Bacillus thuringiensis* in feces of wildlife of Korea. D.H. Lee[1], I.H. Cha[2], D.S. Woo[3] and M. Ohba[1]. [1]Graduate School of Agriculture, Kyushu University, Fukuoka, Japan. [2]College of Natural Sciences, Kyunpook National University, Daegu, Korea. [3]Mt. Jiri Conservationists Club, Gurye-gun, Jeollanamdo, Korea. **(STUDENT POSTER)**

BP9 Development of *Bacillus thuringiensis* formulation for phyto-sensitive crops in North-eastern Asia. C.-Y. Chen and J. Eyal. Certis USA LLC, 9145 Guilford Rd. Columbia, Suite 175, Maryland, USA 21046.

BP10 Cereolysin O: distribution, diversity and potential biological role in the *Bacillus cereus* group. N. Michelet and J.G. Mahillon. Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, Place Croix du Sud, 2/12 B-1348 Louvain-la-Neuve, Belgium.

BP11 The *Bacillus thuringiensis* toxin specificity database. K. van Frankenhuyzen, and C. Nystrom. Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada.

BP12 Behavior of Brazilian *Bacillus thuringiensis* strains when submitted to Sodium Dodecyl Sulphate (SDS) as a plasmid curing agent. C.F.G. Cavados, A.F.M. Santos, L.L. Oliveira, S.V. Azevedo and L. Rabinovitch. Dept. of Bacteriology, Institute Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.

BP13 *In vitro* binding of *Bacillus thuringiensis* Cry11Bb and Cry11Aa toxins shows two different receptors in the midgut of mosquito larvae. L.M. Ruiz, G. Armengol and S. Orduz. Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas (C.I.B.) A.A. 7378, Medellín, Colombia.

BP14 The presence and number of *Bacillus thuringiensis* spores in Colombian soils are determined by the soil physicochemical characteristics. P. Maduell, G. Armengol, and S. Orduz. Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas (C.I.B.) A.A. 7378, Medellín, Colombia.

BP15 Effects of co-expression of Cry11Aa with Cyt1Aa and/or p20 in aquatic bacteria. G. Armengol[1], U. Bialucha[2], O. Guevara[1], S. Orduz[1] and N. Crickmore[2]. [1]Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas (C.I.B), A.A. 7378, Medellín, Colombia [2]School of Biological Sciences, University of Sussex, Brighton, UK.

BP16 *Bacillus thuringiensis* – non target effect of a purified toxin and a commercial formulation. W.P. de Oliveira, L.A.N. Sá, D.M.F. Capalbo and G. Nicolella. Embrapa Environment, P.O. Box 69, Jaguariúna, São Paulo, Brazil.

BP17 Ion-channel activity of the *Bacillus thuringiensis* Cry4B α1-α5 pore-forming fragment. T. Puntheeranurak[1], L. Potvin[2], J.-L. Schwartz[2], C. Krittanai[1], G. Katzenmeier[1], S. Panyim[1] and C. Angsuthanasombat[1]. [1]Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand and [2]Biotechnology Research Institute, National Research Council, 6100 Royalmount Avenue, Montreal, Quebec, H4P 2R2, Canada. **(STUDENT POSTER)**

BP18 Implications for the sustainability of transgenic Bt maize. Monitoring the susceptibility of European Corn Borer in Germany. C. Saeglitz, T. Muecher, C. Zahn, D. Bartsch and I. Schuphan. Aachen University of Technology RWTH, Department of Biology V, Worringerweg 1, 52056 Aachen, Germany.

BP19 Effects of Bt-corn growing on the epigeic and herbaceous layer fauna of different trophic levels. A. Gathmann, M. Rob-Nickoll, C. Saeglitz, D. Bartsch and I. Schuphan. Aachen University of Technology RWTH, Biology V, Worringerweg 1, 52056 Aachen, Germany.

Microsporidia

MP1 Epizootic and enzootic features of microporidia in *Simulium pertinax* (Diptera: Simuliidae) larvae in the state of Rio de Janeiro, Brazil. CJPC Araújo-Coutinho[1], E.S. Nascimento[1], R. Figueiró[1] and J.J. Becnel[2]. [1]Laboratory of Simuliids and Onchocerciasis, Dept. of Entomology, IOC/FIOCRUZ, Rio de Janeiro/RJ, Brazil; [2]U.S. Department of Agriculture/ Agriculture Research Service, USA.

MP2 Changes in reproductive life history patterns of the gerrid, *Aquarius remigis*, alter

trypanosomatid prevalence. K. Gurski, C. and M.A. Ebbert. Department of Zoology, Miami University, Oxford, OH 45056 USA.

Nematodes

NP1 Susceptibility of chilean target pests to native entomopathogenic nematodes A. France, S. Espinoza, and M. Gerding. Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Quilamapu, Chillán, Chile.

NP2 Evolution of the oxygen consumption of *Steinernema feltiae* and *Xenorhbadus nematophilus* in axenic and monoxenic cultures. J. Suárez, Y. Reyes, A. Asaff and M. de la Torre. Centro de Investigación y de Estudios Avanzados del IPN, Departamento de Biotecnología y Bioingeniería, México, D.F., México. **(STUDENT POSTER)**

NP3 Comparative efficacy of different species of entomopathogenic nematodes for the control of guava weevil *Conotrachelus psidii* (Coleoptera: Curculionidae). C. Dolinski and R.I. Sammuels. Universidade Estadual do Norte Fluminense, Laboratório de Proteção de Plantas, Setor de Patologia de Insetos, Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brazil, 28015-620.

NP4 Survey of native populations of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and their symbiotic bacteria from Costa Rica. L. Uribe-Lorío[1], A. Sittenfeld[1], S.P. Stock[2] and M. Mora[1]. [1]Center for Research in Cellular and Molecular Biology, University of Costa Rica.[2]Department of Plant Pathology, College of Agriculture & Life Sciences, University of Arizona, USA.

NP5 Pathogenicity of *Heterorhabditis* spp. and *Steinernema* spp. against the citrus root weevil, *Naupactus* sp. L.A. Machado[1], L.G. Leite[1], R.M. Goulart[1], J.V.C. Guedes[2] and F.M. Tavares[1]. [1]Instituto Biológico, Caixa Postal 70, Campinas, SP, 13001-970, Brazil. [2]UFSM/CCR/DFS, Santa Maria, RS, 97105-900, Brazil.

NP6 Pathogenicity of *Heterorhabditis* sp. and *Steinernema* spp. (Nemata: Rhabditida), in different dosages, against the citrus root weevil. L.G. Leite[1], L.A. Machado[1], R.M. Goulart[1], J.V.C. Guedes[2] and M. Dinardo[3]. [1]Instituto Biológico, Caixa Postal 70, Campinas, SP, 13001-970, Brazil. [2]UFSM/CCR/DFS, Santa Maria, RS, 97105-900, Brazil. [3]Citrovita, Itapetininga, SP, 18200-000, Brazil.

NP7 Biological control of *Tecia solanivora* using *Steinernema feltiae* Colombia strain in potatoes in Cundinamarca, Colombia. L.T. Corredor, J.C. Parada and M.S. Serrano. Facultad de Agronomía. Universidad Nacional de Colombia. Bogotá. D.C., Colombia.

NP8 Geographical distribution of *Steinernema feltiae* in Cundinamarca and Boyacá, Colombia. J.C. Parada. Facultad de Agronomia. Universidad Nacional de Colombia. Bogotá. D. C., Colombia.

Microbial Control

MC1 Bioinsecticide formulations using microencapsulation process. A.L.S. Zimmermann[1], M.I. Ré[2] and N.L. Pereira[1]. [1]Dep. Ciências Farmacêuticas, FCFRP, USP, São Paulo, Brazil. [2]Institute for Technological Research of São Paulo, Brazil.

MC2 Distribution of fungal conidia in the canopy of chrysanthemum using hydraulic and electrostatic sprayers. V. Gouli, B.L. Parker and S. Gouli. Entomology Research Laboratory, University of Vermont, USA.

MC3 Cloning and expression of *Bacillus thuringiensis cry2Aa* gene from Bt66 strain. W. Guo[1,3], J. Zhang[2], F. Song[2], D. Huang[2] and G. Li[3]. [1] State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou, P. R. China. [2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China and [3]Hebei Agricultural, University, Baoding P.R. China. **(STUDENT POSTER)**

MC4 Optimized batch production of *Bacillus thuringiensis* subsp. *Israelensis* based on activity against *Aedes aegypti* larvae. M.G. Maldonado-Blanco, G. Solís-Romero and L.J. Galán-Wong. Departamento de Microbiología e Inmunología, Fac. Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México.

MC5 Field evaluation of polymer-based granular formulations of *Bacillus thuringiensis* subsp. *israelensis* H-14. M.G. Maldonado-Blanco[1], S.A.M. Rodríguez[1], L.J. Galán-Wong[1] and H. Quiroz-Martínez[2]. [1]Laboratorio de Microbiología Industrial and [2]Laboratorio de Entomología., Fac. de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, N. L., México.

MC6 Production of active ingredient (conidia or blastospores) of Beauveria bassiana (Bals.) Vuillemin (Deuteromycotina: Hyphomycetes) in different liquid media. M. J. Chong[1], L.J. Galán[1], M.A. Jackson[2], K. Arévalo-Niño[1], L.H. Morales[1], C.F. Sandoval[1]. [1]Dep. de Microbiología Universidad Autónoma de Nuevo León, México and [2]USDA/ARS/NCAUR , Peoria, USA. **(STUDENT POSTER)**

MC7 Biological control of the phytophagous mite *Tetranychus urticae* (Acari: Tetranychidae) using *Beauveria bassiana*. D.L.A. Coracini, K.F.S. Collier and R.I. Samuels. Laboratory of Plant Protection, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ 28015-620, Brazil. **(STUDENT POSTER)**

MC8 A *Bacillus thuringiensis* autoagglutinating strain highly toxic to their source of isolation, *Simulium pertinax* larvae. C.F.G Cavados[1], R.N. Fonseca[1], J.Q. Chaves[1], L. Rabinovitch[1] and C.J.P.C. Araújo-Coutinho[2]. [1] Dept. of Bacteriology, Institute Oswaldo Cruz/ FIOCRUZ. [2]Dept. of Entomology, Institute Oswaldo Cruz – SUCEN/FIOCRUZ Rio de Janeiro, Brazil.

MC9 Evaluation of three differentes formulation based on *Bacillus thuringiensis* sorovar. *israelensis* to the control of *Culex quinquefasciatus* and *Aedes aegypti*. J.A.C. Zequi[1] and J. Lopes[2]. [1]UEL Doctoral student at the Department of Agronomy. [2]Dept. of Animal and Vegetable Biology, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

MC10 Different substrates in local *Bacillus thuringiensis* var. *israelensis* production. J. Lopes, J.G. Bueno, J.A.C. Zequi, M.T. Suzuki and O.M.N. Arantes. CCB/Universidade Estadual de Londrina, 86051-970–Londrina, PR, Brazil.

MC11 Binding sites of *Bacillus thuringiensis* Insecticidal Crystal Proteins on the midgut of *Anticarsia gemmatalis* (Lepidoptera, Noctuidae) larvae. L.M. Fiuza[1], J.A.P. Henriques[2], R.F.P.Silva[3]. [1]Microbiologia/Centro2/ UNISINOS, [2]Biotecnologia/ UFRGS, [3]Agronomia/ UFRGS, RS, Brazil.

MC12 Pathogenic effect of *Bacillus thuringiensis* isolate from south of Brazil against *Oryzophagus oryzae* (Coleoptera, Curculionidae). L.M. Fiuza[1,2], L.M.N. Pinto[1], A.O. Azambuja[1], C. Steffens[1], V.G. Menezes[2] and J.O. Vargas[2]. [1]Microbiologia, Centro 2, UNISINOS, São Leopoldo, RS, Brasil. [2]EEA-IRGA, Cachoeirinha, RS, Brazil.

MC13 Histopathological study of the fall armyworm parasitated by *Campoletis flavicincta* and infected with *Bacillus thuringiensis aizawai*. S.T. Dequech[1, 2], L.M. Fiuza[3], R.F. P. Silva[2] and S.L. Sieben[2]. [1]Fitosanity Defense Dept. CCR – UFSM, Santa Maria, RS, Brazil. [2]Health Plant Department, School of Agr., UFRGS, Porto Alegre, RS, Brazil. [3]Microbiology, UNISINOS, São Leopoldo, RS and EEA-IRGA, Cachoeirinha, RS, Brazil.

Cross Division

CDP1 Culture of insect cells in airlift reactors: study of the influence of geometrical characteristics and gas flow rate on culture behaviour. G.A. Visnovsky[1], J.D. Claus[2] and J.C. Merchuk[3]. [1]Biotech. Dept. and [3]Chem. Engn. Dept., Ben Gurion University, Beer-Sheva, Israel, [2]INTEBIO, Fac. Bioq. Cs. Biol.-UNL, Santa Fe, Argentina.

CDP2 Elimination of *Wolbachia* from *Urolepis rufipes* (Ashmead) (Hymenoptera: Pteromalidae) with heat and antibiotic treatments: implications for host reproduction. G. Kyei-Poku, B. Benkel, M.S. Goettel and K. Floate. Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta Canada T1J 4B1.

CDP3 Insect digestive biochemistry: A genomics and proteomics perspective. D.D. Hegedus[1], M. Chamankhah[2], L. Braun[1], D. Baldwin[1] and S. Hemmingsen[3]. [1]Agriculture and Agri-Food Canada. [2]University of Saskatchewan. [3]National Research Council of Canada, Saskatoon, Canada.

CDP4 Formulation of biopesticides for application to soil. M. O'Callaghan, V.W. Johnson, E.M. Gerard and T.A. Jackson. AgResearch, P.O. Box 60, Lincoln, New Zealand.

CDP5 Origins of *Yersinia pestis* pathogenesis and investigation of the potential of *Yersinia pseudotuberculosis* as an insect pathogen. V. Pinheiro and D.J. Ellar. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge CB2 1GA, UK.

CDP6 Mucoid secretion is a virulence factor for QPX, a protist pathogenic for the clam *Mercenaria mercenaria*. R.S. Anderson and B.S. Kraus. University of Maryland Center

for Environmental Science, CBL, Solomons, Maryland, USA.

Monday, 20:00 - 22:00
DIVISION BUSINESS MEETINGS

Fungi (Iguaçu I)
Virus (Cataratas I)
Bacteria (Cataratas II)
Nematodes (Araucária)
Microsporidia (Ipê)

TUESDAY, AUGUST 20

Tuesday, 8:00 – 10:15
Iguaçu I
CONTRIBUTED PAPERS – Microbial Control 1
Chair: C. Nielsen **pgs 62 - 65**

8:00 The main features of microbial plant protection in Siberia. M.V.Shternshis and V.V.Gouli. Novosibirsk State Agrarian University, Russia, and University of Vermont, USA.

8:15 Field-scale studies on spatio-temporal relationships between aphids and natural enemies. P.A. Shah, A. Tymon and J.K. Pell. Plant and Invertebrate Ecology Division, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK.

8:30 Lab-scale mass production and field trials with mycoinsecticides for the biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in pulses. M. Hassani, T. Bucher, A. Hadapad, P. Nahar, A. Chandele, U. Tuor, S. Keller and M.V. Deshpande. Swiss Federal Institute of Technology ETH, Institute of Microbiology, ETH Zentrum/LFV, Schmelzbergstr. 7, CH-8092 Zürich, Switzerland. National Chemical Laboratory, Biochemical Sciences Division, Pune 411008, India. Agriculture College, Pune-411055, India. Federal Research Station for Agroecology and Agriculture, CH-8046 Zurich, Switzerland.

8:45 Efficacy of blastospores of *Beauveria bassiana* compared to Mycotrol® and Meta-sin® spores efficacy against colding moth *Cydia pomonella* L. C. García-Gutiérrez, H. Medrano-Roldán and B. González-Maldonado. CIIDIR-COFAA IPN Unidad Durango. Sigma s/n Fracc. 20 de Noviembre II. C.P. 34220. Instituto Tecnologico de Durango ITD. Blvd. Felipe Pescador No. 1830 Ote. C.P. 34250

9:00 Biocompatibility of *Beauveria bassiana* (BotaniGard® 22WP and ES) and the parasitoid *Eretmocerus eremicus* in a silverleaf whitefly control strategy on poinsettia. C. Armstrong and M. Brownbridge. Entomology Research Laboratory, University of Vermont, Burlington, VT 05405, USA.

9:15 Field efficacy of emulsifiable suspensions of *Beauveria bassiana* conidia for control of *Myzus persicae* population on Chinese cabbage. S.-H. Ying, M.-G. Feng, and S.-T. Xu. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou 310029, P. R. China.

9:30 Biological control of weevils in Danish greenery production. C. Nielsen, S. Vestergaard, S. Harding and J. Eilenberg. The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, 1871 Frederiksberg C., Denmark.

9:45 Development of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) for the control of *Lygus lineolaris* (Hemiptera : Miridae) on Wild Host Plants in the Mississippi Delta of the Unites States. J.E. Leland, USDA-ARS, SIMRU, USA.

10:00 Efficiency of *Bt* formulation for genetically distinct lines of potato tubeworm. A.V. Ivashov[1], A.P. Simchuk[1], S.G. Grigoriev[1] and V.Y. Gouli[2]. [1]V.I. Vernadsky National University, Simpheropol, Ukraine. [2]University of Vermont, USA.

Tuesday, 8:00 - 10:00
Cataratas I
CONTRIBUTED PAPERS - Bacteria 2
Chair: P. Butko **pgs 65 - 68**

8:00 Plasma membrane microdomains integrity is essential for pore formation activity of *Bacillus thuringiensis* Cry1Ab toxin. A. Bravo, R. Miranda, I. Gómez and M. Soberón Instituto de Biotecnología, Departamento de Microbiología. Universidad Nacional Atónoma de México. Apdo postal 510-3, Cuernavaca, Morelos 62250, México.

8:15 Cyt toxins - pore formers or detergents? P. Butko, S. D. Manceva, P. S. Russo, and M. P.Carey. Dept. Chemistry and Biochemistry, University of Southern Mississippi, Hattiesburg, MS, USA., Dept. Chemistry, Louisiana State University, Baton Rouge, LA, USA. Dept. Biochemistry, Case Western Reserve University, Cleveland, OH, USA.

8:30 Distribution of Cry1A toxins and receptors in membrane fractions isolated from lepidoptera midgut M. Zhuang, D. Oltean, I. Gomez, M. Soberón, A. Bravo and S.S. Gill . Graduate

Program in Environmental Toxicology, Department of Cell Biology and Neuroscience, University of California, Riverside, CA 92521, USA. Instituto de Biotecnología, Departamento de Microbiología, Universidad Nacional Autónoma de Mexico, Apdo postal 510-3, Cuernavaca, Morelos 62250, Mexico.

8:45 Molecular basis of membrane poré-formation by the *Bacillus thuringiensis* Cry4B mosquito-larvicidal protein C. Angsuthanasombat, I. Sramala, T. Puntheeranurak, Y. Kanintronkul, C. Krittanai, G. Katzenmeier and S. Panyim. Laboratory of Molecular Biophysics, Institue of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom, Thailand 73170.

9:00 The high dose - Refugia management resistance strategy for Bt transgenic crops: the influence of refugia. H. Cerda and D.J. Wright. Imperial College, London University, UK. Universidad Simon Rodriguez, Venezuela.

9:15 *Bacillus thuringiensis* resistance and associated fitness costs in *Trichoplusia ni* populations. A.F. Janmaat and J. Myers. Department of Zoology, University of British Columbia, 6270 University Blvd. Vancouver, British Columbia, Canada, V6T 1Z4.

9:30 Genetic variability for resistance to *Bacillus thuringiensis* toxins: a case study with the diamondback moth J. Ferré, J.González-Cabrera and S.Herrero. Department of Genetics, Universidad de València, 46100 Burjassot, Valencia, Spain.

9:45 BT-r, a novel resistant gene against BT-toxin, Cry1Ab, was mapped on the molecular map based on RFLP of EST-cDNA clones in the silkworm, *Bombyx mori*. W. Hara[1], K. Yonsun[1], K. Miyamoto[1], K. Kanda[2] and M. Goldsmith[3]. [1]National Institute of Agrobiological Sciences, Japan. [2]University of Saga, Japan. [3]University of Rhode Island, RI, USA.

Tuesday, 8:00 - 10:00
Iguaçu II
SYMPOSIUM (Fungi 2) - Microecology of Entomopathogenic Fungi
Chair: M. R. de Faria

8:00 Ecology of entomopathogenic fungi in field soils. A.C. Rath. Valent BioSciences Corporation, Asia-Pacific Research Office, 13 Hynds Road Box Hill NSW 2765, Australia.

8:25 Phyllosphere ecology of terrestrial entomopathogenic fungi. S.P. Wraight. USDA, ARS, Plant, Soil & Nutrition Laboratory, Tower Rd., Ithaca, NY, USA.

8:50 Endophytic fungi as agents for the biological control of insects. W. Maccheroni Jr. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 13400-970 Piracicaba, São Paulo, Brazil.

9:15 Microecology of entomopathogenic fungi from aquatic environments. C. C. L. Lastra, J.J. García & M.V. Micieli. CEPAVE (Centro de Estudios Parasitológicos y de Vectores) (CONICET- UNLP), Calle 2 N° 584 (1900) La Plata, Argentina.

9:40 Discussion

Tuesday, 8:00 - 10:00
Cataratas II
SYMPOSIUM (Virus 2) - Prospects for the Use of Viral Pesticides
Convenor: M.L. Souza

8:00 The successful use of AgMNPV for the control of velvetbean caterpillar, *Anticarsia gemmatalis*, in soybean in Brazil. F. Moscardi[1], L. Morales[2] and B. Santos[3]. [1]Embrapa Soybean, C. Postal 231, Londrina, PR, Brazil, CEP 86001-970. [2]Emater-PR, Londrina, PR, Brazil. [3]Universidade Federal do Paraná, Departamento de Agronomia, Curitiba, PR, Brazil.

8:30 Development of *Spodoptera frugiperda* nucleopolyhedrovirus as a bioinsecticide in Mexico and Central America. T. Williams, Depto. Producción Agrária, Universidade Publica de Navarra, Pamplona 31006, Espanha.

9:00 Development of wild-type and recombinant HaSNPV as viral pesticides for controlling cotton bollworm in China. X. Sun[1,2], X. Chen[1], J. M. Vlak[2] and Z. Hu[1]. Joint-Lab of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China[1]. Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands[2].

9:30 Use of engineered baculovirus as biopesticides: reality and prospects. J. Cory, Mansfield Road, Oxford OX1 3SR, UK.

Coffee break 10:00 - 10:30

Tuesday, 10:30 - 12:45
Iguaçu I
CONTRIBUTED PAPERS - Microsporidia 1
Chair: T. Andreadis **pgs 68 - 71**

10:30 Comparative phylogenetic analysis of *Amblyospora* and related species. C.R. Vossbrinck[1], T.G. Andreadis[1], J. Vavra[2] and J.J. Becnel[3]. [1]The Connecticut Agricultural Experiment Station, New Haven Connecticut USA, and [2]Department of Parasitology and Hydrobiology, Charles University, Prague, Czech Republic [3]USDA/ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA.

10:45 Efficacy of Neem seed extract and an entomopathogenic microsporidian on *Haritalodes derogata* (F.) and *Spodoptera litura* (F.) larvae E.P.S. Chandana and H.C.E. Wegiriya. Department of Zoology, University of Ruhuna, Wellamadama, Matara, Sri Lanka.

11:00 A remnant mitochondrion in the microsporidian *Trachipleistophora hominis*. B.A.P. Williams[1], R.P. Hirt[1], J.M. Lucocq[2] and T. M.Embley[1]. [1]Department of Zoology, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK. [2]School of Life Sciences, WTB/MSI complex, University of Dundee, Dundee DD1 5EH, UK.

11:15 *Mattesia oryzaephili* (Sporozoea: Neogregarinida) host range and virulence. J. Lord. USDA-ARS, Manhattan, Kansas, USA.

11:30 Histopathological observations and bionomical effects of microsporidian infections of the gypsy moth, *Lymantria dispar*. L.A. Linde[1], D. Goertz[1], D. Pilarska[2] and J. Feuerstein[1]. [1]University of Applied Sciences, Alfred-Moeller-Str. 1, 16225 – Eberswalde, Germany. [2]Bulgarian Academy of Sciences, Blvd. Czar Osvoboditel 1, Sofia, Bulgaria.

11:45 A genomic sequence survey of *Nosema locustae*: microsporidian origins, metabolism and variation in genomic structure. N.M. Fast, J. Law, T.S. Lena and P.J. Keeling. Canadian Institute for Advanced Research, Department of Botany, University of British Columbia, Vancouver, B.C. Canada V6T 1Z4.

12:00 Impact of a new species of microsporidia on two weevils, *Neochetina eichhorniae* and *N. bruchi*, biological control agents of water hyacinth (*Eichhornia crassipes*). M.T. Rebelo[1] , J.J. Becnel[2] and T.D. Center[3]. [1]CBA/DZA, Faculdade de Ciencias, Universidade de Lisboa, Portugal. [2]USDA/ ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA. [3]USDA/ARS, Invasive Plant Research Laboratory, Fort Lauderdale, Florida, USA.

12:15 Defense response of *Lymantria dispar* larvae to microsporidian species in the genera *Nosema* and *Vairimorpha*. G. Hoch[1,2], L.F. Solter[2] and A. Schopf[1]. [1]Institute of Forest Entomology, Univ. BOKU Vienna, Hasenauerstraße 38, A-1190 Vienna, Austria. [2]Center for Economic Entomology, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820, USA.

12;30 Two new early-branching microsporidians from crustaceans distinguished using DNA. M.V. Brown. Department of Zoology, University of British Columbia, Canada.

Tuesday, 10:30 - 12:45
Cataratas I
CONTRIBUTED PAPERS - Bacteria 3
Chair: R. Akhurst **pgs 71 - 74**

10:30 Interactions of *B. thuringiensis* subsp. *israelensis* insecticidal proteins with the bloodworm *Chironomus tepperi* and their potential in creating Bloodworm-resistant transgenic rice. P.A. Hughes, M. Stevens, L. Dennis and R. Akhurst. CSIRO Entomology, Canberra ACT 2600, Australia. CSIRO Plant Industry, Canberra ACT 2600, Australia. CRC for Sustainable Rice Production, Yanco, NSW 2703, Australia. NSW Agriculture, Yanco NSW 2703, Australia.

10:45 The research on *cry1Ie1* gene from *Bacillus thuringiensis*. F. Song J. Zhang, Y. Wu, C. Li and L.Han D. Huang . Institute of Plant Protection, Institute of Biotechnology Research Chinese Academy of Agricultural Science, Beijing, 100094, P.R. China.

11:00 Cloning and expression of Bt *cry* genes from BtLy30 strain with high toxicity and wider anti-insect spectrum. Y. Jiang , Z. Jie and Z. Shenghua. H. Dafang State Key Laboratory for Biology of Plant Insect Pests and Diseases, Institute of Plant Protection CAAS Beijing 100094 Biotechnology Research Institute CAAS, Beining 100081, P.R. China.

11:15 Assessing non-target effects of *Bacillus thuringiensis* and neem using *Folsomia candida* (Collembola). M. Brownbridge, H. Kato and M. Broza. University of Vermont, Entomology Research Laboratory, Burlington, Vermont, USA. University of Haifa, Dept. Biology, Oranim, Tivon 36006, Israel.

11:30 Evaluation of non-target insect populations on conventional cotton, and transgenic Bollgard® cotton and Bollgard® II cotton, under two insecticide management regimes. S. Fernandez, C. Jiang, K.A. Hamilton, M.J. McKee, A. Catchot, P. Ellsworth and G. Head. Monsanto Company, St. Louis, Missouri, USA. Monsanto Research Farm, Leland, Mississippi, USA. University of Arizona, Maricopa, Arizona, USA.

11:45 Characterization of newly isolated Cry1A-binding proteins of *B. mori* larva. T. Hayakawa, Y. Shitomi, M. Higuchi, K. Moriyama, K. Miyamoto, R. Sato and H. Hori. Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan. National Institute of Agrobiological Sciences, Kannondai, Tsukuba, Ibaraki 305-8602, Japan. Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-0012, Japan.

12:00 Identification of a mexican strain of *Serratia* spp. phatogenic against larvae of *Phyllophaga* spp (Coleoptera:Scarabaeidae). M.E. Nuñez-Valdez[1], M.A. Calderon[2], E. Aranda[2], L. Hernández[3] R. Gama, R.M.[3] L. Lina[2] and F.J. Villalobos[1]. [1]Centro de Desarrollo y Investigación Agropecuaria, FaCAgr. [2]Centro de Investigación en Biotecnología, Universidad Autonóma del Estado de Morelos. [3]Facultad de Química, Universidad Nacional Autónoma de México, México.

12:15 Field efficacy of formulation based on β-exotoxin *Bt* for Uzbekistan population of Colorado potato beetle. E.N. Abdullaev. Alisher Navoi Samarkand State University, 15 University Avenue, Samarkand, Uzbekistan, 703004.

12:30 Laboratory efficacy of formulation based on β-exotoxin *Bt* for Uzbekistan population of Colorado potato beetle. E.N. Abdullaev. Alisher Navoi Samarkand State University 15 University Avenue, Samarkand, Uzbekistan, 703004.

Tuesday, 10:30 - 12:30
Iguaçu II
SYMPOSIUM (Nematodes 1)- Entomopathogenic Nematodes: Current Status
Convenors: M.M. Aguillera and I. Glazer

10:30 Worldwide production and use of entomopathogenic nematodes. H.K. Kaya and P. Grewal. Department of Nematology, University of California, Davis, CA 95616 USA and Department of Entomology, Ohio State University, Wooster, OH 44691, USA.

10:50 Entomopathogenic nematode diversity in South America: Opportunities for exploration. S.P. Stock. Department of Plant Pathology. University of Arizona. 1140 E. South Campus Dr. Tucson, AZ 85721-0036. USA.

11:10 Development of entomopathogenic nematodes as a management tactic for citrus root weevils in Florida. C.W. McCoy[1], L.W. Duncan[1], R.J. Stuart[1], and D.I. Shapiro[2]. [1]University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, Florida, 33850, USA. [2]USDA-ARS, SE Fruit and Tree Nut Research Lab, Byron, Georgia, 31008, USA.

11:30 Advances in the use of entomopathogenic nematodes for the management of scarab pests. A.M. Koppenhöfer and E.M. Fuzy. Dept. Entomology, Rutgers University, Blake Hall, 93 Lipman Dr., New Brunswick, NJ 08901. USA.

11:50 Entomopathogenic Nematodes: Research and implementation in South America countries. M. M. Aguillera, E. A. B. De Nardo , UFSCar, São Carlos, Brazil.

12:10 Entomopathogenic nematodes: Research and implementation in Mexico and Central America countries.J.V. Ruiz, R. Alatorre-Rosas and H. C. Arredondo- Bernal Dept. Entomology, Rutgers University, Blake Hall, 93 Lipman Dr., New Brunswick, NJ 08901. USA.

Tuesday, 10:30 - 12:30
Cataratas II
WORKSHOP - The Future of Scientific Publications
Convenors: M.S. Goettel and D. Onstad

10:30 Introduction and Scientific Society's Viewpoint. M.S. Goettel, Assistant Editor, SIP Newsletter and Research Scientist, Lethbridge Research Centre, Agriculture & Agri-Food Canada. D. Onstad, Chair, SIP

Publications Committee and Research Scientist, Department of Economic Entomology, Illinois Natural History Survey, USA.

10:55 The Publisher's Viewpoint. A. Richford, Executive Editor, Science and Technology, Elsevier Science, Netherland.

11:20 The Electronic Publishing Viewpoint. D.A.L. Canhos, Project Director, Centro de Referência em Informação Ambiental (CRIA), Brazil.

11:45 The Scientist's Viewpoint. J.D. Vandenberg, Research Scientist, US Plant, Soil & Nutrition Laboratory, USDA, USA.

12:10 The Librarian's Viewpoint. D. Schmidt, Biology Librarian, University of Illinois, USA.

Tuesday, 14:00 - 16:00
POSTER SESSION II **pgs 74 - 93**

Fungi

FP19 Compatibility between entomopathogenic *Beauveria bassiana* and pesticides used in coffee crop protection. C.N.de Oliveira, P.M.O.J. Neves, L.S. Kawazoe, and R.C. de Oliveira. Universidade Estadual de Londrina – Dep. de Agronomia C.P. 6001 CEP 86051-970, Londrina, PR, Brazil.

FP20 Influence of temperature in germination, vegetative growth and conidia prodution of *Beauveria bassiana* (Bals.) Vuill. P.M.J.O. Neves[1], E.T. Ito[1], E.I. Yuki [2], P.H. Santoro[1], V.R. Chocorosqui[1], L. Koguishi[1] and J.G.Z. Vieira[1]. [1]Dep. de Agronomia, [2]Dep. de Biologia, Universidade Estadual de Londrina, C. P. 6001 CEP 86051-970, Londrina, PR, Brazil.

FP21 Inhibition of coffe anthracnose *Colletotrichum* spp. by the entomopathogenic fungus *B. bassiana* (Bals.) Vuill. *in vitro*. D.C. Ribeiro[1], P.M.J.O. Neves[1], N. Massola[2] and P.H. Santoro[1]. [1]Universidade Estadual de Londrina, Dep. de Agronomia C. P. 6001, CEP 86051-970, Londrina, PR, Brazil. [2]ESALQ, C. P. 9, 13418-900, Piracicaba, SP, Brazil. **(STUDENT POSTER)**

FP22 *Evlachovaea*: First reports of an unusual and little known entomopathogenic fungal genus from the New World. R.A. Humber[1], M.R. Tanzini[2] and S.B. Alves[2]. [1] USDA-ARS Plant, Soil & Nutrition Laboratory, Ithaca, New York, USA and [2]Universidade de São Paulo, ESALQ, Piracicaba, Brazil.

FP23 Virulence of *Verticillium lecanii* varies with different developmental stages of cotton aphid (*Aphis gossypii*). J. J. Kim[1,4], D. R. Choi[1], K.C. Kim[2], C.S. Yoon[3], and D.W. Roberts[4]. [1]Division of Entomology, NIAST, RDA. [2]Dept.Agrobiology, Chonnam National University. [3]Research Institute of Engineering and Technology, Korea University, Korea. [4]Dept. Biology, Utah State University, USA.

FP24 Entomopathogens associated with soybean/wheat production systems in Brazil and Argentina. D.R. Sosa-Gomez[1], R.A. Humber[2] and F. Moscardi[1]. [1]Embrapa Soja. C.P. 231, Londrina, PR. 86001-970. [2]USDA-ARS, Plant Protection Research Unit US Plant, Soil & Nutrition. Tower Road. Ithaca, NY 14853-2901, USA.

FP25 Compatibility of entomopathogenic fungi with pesticides. R.C. Oliveira[1] and P.M.O.J. Neves[2]. [1]Depto. de Ciências Biológicas, UNIPAR, C.P. 4515, CEP: 85801-470, Cascavel, PR, Brazil. [2]Depto. de Agronomia, UEL, C. P. 6001, CEP: 86051-990, Londrina, PR, Brazil.

FP26 Susceptibility of the Paraguay-tea borer *Hedyphates betulinus* (Coleoptera: Cerambycidae) to entomopathogenic fungi. R.C. Oliveira[1], P.M.O.J. Neves[2] and L.F.A. Alves[3]. [1]Depto. de Ciências Biológicas, UNIPAR, C.P. 4515, CEP: 85801-470, Cascavel, PR, Brazil. [2]Depto. de Agronomia, UEL, C.P. 6001, CEP: 86051-990, Londrina, PR, Brasil. [3]CCBS – UNIOESTE, Rua Universitária, 2069, CEP: 85814-110, Cascavel, PR, Brazil.

FP27 Effect of the fungus Metarhizium anisopliae var. acridum on non-target arthropods in Brazil. A. Foucart[1], M. Lecoq[1], B.P. Magalhães[2], M. Faria[2], F.G.V. Schmidt[2], and J.B.T. da Silva[2]. [1]CIRAD-AMIS, Montpellier, France. [2]Embrapa Recursos Genéticos and Biotecnologia, Brasília, DF, Brazil.

FP28 Hydrophobicity of conidia of *Metarhizium anisopliae* produced by submerged fermentation. T. Scopa[1], M. Turner[1], and N. Jenkins[2]. [1]Dept. Biochemical Engineering, University College London, UK; [2]CABI, Bioscience, Ascot, UK, Silwood Park, Buckhurst Rd, Ascot, UK. **(SUDENT POSTER)**

FP29 *Beauveria bassiana* yeast phase on agar medium and its pathogenicity against *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Tetranychus urticae* (Acari: Tetranychidae). S.B. Alves[1], L.S. Rossi[1],

R.B. Lopes[1], M.A. Tamai[1] and R.M. Pereira[2]. [1]Dept. de Entomol., Fitopatol. e Zool. Agric., ESALQ/USP, CP 9, 13418-900 Piracicaba, SP, Brazil. [2]USDA-ARS, CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, USA.

FP30 A new record of the *Entomophaga* (?) *grylli* (Fresenius) Batko species complex on Acrididae (Orthoptera) in southern Bahia, Brazil. S.E.M.Sánchez[1], R.A.Huber[2], A.L Freitas.[3] and E.F. Nunes[3]. [1] Depto. de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz - UESC, km. 16 Rod. Ilhéus/ Itabuna 45650-000 Ilhéus - Bahia, Brazil. [2] USDA-ARS, Ithaca, NY. USA. [3]Depto. de Ciências Biológicas, Universidade Estadual de Santa Cruz – UESC, Brazil.

FP31 Study of *Verticillium lecanii* culture at different temperatures and growth media. A.do C.B. Correia, D.E.N. Rangel and T. Nucci. Departamento de Fitossanidade, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil.

FP32 Determination of growth media for the viability test of entomopathogenic fungi. E. A. Francisco[1], D.A. Mochi[2], A.do C.B. Correia[1] and A.C. Monteiro[2]. [1]Departamento de Fitossanidade e [2]Departamento de Produção Vegetal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil.

FP33 Water sorption isotherms of *Beauveria bassiana* (Bals.) Vuill. Formulation. R.Z. da Silva[1], P.M.J.O. Neves[1], F. Yamashita[2] and P.H. Santoro[1]. Londrina State University, [1]Department of Agronomy - [2]Department of Food and Drugs Technology, CP 6001 CEP 86051-970, Londrina, PR, Brazil.

FP34 Pathogenesis of *Aphanocladium album on Leptopharsa heveae* (Hemiptera: Tingidae). A. do C.B. Correia, J.B. Negrão Neto and J.M. dos Santos. Departamento de Fitossanidade, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil.

Viruses

VP22 How Cotton Foliage Impedes Lethal Infection of *Heliothis virescens* by *Ac*MNPV. R. Plymale, T. Pomicter and K. Hoover. Department of Entomology, Pennsylvania State University, University Park, PA 16802 USA. **(STUDENT POSTER)**

VP23 Biological relevance of intrastadial developmental resistance of *Lymantria dispar* to LdMNPV. K. Hoover and M. Grove. Department of Entomology, Pennsylvania State University, University Park, PA 16802 USA.

VP24 Isolation of new geographic variants of *Cydia pomonella* granulovirus (CpGV) from the Iran. M. Rezapanah[1,2], S. Shojai-Estrabragh[3], A. Kharrazi-Pakdel, J. Huber[4] and J.A. Jehle[5]. [1]Biocontrol Dept., Plant Pests and Diseases Research Institute, Tehran, Iran. [2]Tarbiat Modarres University, Tehran, Iran. [3]National Research Center of Genetic Engineering & Biotechnology, Tehran, Iran. [4]Institute for Biological Control, BBA Darmstadt, Germany. [5]State Education and Research Center for Agriculture Viticulture and Horticulture, Neustadt/Wstr., Germany. **(STUDENT POSTER)**

VP25 Identification and analysis of two putative DNA-binding proteins from *Cf*MNPV. J. de Jong,[1] B. Arif,[2] and P. Krell,[1]. [1]Department of Microbiology, University of Guelph, Guelph, ON, Canada, N1G 2W1. [2]Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada, P6A 2E5.

VP26 Construction of a baculovirus expression system based on gp50 of CfDEFNPV. Xiao. Wen. Cheng[1], T.R. Henriques[1], E.J. Lingohr[1], P.J. Krell[2] and B.M. Arif[1]. [1]Laboratory for Molecular Virology, Great Lakes Forestry Center, 1219 Queen St E, Sault Ste. Marie, Ontario, P6A 2E5, Canada. [2]Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

VP27 Genetic stability and virulence of *Anticarsia gemmatalis* nucleopolyhedrovirus used as bioinsecticide in Brazil. M.L. Souza[1], M.E.B. Castro[1], W. Sihler[1], L.O. Leitão[1], Z.M. de A. Ribeiro[1] and F. Moscardi[2]. [1]Embrapa Recursos Genéticos e Biotecnologia, C. Postal 02372, CEP 70849-970, Brasília-DF, Brazil. [2]Embrapa Soja, C. Postal, 231, CEP 86001-970, Londrina-PR, Brazil.

VP28 Molecular characterization and sequence analysis of the *Anticarsia gemmatalis* Multicapsid Nuclear Polyhedrosis Virus (AgMNPV) glycoprotein GP64. M.G. Pilloff, M.E. Lozano and P.D. Ghiringhelli. LIGBCM, Departamento de Ciencia y Tecnología, Universidad Nacional de

Quilmes, Argentina. **(STUDENT POSTER)**

VP29 A transfer vector for the generation of recombinant AgMNPVs containing large insertions of foreign DNA. R.F. Méndez[1], C. Karacsonyi[1], M.A.Manzán[1], E.I. Arana[1], A. Sciocco-Cap[2], P.D. Ghiringhelli[2] and V. Romanowski[1,3]. [1]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata; [2]IMYZA, INTA, Castelar, [3]Universidad Nacional de Quilmes, Argentina.

VP30 Identification and sequence analysis of a putative EpapGV envolope fusion protein. A. Goldberg[1,2], M.A. Manzán[2], P.D. Ghiringhelli[3], A. Sciocco-Cap[1], and V. Romanowski[2,3]. [1]Instituto de Microbiología y Zoología Agrícola (INTA). [2]IBBM, Facultad de Ciencias Exactas, UNLP. [3]Departamento de Ciencia y Tecnología, UNQ, Argentina.

VP31 A quick and simple method for the analysis of putative recombinants during plaque purification. C.B. McCarthy[1] and V. Romanowski[1,2]. [1]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. [2]Dto. Ciencia y Tecnología, Universidad Nacional de Quilmes, Argentina.

VP32 Identification and molecular characterization of *Epinotia aporema* granulovirus helicase genes. M.A. Manzán[1], A. García[1], E. Arana[1], A. Sciocco-Cap[2], P.D. Ghiringhelli[3], and V. Romanowski[1,3]. [1]IBBM, Facultad de Ciencias Exactas, UNLP. [2]Instituto de Microbiología y Zoología Agrícola INTA). [3]Departamento de Ciencia y Tecnología, UNQ, Argentina.

VP33 Localization and sequence analysis of an inhibitor of apoptosis gene in the EpapGV genome. A.D. Parola[1,2] and V. Romanowski[1,2]. [1]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. [2]Universidad Nacional de Quilmes, Argentina.

VP34 Comparison of DNA sequences of polyhedrin gene of the Latvian isolates of *Malacosoma neustria* nucleopolyhedrovirus. L. Jankevica[1], M. Kropa[1] and E. Jankevics[2]. [1]Institute of Biology, University of Latvia, Latvia, and [2]LU Biomedical Research and Study Center, Latvia.

VP35 Further results with optical brighteners as enhancers to *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus activity. L. Morales[1], F. Moscardi[2] and D.R. Sosa-Gómez[2]. [1]EMATER-Paraná/UFPR, Londrina-PR, Brazil. [2]Embrapa-Soja, Londrina-PR, Brazil.

VP36 Characterization of the ecdysteroid UDP-glucosyltransferase (*egt*) gene of *Spodoptera frugiperda* nucleopolyhedrovirus. V.F. Tumilasci[1], É. Leal[2], T. Luque[3], P.M.A. Zanotto[2] and J.L.C. Wolff[4]. [1]ICB, Universidade de São Paulo, Brazil. [2]ICB, Universidade de São Paulo, Brazil. [3]Department of Biological Sciences, Imperial College, London UK. [4]NIB, Universidade de Mogi das Cruzes, SP, Brazil.

VP37 A recombinant *Cydia pomonella* granulovirus expressing green fluorescent protein. S.L. Wormleaton, G. Keane, N. Naish and D. Winstanley. Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK.

VP38 Susceptibility of chilean target pests to entomopathogenic fungi. M.G. Gerding[1], M. Rodríguez[1], A. France[1] and M. Gerding. [1]Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Quilamapu, Chillán, Chile

VP39 Identification of a *Bombyx mori* multiple nucleopolyhedrovirus isolate (BmMNPV) in Parana State, Brazil. R.M.C. Brancalhão[1], E.F.B. Torquato[1] and M.E.B. Castro[2]. [1]Universidade Estadual do Oeste do Paraná, Cascavel, PR, Brazil. [2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.

VP40 Enhancement of *Agrotis ipsilon* nucleopolyhedrovirus activity by an optical brightener and potential for control of the black cutworm. A.J. Boughton, L.C. Lewis and B.C. Bonning. Department of Entomology, Iowa State University, Ames, IA 50011, USA.

VP41 Improvement of the pathogenicity of the *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) by inactivating the *egt* gene. F.J.R. Pinedo[1]; F. Moscardi[2]; D. O'Reilly[3] and B.M. Ribeiro[1]. [1]Departamento de Biologia Celular, Universidade de Brasília, Brazil. [2]Embrapa-Soja, Londrina, Paraná-Brazil. [3]Department of Biology, Imperial College of Sciene, Technology and Medicine, London, UK.

VP42 Identification, location and partial sequencing of an *Anticarsia gemmatalis* nucleopolyhedrovirus DNA polymerase gene. M.E.B. Castro[1], C.C. Dalmolin[2], A.C.

B. dos Santos[1], Z.M. de A. Ribeiro[1], B.M. Ribeiro[2] and M.L. Souza[1]. [1]Embrapa Recursos Genéticos e Biotecnologia, C. Postal 02372, CEP 70770-900, Brasília, DF, Brazil. [2]Departamento de Biologia Celular, Universidade de Brasília, Brazil.

VP43 Sequence and transcription analysis of the *Anticarsia gemmatalis* MNPV *p74 locus*. M.N. Belaich.; V.A. Rodriguez, V. Romanowski and P.D. Ghiringhelli. [1]LIGBCM, Departamento de Ciencia y Tecnología; Universidad Nacional de Quilmes. [2]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina.

Bacteria

BP18 Activity of chimeric Cry toxins against *Helicoverpa armigera*. C. Beard[1], C. Rang[2], R. Frutos[2] and R. Akhurst[1]. [1]CSIRO Entomology, GPO Box 1700, Canberra , ACT, 2601, Australia. [2]CIRAD, TA 40/PS1, Boulevard de la Lironde, 34398 Montpellier Cedex 5, France.

BP19 *Ex vivo* toxic potency of the *Bacillus thuringiensis* Cry4B protein on isolated midguts of *Aedes aegypti* larvae. S. Barusrux[1], I. Sramala[1], S. Sakdee[1], A. Bunyaratvej[2], P. Wilairat[3], S. Panyim[1,3] and C. Angsuthanasombat[1]. [1]Institue of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom. [2]Department of Pathology, Faculty of Medicine (Ramathibodee), [3]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand. **(STUDENT POSTER)**

BP20 Bioassay of *Bacillus thuringiensis* toxins against two major coffee pests, i.e. coffee berry borer (*Hypothenemus hampei*) and coffee white stem-borer (*Xylotrechus quadripes*). K. Surekha[1], M. Royer[2], R. Naidu[1], J.-M. Vassal[2], R. Philippe[2], I. Jourdan[2], C. Fenouillet[2], T. Leroy[2] , M. Dufour[2]. [1]Coffee Board, No. 1, Dr. B.R. Ambedkar Veedhi, Bangalore-560 001, India. [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Avenue d'Agropolis, TA 40/02, 34098 Montpellier Cedex 5, France.

BP21 Comparison of the expression of *Bacillus thuringiensis* full-length and N-truncated vip3A gene in *Escherichia coli*. J. Chen, J. Yu, L. Tang, M. Tang, Y. Shi and Y. Pang. State Key Laboratory for Biocontrol & Institute of Entomology, Zhongshan. University, Guangzhou 510275, P.R. China. **(STUDENT POSTER)**

BP22 Characterization of strains of *Bacillus thuringiensis* effective against bolweevil *Anthonomus grandis* Boema, 1843. É.S. Martins, L.B. Praça and R. Monnerat. Laboratory of Bacteriology/Embrapa Recursos Genéticos, P.O.Box 02372, 70849-970, Brasilia, DF, Brazil.

BP23 Evaluation and characterization of *Bacillus thuringiensis* for *Culex quinquefasciatus* and *Aedes aegypti* larvae control. D.G.S. Dias[1], S.F. Silva[1], C.M.S. Soares[2] and R.G. Monnerat[1]. [1]Embrapa Recursos Genéticos e Biotecnologia, P.O. Box 02372, 70849-970 Brasília, Brazil. [2]Bthek Biotecnologia Ltda., SAAN Q. 3 – Lt. 240 (fundos), 70220-000 Brasília, Brazil.

BP24 How vast is the *Bacillus thuringiensis* toxin arsenal to control the geranium bronze? S. Herrero[1], B. Escriche[1], M. Borja[2], and J. Ferré[1]. [1]Department of Genetics, University of Valencia, 46100-Burjassot, Valencia, Spain. [2]Department of R+D, Fundación PROMIVA, 28660-Boadilla del Monte, Madrid, Spain. **(STUDENT POSTER)**

BP25 Four aminopeptidase N loci are involved as Cry1A receptors in Lepidoptera. M. de S. Ibiza-Palacios and B. Escriche. Department of Genetics, University of Valencia. 46100-Burjassot, Valencia, Spain.

BP26 *Bacillus thuringiensis* survey in Brazil: Geographical distribution and insecticidal activity against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). M.R. Barreto[1], F.H. Valicente[2] and E. Paiva[2]. [1]Departamento de Zoologia, UFPR, C.P. 19020, Curitiba-PR, 81531-990. [2]Embrapa Maize & Sorghum, C.P. 151, Sete Lagoas-MG, 35701-970, Brazil. **(STUDENT POSTER)**

BP27 Identification and analysis of *Bacillus thuringiensis* virulence genes. J.R. Steggles and D.J. Ellar. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge CB2 1GA, UK. **(STUDENT POSTER)**

BP28 Environment stress and virulence in *B. thuringiensis* and *B. cereus*. D. Harvie and D.J. Ellar. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge CB2 1GA, UK. **(STUDENT POSTER)**

BP29 Local alternatives to produce *Bacillus thuringiensis* subsp. *israelensis*. P.

Ventosilla, J. Merello, J. Chauca, H. Guerra and B. Infante. Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia. A.P.4314, Lima 100, Peru.

BP30 Studies of *vip3a* gene of vegetative insecticidal protein form *Bacillus thuringensis*. Y. Wu and X. Guan. Biotechology Center, Fujian Agriculture and Forestry University, Fuzhou 350002, P.R. China.

BP31 *Bacillus thuringiensis* serovar *oswaldocruzi* (H-38) and serovar *brasiliensis* (H-39): challenge against *Culicidae* family and crystal's protein profile. L.R. Carvalho[1,2], B.S. Santos[2], J.Q. Chaves[1], C.A.S. Lima[2], M.A. Lamounier[2], A.T.S.M. Corsino[4], M.E.B. Margutti[4], J.B.P. Lima[1,3], V.A.C. Azevedo[4], M.C. Resende[2] and L. Rabinovich[1]. [1]Fundação Oswaldo Cruz, Rio de Janeiro – RJ, [2]Fundação Nacional de Saúde, Belo Horizonte – MG, [3]Instituto de Biologia do Exército, Rio de Janeiro – RJ, [4]Universidade Federal de Minas Gerais, Belo Horizonte, MG., Brazil.

BP32 Persistence of spores of *Bacillus thuringiensis* serovar *israelensis* in various biotopes of a saltmarsh ecosystem in Southern France. M. Hajaij[1], A. Carron[2], J. Deleuze[2], B. Gaven[2], G. Vigo[2,1*], I. Thiéry[3], C.Nielsen-LeRoux[1*] and C. Lagneau[2]. [1]Institut Pasteur, Bactériés Entomopathogènes, (present :* Biochimie Microbienne); [3]Institut Pasteur, Biologie et Génétique du Paludisme, 25, Rue du Dr. Roux, 75724, Paris, France. [2]E.I.D. Méditerranée, 165 Rue Paul Rimbaud BP 6036, F-34030 Montpellier Cedex 1, France.

BP33 Genetic divergence between *Bacillus thuringiensis* and *Bacillus cereus*. G. Vilas-Boas[1,2], V. Sanchis[1,3], D. Lereclus[1,3], M.V. Lemos[2] and D. Bourguet[1]. [1]Institut National de la Recherche Agronomique, La Minière, Guyancourt, France. [2]FCAV/UNESP, Jaboticabal/SP, Brazil. [3]Unité de Biochimie Microbienne, Institut Pasteur, Paris, France.

BP34 Association of PCR and feeding bioassays as first-tier screening method for *Bacillus thuringiensis* strains more efficient in the control of tropical fall armyworm larvae. K.G.B. Boregas[1], M.R. Barreto[2], C.T. Guimarães[1], F.H. Valicente[1] and L.L. Loguercio[3]. [1]Embrapa Maize & Sorghum, Sete Lagoas-MG. [2]Dept. Zoology, UFPR, Curitiba-PR. [3]Depto. Ciências Biológicas, UESC, Ilhéus-BA, Brazil.

BP35 Toxicity of *Bacillus thuringiensis kurstaki* HD68 ICPs to the *Spodoptera frugiperda* (Lepidoptera, Noctuidae) larvae from south Brazil. L.M.N. Pinto[1] and L.M. Fiuza[1,2]. [1]Microbiologia, Centro 2, UNISINOS, RS, Brazil. [2]EEA-IRGA, Cachoeirinha, RS, Brazil.

Microbial Control

MC14 Entomopathogens isolated from field collected termites including *Coptotermes formosanus*. W.G. Meikle, G. Mercadier, A. Kirk, F. Derouané, and S. Gras. USDA-ARS, European Biological Control Laboratory, Campus International de Baillarguet, Montferrier-sur-Lez, 34988, St. Gely du Fesc, CEDEX, France.

MC15 Susceptibility of 8 Types of Corn Grown in Northeast Mexico to Armyworm (*Spodoptera exigua* Hübner), and the potency of *Bt*-based Products (Xen Tari® and Lepinox®). P. Tamez-Guerra, M. Quintanilla, C. Rodriguez-Padilla, L.J. Galan-Wong, R. Tamez-Guerra, R. Gomez-Flores, G. Damas and V. Zamudio. Lab. de Inmunologia y Virologia. Dep. de Microbiologia e Inmunologia. Fac de Ciencias Biológicas, Universidad Autonoma de Nuevo Leon. AP 46-F. San Nicolás de los Garza, N. L. México. 64451.

MC16 Comparative studies of *Bacillus thuringiensis* var. *israelensis* growth and spore production in tradicional and alternative media (Manipueira). S. Ernandes[1], K. Yamaoka[1], A. Oshiro[1], J.C.C. Oliveira[1], V.L. Del Bianchi[1] and I.de O. Moraes[2]. [1]Dept. Food Engineering and Technology - UNESP – São José do Rio Preto, Brazil. [2]Universidade de Guarulhos – Guarulhos, Brazil.

MC17 Biochemical and molecular characterization of a native mosquitocidal strain of *Bacillus thuringiensis* from Argentina. C. Berón and G. Salerno. Centro de Investigaciones Biológicas – Fundación para Investigaciones Biológicas Aplicadas (FIBA). Vieytes 3103 – 7600 Mar del Plata, Argentina.

MC18 PCR-based strategy for cloning a wide spectrum of new *Bacillus thuringiensis* toxin genes. C. Berón, L. Curatti and G. Salerno. Centro de Investigaciones Biológicas – Fundación para Investigaciones Biológicas Aplicadas (FIBA). Vieytes 3103 – 7600 Mar del Plata, Argentina.

MC19 Soybean extracts and their interactions with the nucleopolyhedrovirus of *Anticarsia gemmatalis* in *Ag*MNPV susceptible and resistant populations of the insect. G.C. Piubelli[1], C.B. Hoffmann-Campo[2], F. Moscardi[2], F. Paro[2], A.M. Toledo[3] and R.M. Monte[4]. [1]UFPR, Dept. of Zoology, Curitiba, PR, Brazil. [2]Embrapa Soybean, Londrina, PR, Brazil. [3]UNIFIL, Londrina, PR Brazil. [4]UNOPAR, Londrina, PR, Brazil. **(STUDENT POSTER)**

MC20 Pathogenicity of *Beauveria bassiana* (Moliniaceae), *Steinernama glaseri* and *S. carpocapsae* (Steinernematidae) Brazilian isolates against the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). L.F.A. Alves[1], S.B. Alves[2], V.S. Alves[1] and E.C. Guzzo[1]. [1]UNIOESTE/ CCBS, R. Universitária, 2069, CEP: 85814-110, Cascavel, PR, [2]ESALQ/USP, Depto. de Entomologia, Fitopatologia e Zoologia Agrícola, Piracicaba, SP, Brazil.

MC21 Embrapa's culture collections of microorganisms potentially useful for biological control of agricultural pests. S.C.M. de Mello & J.B.T. da Silva. EMBRAPA-Genetic Resources and Biotechnology (Cenargen), Brasilia, DF, Brazil.

MC22 Microbial control of *Triatoma infestans* with *Beauveria bassiana* in field conditions. R.E. Lecuona, J.A.L. Rodríguez, F.R. La Rossa and M.F. Berretta. IMYZA INTA Castelar. C.C. 25 (1712) Castelar, Bs.As. Argentina.

MC23 Study of the production of *Anticarsia gemmatalis* nucleopolyhedrovirus in airlift reactors of different geometrical design. G.A. Visnovsky[1], J.D. Claus[2] and J.C. Merchuk[3]. [1]Biotech. Dept. and [3]Chem. Eng. Dept., Ben Gurion University, Beer-Sheva, Israel, [2]INTEBIO, Fac. Bioq. Cs. Biol.-UNL, Santa Fe, Argentina.

No abstract available

WORKSHOP (POSTER FORMAT)
Techniques in Microsporidia Research
Convenor: R. Wegensteiner (Poster Session II)

1. Preparation of microsporidia and diseased specimens for detailed study using the electron microscope. J.J. Becnel and S. Shapiro. USDA/ARS, CMAVE, PO Box 14565, Gainesville, FL 32604, USA.

2. Isolation, purification and storage of microsporidian spores. L. Solter, J.J. Becnel and S. White. Illinois Natural History Survey, 140 NSRC, 1101 W. Peabody Dr., Urbana, IL 61801 and USDA/ARS, CMAVE, PO Box 14565, Gainesville, FL 32604, USA.

3. Collection and diagnosis of microsporidia. R. Wegensteiner[1], G. Hoch and L. Solter. [1]Institute of Forest Entomology, Forset Pathology and Forest Protection, Univ.-BOKU-Vienna, Hasenauerstr. 38, A-1190, Vienna, Austria. [2]Illinois Natural History Survey, 140 NSRC, 1101 W. Peabody Dr., Urbana, IL61801, USA.

4. Methods of molecular phylogeny. C. Vossbrinck. The Connecticut Agricultural Experiment Station, New Haven Connecticut, USA.

5. Bioassays. L. Bauer. Michigan State University, East Lansing MI 48824, USA.

Coffee break, 16:00 - 16:30

Tuesday, 16:30 – 17:45
Iguaçu I
CONTRIBUTED PAPERS - Fungi 1
Chair: I. Delalibera Jr. **Pgs. 93 - 95**

16:30 Dose acquisition by second-instar and adult female western flower thrips exposed to leaf disks and impatiens plants treated with *Beauveria bassiana* T.A. Ugine, S.P. Wraight, J.P. Sanderson, M. Brownbridge Cornell University, Ithaca, NY, University of Vermont, Burlington, USA. **(STUDENT PAPER)**

16:45 Studies on the use of entomopathogenic fungi for biological control of the sheep scab mite *Psoroptes ovis*. M.A. de Muro, P. Bates, A.J..Brooks, D. Moore, M.A Taylor and R. Wall, CABI Bioscience, Egham, UK, , Veterinary Laboratories Agency, Addlestone, UK and UK, and School of Biological Sciences, University of Bristol, UK.

17:00 Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to entomopathogenic fungi from Ethiopia. A. Kassa, G. Zimmermann, D. Stephan, and S. Vidal. Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany. Institute for Plant Pathology and Plant Protection, Entomology Section, Georg-August-University, Grisebachstr. 6, D-37077 Goettingen, Germany. **(STUDENT PAPER)**

17:15 Colonization of the Colorado potato beetle following infection by *Beauveria bassiana*. C. Noronha and M.S. Goettel. Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, Charlottetown PEI. Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge Alberta, Canada.

17:30 Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to 4th instar and adult *Blissus antillus* (Hemiptera: Lygaeidae). D.L.A. Coracini, C.A.T.Gava. and R.I. Samuels. Laboratory of Plant Protection, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ 28015-620, Brazil.

17:45 Caged field trials with Hyphomycetes against the Asian longhorned beetle, *Anoplophora glabripennis*. T. Dubois, A.E. Hajek, H. Jiafu and Z. Li. Department of Entomology, Cornell University, Ithaca, NY, USA Forest Bureau of China, Shengyang, Liaoning, China Anhui Agricultural University, Hefei, Anhui, China. (STUDENT PAPER)

Tuesday, 16:30 - 18:30
Iguaçu II
CONTRIBUTED PAPERS - Virus 1
Chair: J.E. Maruniak **pgs 95 - 98**

16:30 Evolution of the *baculoviridae*. E.A. Herniou, J.A. Olszewski, J.S. Cory and D.R. O'Reilly. Department of Biological Sciences, Imperial College, London SW7 2AZ, UK. Ecology and Biocontrol group, CEH – Oxford, Mansfield Road, Oxford OX1 3SR, UK. Syngenta, Jealotts Hill International Research Station, Bracknell RG42 6EY, UK. **(STUDENT PAPER)**

16:45 Characteristics and genome sequence of *Adoxophyes honmai* nucleopolyhedrovirus. M. Nakai, C. Goto, W.K. Kang, M. Shikata, T. Ishii, J. Takatsuka, S. Okuno and Y. Kunimi. Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan. National Agricultural Research Center, Tsukuba, Japan; RIKEN, Saitama, Japan. Shimadzu Corporation, Kyoto, Japan.

17:00 Sequence analysis of the potato tuber moth, *Phthorimaea operculella* granolovirus. L. Croizier, A. Taha, G. Croizier, and M. L. Ferber. Laboratoire de Pathologie Comparée, UMR 5087 and IRD, UR 132, 30380 Saint Christol-les-Alès, France.

17:15 The sequence of the *Rachiplusia ou* multi-nucleocapsid nucleopolyhedrovirus genome. R.L. Harrison and B.C. Bonning. Department of Entomology and Interdepartmental Program in Genetics, Iowa State University, Ames, IA 50011, USA.

17:30 Construction of a random insertion mutant library of HaSNPV and target screening of specific mutants. S. Hou, X. Chen and Z. Hu. Joint-laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese.Academy of Sciences, Wuhan, 430071, P.R. China. Key Laboratory of Agricultural Department, Huazhong Agriculture University, Wuhan, 430072, P.R. China.

17:45 Construction of a transposon-mediated baculovirus vector hanpvid and a new cotton worm cell line AM1-NB for stably expressing barnase gene. Y. Qi , Y. Liu , Y. Zhu and M.N. Joshua. Institute of Virology, Wuhan University, Wuhan 430072, P.R China.

18:00 Diferencial expression of host cellular genes that are up-regulated at early times of infection, from Sf9 cells infected with AcMNPV. I. Nobiron, D.R. O'Reilly and J.A. Olszewski. Department of Biological Sciences, Imperial College of Science, Technology and Medicine, London, United Kingdom, SW7 2AZ. Syngenta, Jealotts Hill International Research Centre, Bracknell, Berks, RG42 6EY, UK.

18:15 Different isolates of shrimp white spot syndrome virus are distinguishable by hypervariable genomic regions. H. Marks, J.M. Vlak and M.C.W. van Hulten. Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands.

Tuesday, 16:30 - 18:30
Cataratas I
SYMPOSIUM (Bacteria 1) - Bacterial Insecticidal Proteins: Specificity, Improvement and Novel Toxins
Convenor: J. Baum

16:30 The diverse armoury of the *B. thuringiensis* crystal. N. Crickmore, University of Sussex, UK.

17:00 The toxin-coding plasmid of *Bacillus thuringiensis* subsp. *israelensis:* host regulation and a new toxin gene. C. Berry[1], S. O'Neil[2], E. Ben-Dov[3], A.F. Jones[1], L. Murphy[2], M.L.A. Quail[2], D. Harris[2], A. Zaritsky[3] and J. Parkhill[2]. [1]Cardiff School of

Biosciences, Cardiff University, Wales, UK. [2]The Sanger Institute, Wellcome Trust Genome Campus, Hinxton Cambridge, UK. [3]Department of Life Sciences, Ben-Gurion University of the Negev, Israel.

No paper available

17:30 Structure of the 51 kDa component of the binary toxin from *B. sphaericus*. J. Allen. Arizona State University, USA.

18:00 DNA shuffling of *B. thuringiensis* crystal proteins. T. Yamamoto, R. Cong, D. Cerf, and K. McBride. Maxygen, Inc., Redwood City, California 94063, USA.

Tuesday, 16:30 - 18:30
Cataratas II
SYMPOSIUM (Fungi 3) - Genetic Structure of Fungal Populations
Convenor: D.R. Sosa Gomez

No paper available

16:30 Genetic structure of Phytopathogenic Fungi. M. Milgroom. Department of Plant Pathology. Cornell University. 334 Plant Science Building, Ithaca, NY 14853-4203 USA.

17:10 The genetic structure of members from the *Entomophthora muscae* species complex proposes high host specificity and clonal life history strategies. A.B. Jensen*, J. Eilenberg and L.Thomsen. Department of Ecology, The Royal Veterinary and Agricultural University, Copenhagen, Thorvaldsensvej 40, 1871 Frb. C., Denmark. *Present address: Department of Forensic Genetic, The University of Copenhagen, Fr. V´s vej 11, 2100 Kbh. Ø, Denmark.

17:50 Parasexuality and its significance in natural populations of entomopathogenic Fungi. J.L. Azevedo. ESALQ-USP. P.O Box 83, 13400-970 Piracicaba, São Paulo, Brazil. Núcleo Integrado de Biotecnologia. Universidade de Mogi das Cruzes. Mogi das Cruzes, São Paulo, Brazil.

Dinner 19:00 - 20:00

Tuesday, 20:00 - 22:00
Cataratas II
DIVISION BUSINESS MEETING
Microbial Control

WEDNESDAY, AUGUST 21

Wednesday, 8:00 – 9:30
Iguaçu I
CONTRIBUTED PAPERS - Fungi 2
Chair: R. Samuels **pgs 98 – 100**

8:00 Polyclonal antibodies production to detection of Beauvericin from the entomopathogenic fungus: *Beauveria bassiana*. V. Arboleda, W. Jorge, F.B. Delgado and A.J. Valencia. Disciplina de Entomología, Cenicafé, Chinchina,Colombia. Dpto. de Química, Universidad de Caldas, Manizales, Colombia.

8:15 Production and characterisation of Pr1 by fungus *Tolypocladium*. A.R. Bandani and T.M. Butt. Plant Protection Department, University of Zabol, Zabol, Iran. Biological Science Departmet, University of Swansea, Swansea, UK.

8:30 Impact of genetic diversity of *Beauveria bassiana* on sustainable control of Masson's pine caterpillars in forest ecosystem. Z. Li, M. Fan, B. Han, D. Ding and B. Wang. Department of Forestry, Anhui Agricultural University, Hefei, Anhui 230036, P.R. China.

8:45 Molecular variation among isolates of the mite pathogenic fungi *Neozygites tanajoae* and *N. floridana*: development of RAPD, AFLP and SCAR markers. I. Delalibera Jr., A.E. Hajek, A. Cherry, B. Briggs, F. Hountondji, R. Hanna and R.A. Humber. University of Wisconsin, Madison, WI, USA, Cornell University, Ithaca, NY, USA, IITA, Cotonou, Benin and USDA/ARS Ithaca, NY, USA.

9:00 Risk assessment of genetic recombination between introduced and indigenous strains of Beauveria bassiana in agricultural fields. L.A. Castrillo, J.D. Vandenberg, and M.H. Griggs. USDA Agricultural Research Service, US Plant, Soil, & Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA.

9:15 Using Entomophthorales for control: maturation, dormancy and survival of laboratory-produced *Entomophaga maimaiga*. A.E. Hajek, J.R. McNeil and M.J. Filotas. Department of Entomology, Cornell University, Ithaca, New York, USA.

Wednesday, 8:00 - 10:00
Iguaçu II
CONTRIBUTED PAPERS - Virus 2
Chair: B. M. Ribeiro **pgs 100 - 102**

8:00 The Op-*iap* gene is required to prevent OpMNPV-induced apoptosis during infection of LD652Y cells. J.C. Means and R.J. Clem. Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506 USA.

8:15 Extensive tissue damage associated with apoptosis in *Spodoptera frugiperda* caterpillars infected with *p35* mutant AcMNPV. T.E. Clarke and R.J. Clem. Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506 USA.

8:30 Molecular characterization of an *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) mutant and the *iap-3* gene of AgMNPV. M.P. Carpes, M.E.B. Castro, E.F. Soares, A.G. Villela and B.M. Ribeiro. Departamento de Biologia Celular, Universidade de Brasília, DF, Brazil. Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.

8:45 Baculovirus apoptotic suppressors trigger global translation arrest in AcMNPV-infected Ld652Y by stimulating viral DNA replication. S.M. Thiem and N. Chejanovsky. Departments of Entomology and Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, USA. Institute of Plant Protection, The Volcani Center, Bet Dagan, Israel.

9:00 Induction of apoptosis by wild-type AcMNPV in infected *Spodoptera litura* larvae blocks viral propagation. P. Zhang, K. Yang, X. Dai, Y. Pang and D. Su. State Key Laboratory for Biocontrol and Institute of Entomology, Zhongshan University, Guangzhou 510275, P.R. China and Virology Research Unit, Fudan University, Shanghai 200433, P.R. China. **(STUDENT PAPER)**

9:15 Identification of six *Autographa californica* M NPV early genes that mediate nuclear localization of G-actin. T. Ohkawa, A.R. Rowe and L.E. Volkman. Department of Plant and Microbial Biology, 251 Koshland Hall, University of California, Berkeley, California, 94720-3102, USA.

9:30 Interaction of HaNPV capsid protein with host actin. L. Songya, Q. Yipeng, G. Guoqiong, L. Lingyun,Institute of Virology, Wuhan University, Wuhan 430072, P.R. China.

9:45 Study of the effect of deletions of ORF145 and ORF150 11K gene homologues in *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). R. Lapointe, J.A. Olszewski, and D.R. O'Reilly. Department of Biological Sciences, Imperial College of Science, Technology and Medicine, London, UK, SW7 2AZ. Syngenta, Jealotts Hill International Research Centre, Bracknell, Berks, RG42 6EY, UK.

Wednesday, 8:00 - 11:00
Cataratas I
SYMPOSIUM (Cross-Division 1) - Bacteria /Insect Interactions: Virulence Aspects
Convenor: D. Lereclus

8:00 Environmental sensing in Bacilli: a basis for host specificity. D.R. Harvie, J.R. Steggles and D.J. Ellar. University of Cambridge, Tennis court Road, Cambridge, CB2 1QW, UK.

8:30 Identification of new *Bacillus thuringiensis* virulence factors by genetic approaches. S. Fedhila-Hamza, P.Nel, T. Msadek, M. Gohar, and D. Lereclus. INRA/La Minière, 78285 Guyancourt cedex, France.

No paper available

9:00 An ABC guide to the toxin complexes of Photorhabdus: novel alternatives for *B. thuringiensis*. R. Ffrench-Constant, University of Bath, Bath BA2 7AY, UK.

9:30 *Xenorhabdus* and *Photorhabdus* virulence factor and their impact on insect cellular immunity. R. Zumbihl, A. Lanois, K. Brugirard, J. Brillard, E. Duchaud, F. Kunst and A. Givaudan. Laboratoire EMIP, Université Montpellier II, INRA, 34095 Montpellier Cedex 05, France and Laboratoire de Génomique des Microorganismes Pathogènes, Institut Pasteur, 25 rue Dr Roux, 75724 Paris Cedex 15, France.

10:00 Insect/Serratia interactions: the question of virulence. T.A. Jackson, M.R.H. Hurst and T.R. Glare. AgResearch, PO Box 60, Lincoln, New Zealand.

10:30 Discussion

Wednesday, 8:00 – 10:00
Cataratas II
WORKSHOP - Bioinsecticide Production Issues, with a Focus on Latin America
Convenors: J. Lord and T. Jackson

8:00 Bacteria production and use in some Latin American countries. D.M.F. Capalbo[1], I.O.Moraes[2] , O. Arantes[3], L. Regis[4] and L Rabinovith[5]. [1]Embrapa Environment, CP 69, Jaguariúna, SP, [2]Universidade Guarulhos, SP, [3]Universidade Estadual de Londrina, PR, [4]CpqAM/FIOCRUZ, Recife-PE, [5] Instituto Oswaldo Cruz, Rio de Janeiro -RJ, Brazil.

8:20 Mass production of Nucleopolyhedrovirus for the control of the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, in soybeans. B. Santos. Universidade Federal do Paraná, Curitiba, Paraná, Brazil.

8:40 Technical aspects of the industrial production of entomopathogenic fungi in Brazil. L.G. Leite[1], A. Batista Filho[1], J.E.M. Almeida[1] and S.B. Alves[2]. [1]Instituto Biológico, Caixa Postal 70, Campinas, SP, 13001-970, Brazil. [2]ESALQ/USP, Depto. De Fitopatologia e Entomologia, Caixa Postal 9, Piracicaba, SP, 13418-900, Brazil.

9:00 Fungi for coffee berry borer. F.J.P. Flórez. Colombia

No paper available

9:20 Small-scale community production of Beauveria in Peru. A. Lagnaoui. Peru.

9:40 Thirty years of massproduction and extensive application of entomogenous fungi in China. Z. Li. Department of Forestry, Anhui Agricultural University, Hefei, Anhui, P.R. China 230036. (328)

Coffee break, 10:00 - 10:30

Wednesday, 10:30 - 12:30
Iguaçu I
SYMPOSIUM (Cross-Division 2) - Microbial Germplasm Repositories: The Legacy, the Problem, the Future
Convenor: R. A. Humber

No paper available

10:30 From Culture Collections to Biological Resource Centers: local and international initiatives. V.P. Canhos, Centro de Referencia em Informaçao Ambiental, Av. Romeu Tortima, 388, 13084-520, Campinas, SP, Brazil.

10:55 Global perspectives on the discovery, isolation, preservation, and exploitation of entomopathogenic fungal germplasm. R.A. Humber and M.S. Tigano USDA-ARS Plant, Soil & Nutrition Laboratory, Tower Rd., Ithaca, NY, 14853-2901, U.S.A., Embrapa, Recursos Genéticos e Biotecnologia, Av. W5 Norte, Brasília, DF. 70700-900, Brazil.

11:20 Managing microsporidian germplasm. L.F. Solter[1] and J.J. Becnel[2]. [1]Illinois Natural History Survey, 140 NSRC, 1101 W. Peabody Dr., Urbana, IL 61801, [2]USDA/ARS, CMAVE, PO Box 14565, Gainesville, FL 32604, USA.

11:45 Entomopathogenic bacterial repositories. R.G. Monnerat, Embrapa Recursos Genéticos e Biotecnologia, Av. W5 Norte, Brasília, DF. 70700-900, Brazil.

12:10 Perspectives and challenges facing insect viral germplasm repositories. M.L. de Souza. and F. Moscardi. Embrapa Recursos Genéticos e Biotecnologia, Av. W5 Norte, Brasília, DF. 70700-900, Brazil, Embrapa Soja, Rodovia Carlos João Strass, Londrina PR, 86001-970, Brazil.

Wednesday, 10:30 - 12:30
Iguaçu II
SYMPOSIUM (Nematodes 2) - Entomopathogenic Nematodes: Research Trends
Convenors: E. De Nardo and P. Grewal

No paper available

10:30 Novel insecticidal toxins and other metabolites of *Xenorhabdus and Photorhabdus*. D. Bowen, USA.

11:00 Ecological genetics of entomopathogenic nematodes: Are there metapopulations? P. Grewal. Department of Entomology, Ohio State University, Wooster, OH 44691, USA.

11:30 Evaluating nontarget effects on below ground invertebrates. E.A.B. De Nardo[1], P S. Grewal[2] and N. Somasekhar[2]. [1]Embrapa Meio Ambiente, Jaguariuna, São Paulo, Brazil. [2]Dept. of Entomology, Ohio Agricultural Research and Development Center (OARDC), Ohio State University, Wooster, Ohio, USA.

12:00 Virulence mechanism of a slug-parasitic nematode and its associated bacterium. L. Tan and P.S. Grewal. Department of Entomology, Ohio State University, Wooster, Ohio 44691, USA.

Lunch, 12:30 - 14:00

Wednesday, 14:00 - 18:00
EXCURSION

18:30 - 21:30
BARBECUE

THURSDAY, AUGUST 22

Thursday, 8:00 - 10:00
Iguaçu I
CONTRIBUTED PAPERS - Fungi 3
Chair: J. Pell **pgs 102 - 104**

8:00 Quantitative measurements for the fecundity of *Myzus persicae* apterae infected by the entomophthoraceous fungus *Zoophthora anhuiensis*. H.-P. Li. and M.-G. Feng. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou 310029, P.R. China.

8:15 Time-specific infection rate of *Beauveria bassiana* conidia on *Myzus persicae* under controlled conditions. S.-T. Xu, M.-G. Feng and S.-H. Ying. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou 310029, P.R. China.

8:30 Phenoloxidase activity of diamondback moth *Plutella xylostella* and its changes during invasion of *Zoophthora radicans*. Q.-E. Liu, J.-H. Xu, and M.-G. Feng. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou, 310029, P.R. China.

8:45 Temperature gradients and viability of *Beauveria bassiana* conidia on leaf surfaces. S.Y. Gouli, W. Reid and V.V. Gouli. Entomology Research Laboratory, University of Vermont, USA.

9:00 Molecular studies on intra-specific variation in the aphid pathogenic fungus *Erynia* (= *Pandora*) *neoaphidis*. A. Tymon, P.A. Shah and J.K. Pell. Plant and Invertebrate Ecology Division, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK.

9:15 A tiered approach for evaluating *Erynia neoaphidis* isolates against seven aphid species. P.A. Shah, S.J. Clark† and J.K. Pell Plant and Invertebrate Ecology Division, † Bioinformatics Unit IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK.

9:30 Effect of dissolved oxygen concentration in submerged culture of entomopathogenic fungus *Paecilomyces fumosoroseus*. P. Fernández, M. Gutiérrez and M. de la Torre. Dept. of Biotechnology, CINVESTAV-IPN, Mexico City, Mexico and Dept. of Biotechnology, UAM-I, Mexico City, Mexico.

Thursday, 8:00 - 10:00
Iguaçu II
CONTRIBUTED PAPERS - Virus 3
Chair: J.L.C. Wolff **pgs 104 - 107**

8:00 Involvement of IE0 and IE1 in the replication of the *Autographa californica* nucleopolyhedrovirus in *Spodoptera littoralis*. L. Lu and N. ChejanovskyÆ. Department of Entomology, Inst. of Plant Protection, The Volcani Center, POB 6, Bet Dagan, Israel.

8:15 Interchange of OpMNPV and AcMNPV replication factors restores the replication function of chimeric IE1 proteins. J. Pathakamuri and D.A. Theilmann . Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C., Canada V0H 1Z0, Department of Plant Science, Agricultural Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4.

8:30 Deletion of the baculovirus non-*hr* origin of DNA replication prevents the accumulation of defective interfering particles. G.P. Pijlman, J.C.F.M. Dortmans, D.E. Martens, R.W. Goldbach and J.M. Vlak. Laboratory of Virology and Food and Bioprocess Engineering Group, Wageningen University, Wageningen, The Netherlands. **(STUDENT PAPER)**

8:45 Analysis of the impact of deletion of the LdMNPV p24, 25K FP, and PE genes on polyhedron synthesis and morphology. J.M. Slavicek, N. Hayes-Plazolles and M.E. Kelly. USDA Forest Service, Forestry Sciences Laboratory, 359 Main Road, Delaware, Ohio, USA 43015.

9:00 Cloning *Panolis flammea* nucleopolyhedrovirus genotypes from ixed populations. R.B. Hitchman, L.A. King[2] and R.D. Possee . NERC Institute of Virology and Environmental Microbiology, Oxford, OX1 3SR. School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK.

9:15 Functional analysis of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus envelope fusion protein. G. Long, M. Westenberg, H. Wang, M. Usmany, H. Wang , X. Chen, D. Zuidema, Zhi-Hong Hu, and J.M. Vlak. Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD, Wageningen, The Netherlands. Joint-laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, the People's Republic of China.

9:30 Genetic requirements for homologous recombination in *Autographa californica* nucleopolyhedrovirus. E.A. Crouch and A.L. Passarelli. Division of Biology, Molecular, Cellular, and Developmental Biology Program, Kansas State University, Manhattan, Kansas 66506, USA.

9:45 Characterization of *Mamestra configurata* nucleopolyhedrovirus enhancin and its functional analysis via expression in an AcMNPV recombinant. Q. Li, C. Donly, L. Li, K. Moore, D.A. Theilmann and M. Erlandson. Saskatoon Research Centre, AAFC-Saskatoon, SK; Southern Crop Protection and Food Research Centre, AAFC, London, Ont. and Pacific Agri-Food Research Centre, AAFC, Summerland, B.C.

Thursday, 8:00 - 10:00
Cataratas I
SYMPOSIUM (Bacteria 2) - Bt Transgenic Plants and Insect Resistance to Bt Toxins
Convenor: J. Ferre and J. Van Rie.

8:00 Current status of *B. thuringiensis* resistance and *B. thuringiensis* resistance-management in Bt cotton in the U.S. W.J. Moar. Auburn University, Auburn, AL 36849, USA.

8:30 *Bacillus thuringiensis* toxin and nematodes: mechanisms of resistance and toxicity. R Aroian, J. Griffitts, J. Wei, K. Hale, J. Whitacre, D. Huffman, K. Chien and K. McDonald. University of California, San Diego, CA 92093-0349, USA.

9:00 Managing resistance to Bt plants through use of gene and promoter strategies and field tactics. A.M. Shelton, J.-Z. Zhao, E.D. Earle, R.T. Roush and J. Cao. Cornell University, USA.

9:30 Transgenic Bt rice expressing a synthetic *cry*1B Gene: Expression strategies and field protection against the striped stem borer. J.C. Breitler, M. Royer, J.M. Vassal, J. Messeguer,V.Marfa, M.Del Mar Catala, B. San Segundo, JA. Martinez-Izquierdo, D. Meynard and E. Guiderdoni. BIOTROP and Crop Protection, CIRAD-AMIS, F-34398-Montpellier cedex 5, France.

Thursday, 8:00 - 10:00
Cataratas II
SYMPOSIUM (Microbial Control 1) - Solar Irradiation of Fungal Pathogens: Deleterious Effects, and Mitigation through Genetics and Formulation
Convenors: D. Roberts and D. Moore

No paper available

8:00 Tools of the UV trade: Light sources, filtering, measuring irradiance, and selecting biological weighting factors (action spectra). D.W. Roberts[1] and S.D. Flint[2]. [1]Department of Biology and [2]Department of Rangeland Resources, Utah State University, Logan, Utah 84322 USA.

No paper available

8:30 Damage to fungi from solar/UV exposure, and genetic and molecular-biology approaches to mitigation. G.U.L. Braga[1] , S.D. Flint[2], D.E.N. Rangel[1], C.D. Miller[1], F. Freimoser[3], R.J. St. Leger[3], A.J. Anderson[1] and D.W. Roberts[1]. [1]Dept. of Biology and [2]Dept. of Rangeland Resources and the Ecology Center, Utah State University, Logan, UT 84322-5305, USA. [3]Dept. of Entomology, University of Maryland, College Park, MD 20742-4454, USA.

9:20 Mitigation of solar damage to microbial control agents through formulation and application technology R. Bateman and D. Moore. CABI Bioscience, Silwood Park, Ascot, Berkshire SL5 7TA UK.

Coffee break 10:00 - 10:30

Thursday, 10:30 - 12:30
SIP GENERAL BUSINESS MEETING

Lunch, 12:30 - 14:00

Thursday, 14:00 - 16:00
Iguaçu II
SYMPOSIUM (Cross-Division 3) - Microsporidia within Entomophthorales
Convenors: J. Becnel and R. Humber

14:00 Origin and metabolic adaptation of microsporidia. P. Keeling. Canadian Institute for Advanced Research, Department of Botany, University of British Columbia, 3529-6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada.

No paper available

14: 30 Molecular phylogenetics of Microsporidia: why do different genes tell us different stories. R.P. Hirt. Molecular Biology Unit, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK.

15:00 Characteristics of the Microsporidia: Reasons to ponder that Microsporidia are highly evolved fungi. J.J. Becnel. USDA, ARS, CMAVE, 1600 S.W. 23rd Drive, Gainesville, FL, USA.

15:30 Microsporidian roots and branches within the Zygomycetes? Take a number and step in line! R.A. Humber. USDA-ARS, Plant Protection Research Unit, US Plant, Soil & Nutrition Laboratory, Tower Road, Ithaca, NY, USA.

Thursday, 14:00 - 17:00
Cataratas II
SYMPOSIUM (Microbial Control 2) - Microbial Control of Insect Pests of Potato; from Tiera del Fuego to the Great White North
Convenors: L.A. Lacey and J.D. Vandenberg

14:00 Insect pests os potatoes in the Western Hemisphere and the potential for their control using entomopathogens. L.A. Lacey, USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, USA.

14:25 Microbial control of potato tuber moth and Andean potato weevil in South America. A. Lagnaoui, J. Alcazar and A. Vera. International Potato Center, Apartado 1558, Lima 12, Peru.

14:50 The discovery, development and death of *Bacillus thuringiensis* var. *tenebrionis* as a microbial control product for the Colorado potato beetle. W D Gelernter. PACE Consulting, 1267 Diamond Street, San Diego, CA, 92109 USA.

15:15 Microbial control of the Colorado potato beetle in rain-fed potato agroecosystems in the Northeastern US. E.Groden,S.P. Wraight,and F A Drummond, USDA-ARS, Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA and E. Groden, Department of Biological Sciences, University of Maine, 5722 Deering Hall, Orono, Maine, 04469-5722 USA.

15:40 Microbial control of insect pests of potato in Canada and the Western United States. M.S. Goettel, L.A. Lacey,C. Noronha and D. Hunt,Lethbridge Research Centre, Agriculture and Agri-Food Canada, Box 3000, Lethbridge, Alberta, T1J 4B1 Canada, USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, USA,. Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Ave, Charlottetown, PEI, C1A 7M8, Canada, Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Centre, 2585 Highway 20 E, Harrow, ON, N0R 1G0, Canada.

16:05 Integration of insect-resistant transgenic plants, predators and parasitoids, and microbial agents for the control of potato pest insects. C. Cloutier, D..Michaud and J. Brodeur Département de biologie, Université Laval, Quebec City, Quebec, Canada, Département de phytologie, Université Laval, Quebec City, Quebec, Canada., Département de phytologie, Université Laval, Quebec City, Quebec, Canada.

16:30 Summary/Discussion. J.D. Vandenberg, USDA-ARS, Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA.

Thursday, 14:00 - 16:00
Cataratas I
WORKSHOP - Ethics, Legal and Regulatory Concerns of Transgenic Plants
Convenors: J.-L. Schwartz and W. Moar

14:00. Development of international scientific biosafety testing guidelines for transgenic plants. A. Hilbeck[1], D. Andow[2], D.M.F. Capalbo[3], E. Underwood[1] and the Steering Committee[4]. [1]Swiss Federal Institute of Technology, Geobotanical Institute, Zurich. [2]Department of Entomology, University of Minnesota. [3]Embrapa Environment, Jaguariúna, SP, Brazil. [4]Steering Committee nominated at the end.

Paper not available
14:10. Bt trangenic plant registration in Brazil. Eliana Fontes, Embrapa, Brazil.

Paper not available
14:20 Ethics of Bt trangenic plants .Les Levidow, The Open University, UK.

Paper not available
14:30. Bt trangenic plant registration in Canada. Philip MacDonald, Canadian Food Inspection Agency, Canada.

Paper not available
14:40. Bt trangenic plant registration in Germany. Christiane Saeglitz, University of Aachen, Germany.

14:50. Considerations for research in agricultural biotechnology. A.M. Shelton. Department of Entomology, Cornell University/NYSAES, Geneva, NY, USA Cornell University, USA.

15:00 Discussion

Coffee break, 16:00 - 16:30

Thursday, 16:30 - 18:30
Iguaçu II
CONTRIBUTED PAPERS - Virus 4
Chair: A.S. de Cap **pgs 107 - 110**

16:30 Origin and host range of the *Choristoneura fumiferana* defective nucleopolyhedorvirus, CfDEFNPV. H.A.M. Lauzon, L. Pavlik, P.D. Ghiringhelli, A.S. de Cap, P. Krell and B.M. Arif. Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Canada. Departamento de Ciencia y Tecnologia-CEI, Universidad Nacional de Quilmes, Bernal, Argentina. Dept. of Microbiology, University of Guelph, Canada.

16:45 Construction of HaSNPV polyhedra with polyhedrin-fusolin fusion protein. F. Deng, X. Pan, D. Wu, X. Chen, B.M. Arif and Z. Hu . Joint-laboratory of Invertebrate Virology, Wuhan Institute of Virology, CAS, Wuhan 430071, Wuhan Institute of Hydrobiology, CAS, Wuhan 430072, P.R. China . Canadian Forest Service, Great Lake Forestry Center, Sault Ste. Marie, Ontario P6A 5M7 Canada.

17:00 Further characteristics of developmental resistance of *Lymantria dispar* (Lepidoptera, Lymantriidae) to its baculovirus, *Ld*MNPV. M. Grove, L. Behrendt, M. Beercheck, O. Thompson, B. Pundiak, K. O'Connor, A. Draeger, M. Geleskie and K. Hoover. Department of Entomology, The Pennsylvania State University, 501 ASI Building, University Park, PA, 16802, USA.

17:15 Vertical transmission of HaNPV marked with green fluorescent protein gene in the larvae of cotton bollworm and its infectious course in the host L.Zuqiang, Q. Yipeng, L.Lingyun, Y. Fuhua, L.Songya Institute of Virology, Wuhan University, Wuhan, 430072, China.

17:30 Vertical transmission of nucleopolyhedrovirus in *Spodoptera exempta*. L. Vilaplana, K. Wilson and J. Cory . Molecular Ecology and Biocontrol Group, NERC - Centre for Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR and Institute of Biological Sciences, University of Stirling, Stirling FK9 4LA, UK.

17:45 Developments in the use of endemic baculoviruses of *Plutella xylostella* (diamondback moth) for control of DBM in East Africa. M. Parnell , D. Grzywacz, G. Kibata, G. Odour, W. Ogutu, D. Miano and D. Winstanley. Sust. Agric. Group, Nat. Res. Inst., Univ.of Greenwich Central Ave., Chatham Maritime, Kent ME4 4TB, UK. Kenya Agric. Res. Inst., Waiyaki Way, PO Box 14733, Nairobi, Kenya. CAB Intl., Africa Reg. Centre, PO Box 633, Village Market, Nairobi Kenya. Hort. Res. Intl., Wellesbourne, Warwickshire, CV35 9EF UK.

18:00 Research on the environmental release and ecological effects of gene manipulation bacoluviruspesticide application in vegetable pests control. L. Yao, W. Zhou, F. Yang, L. LI and Y. Qi. Institute of Virology, Wuhan University, Wuhan 430072, P.R. China.

18:15 Incidence and impact of *Entomophaga aulicae* and a Nucleopolyhedrovirus in an outbreak of the whitemarked Tussock Moth (Lepidoptera: Lymantriidae). K. van Frankenhuyzen[1], P. Ebling[1], G. Thurston[2], C. Lucarotti[2], T. Royama[2], R. Guscott[3], E. Georgeson[3] and J. Silver[4]. [1]Canadian Forest Service, Sault Ste. Marie, Ontario, and [2]Fredericton, New Brunswick, [3]Nova Scotia Department of Natural Resources, Shubenacadie, Nova Scotia, [4] University of Toronto, Scarborough, Ontario, Canada.

Thursday, 16:30 - 18:30
Cataratas I
CONTRIBUTED PAPERS - Bacteria 4
Chair: J.E. Ibarra **pgs 110 - 112**

16:30 Phylogenetic relationship between the *Bacillus thuringiensis* type strains, based on the sequence of the flagellin gene. M. Santos-Mendoza, J.E. Ibarra, A. Delecluse, and V. Juarez-Pérez. Departamento de Biotecnología y Bioquímica, CINVESTAV-I.P.N., Irapuato, Gto. Mexico. Laboratoire des Bactéries et Champignons Entomopathogènes, Institut Pasteur, Paris, France.

16:45 Molecular analysis of a Brazilian *Bacillus thuringiensis* strain collection. M.V.F. Lemos, G. Vilas-Boas, J.A.D. Sena and E.G.M. Lemos. Departamento de Biologia Aplicada à Agropecuária/ FCAV/ UNESP, Jaboticabal/SP, Brazil. Departamento de Tecnologia/FCAV/UNESP, Jaboticabal/SP, Brazil.

17:00 Could the low concentration of Cry protein be the consequence of accumulation of citrate or lactate in fed batch cultures? U. Gaona, E. López-y-López and M. de la Torre. Centro de Investigación y de Estudios Avanzados del IPN, Departamento de

Biotecnología y Bioingeniería, México D.F., México.

17:15 Does exist a relationship between enthalpy consumed, PHB accumulated and Cry production in *Bacillus thuringiensis*? K. Navarro, R.R. Farrera and F. Pérez-Guevara. Dept. of Biotechnology, Centro de Investigación y de Estudios Avanzados del IPN and Dept. of Biochemical Engineering, Escuela Nacional de Ciencias Biológicas, Mexico.

17:30 Shaking flask fermentation of *Bacillus thuringiensis*. H. Tianpei, Q. Sixin, H. Zhipeng, H. Biwang and G. Xiong. Biotechnology Center, Fujian Agriculture and Forestry University, Fuzhou 350002, China.

17:45 Comparative studies between *Bacillus thuringiensis* subsp.*thuringiensis* and *Bacillus thuringiensis* subsp. *israelensis* production by solid state fermentation to use as a bioinsecticide. I.O. Moraes, R.O.M. Arruda, D.M.F Capalbo, R.O. Moraes, V.L. Del Bianchi. Universidade Guarulhos, Laboratório de Bioprocessos/ CEPPE. Praça Tereza Cristina, 01 Centro, CEP 07023-070 Guarulhos/SP. CNPMA/ EMBRAPA, Jaguariúna/SP. UNESP/ S. José do Rio Preto, SP. Brazil.

18:00 Production and commercialization of biological insecticides in Colombia. E. Morales[1] and S. Orduz[2]. [1]Live System Technology. Bogotá, Colombia. [2]Unidad de Biotecnología y Control Biológico. Corporación para Investigaciones Biológicas, Medellín, Colombia.

18:15 The expression of *gfp* gene in *Bacillus thuringiensis*. Z. Qin, Z. Qiong, S. Ming, Z. Junchu and Y. Ziniu. Huazhong Agricultural University, Key Laboratory of Agricultural Microbiology of Ministry of Agriculture, National Engineering Research Center of Microbial Pesticides, Wuhan, Hubei, 430070, P.R. China.

Thursday, 16:30 - 18:30
Iguaçu I
WORKSHOP - Preservation of Entomopathogenic Fungi
Convenor: R. Humber

1. Workshop in methods for the preservation of fungal cultures. R. A. Humber. USDA-ARS Plant, Soil & Nutrition Laboratory, Ithaca, New York, USA.

Thursday, 16:30 - 18:30
Cataratas II
WORKSHOP – Microbiol Control of the Coffee Berry Borer by Entomopathogens Fungi
Convenor: P. Neves

16:30 Microbiol control of the coffee berry borer in Colombia. F J P Flórez, Cenicafé, Colômbia.

17:00 Microbiol control of the coffee berry borer in Nicarágua. F. Guharay, CATIE/MIP-AF (NORAD), Managua, Nicaragua.

17:30 Microbiol control of the coffee berry borer in Brazil. P M J O. Neves, S. B Alves and A. Moino Jr., Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

18:00 Use of fungal pathogens for the management of coffee berry borer, *Hypothenemus hampei* – the Indian Experience. K. Sreedharan, M.M. Balakrishnan, C.B. Prakasan and R.. Naidu. Central Coffee Research Institute, Coffee Research Station 577 117, Chikmagalur Dt., Karnataka, India.

Thursday, 19:30
BANQUET

FRIDAY, AUGUST 23

Friday, 9:00 - 11:00
Iguaçu I
CONTRIBUTED PAPERS - Fungi 4
Chair: A. Hajek **pgs 112 - 115**

9:00 Importance of initial inocula and host density for development of *Zoophthora anhuiensis* induced epizootic in *Myzus persicae* colonies. M.-G. Feng and H.-P. Li. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou, 310029, P. R. China.

9:15 Interactive effect of water content and temperature on viability of *Beauveria bassiana* conidia and metabolism of internally reserved nutrients in storage. M.-G. Feng and S.-H. Ying. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou 310029, P.R. China.

9:30 Impact of temperature and humidity regimes on the development of *Zoophthora anhuiensis* induced epizootic in *Myzus persicae* population. M.-G. Feng and H.-P. Li. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou 310029, P.R. China.

9:45 Isolation of two β-1,3-glucan binding proteins from hemolymph of *Plutella xylostella* larvae. J.-H. Xu, Q.-E. Liu, M.-G. Feng. Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou, 310029, P.R. China.

10:00 Preliminary studies on bioactivities of metabolites from the entomogenous Fungus *Aschersonia aleyrodis*. J. Qiu, T. Huang and X. Guan. Biotechnology Center, Fujian Agricultural and Forestry University, Fuzhou 350002, China.

10:15 Diversity of subterranean entomopathogenic fungi in Southern coastal belt of Sri Lanka. H.C.E. Wegiriya. Department of Zoology, University of Ruhuna, Wellamadama, Matara, Sri Lanka.

10:30 Impact of species diversity on sustainable control of Masson's pine caterpillars with *Beauveria bassiana* in forest ecosystem. Z. Li, M. Fan, B. Han, D. Ding and B. Wang. Department of Forestry, Anhui Agricultural University, Hefei, Anhui 230036, P.R. China.

10:45 Impact of *Beauveria bassiana* on species diversity and sustainable control of Masson's pine caterpillars in forest ecosystem. Z. Li, M. Fan, B. Han, D. Ding and B. Wang. Department of Forestry, Anhui Agricultural University, Hefei, Anhui 230036, P.R. China.

Friday, 9:00 - 11:00
Cataratas I
SYMPOSIUM (Bacteria 3) - *B. thuringiensis* and *B. sphaericus* Mosquitocidal Strains: Use and Necessities
Convenor: C.N. LeRoux.

9:00 *Bacillus thuringiensis* and *Bacillus sphaericus* useful tools for mosquito and blackfly control and a short history of two insecticides development. L. Rabinovitch[1], R.S.A. Alves[1], C.M.B. Silva[1], C de.F.G. Cavados[1], Jeane Q.J.[1], B.S. Santos[2], M.A. Lamounier[2] and M.C. Resende[2]. [1]FIOCRUZ, IOC – Depart. of Bacteriology, Rio de Janeiro, [2]FUNASA, Entomology staff, Belo Horizonte, Brazil.

9:30 *Bacillus thuringiensis israelensis*: a model for improving microbial insecticides for mosquito control. M.C. Wirth, W.E Walton, and B. Federici. University of California, Riverside, CA92506, USA.

10:00 Strains and application strategies for improving the use of *B. sphaericus* and *B. thuringiensis* against mosquitoes. L. Regis[1], M.H.Silva-Filha[1],M.A.V.M.-Santos[1], C.M.F. Oliveira[1] and C.N.-LeRoux[2]. [1]Dept of Entomology, CPqAM, FIOCRUZ, Recife Brazil. [2]Institut Pasteur, Paris, France.

10:30 Molecular characterization of a resistance mechanism to the *B. sphaericus* binary toxin in *Culex pipiens*. I. Darboux, Y. Pauchet, C. Castella, M.H. Silva-Filha, C.N. LeRoux, J.F. Charles and D. Pauron. INRA UMR 1112 "ROSE" BP2078 06606 Antibes, France.

END OF THE MEETING

ABSTRACTS

Monday, 14:00 - 16:00
Cataratas I
CONTRIBUTED PAPERS - Bacteria I
Chair: A. Bravo

Hydropathic complementarity determines interaction of epitope ^{869}HITDTNNK^{876} in *Manduca sexta* Bt-R_1 receptor with loop 2 of domain II of *Bacillus thuringiensis* Cry1A toxins

I.Gomez[1], J. Miranda-Rios[1], E. Rudiño-Piñera[2], D. I. Oltean[3], S. S. Gill[3], A. Bravo[1], and M. Soberón[1]

1,2Instituto de Biotecnología, 1 Departamento de Microbiología Molecular, 2 Departamentode Reconocimiento Molecular y Bioestructura. UNAM. Apdo postal 510-3, Cuernavaca, Morelos 62250, México. 3 Department of Cell Biology and Neuroscience, University of California, Riverside, CA 92521, USA

In susceptible insects, Cry toxin specificity correlates with receptor recognition. In previous work, we characterized a scFv antibody (scFv73) that inhibits binding of Cry1A toxins to cadherin-like receptors . CDR3 region of scFv73 shared homology with an eight amino acid epitope (^{869}HITDTNNK^{876}) of the *M. sexta* cadherin-like receptor Bt-R_1 (Gomez, I., Oltean, D., Gill, S., Bravo, A., and Soberón M. (2001). J. Biol. Chem. 276: 28906-28912). In this work, we show that the previous sequence of scFv73 CDR3 region was obtained from the noncoding DNA strand. However, most importantly, both scFv73 CDR3 amino acid sequences of the coding and noncoding DNA strands have similar binding capabilities to Cry1Ab toxin as Bt-R_1 ^{869}HITDTNNK^{876} epitope, as demonstrated by the competition of scFv73 binding to Cry1Ab with synthetic peptides with amino acid sequences corresponding to these regions. Using synthetic peptides corresponding to three exposed loop regions of domain II of Cry1Aa and Cry1Ab toxins we found that loop 2 synthetic peptide competed binding of scFv73 to Cry1A toxins in Western blot experiments. Also, loop 2 mutations that affect toxicity are affected in scFv73 binding. Toxin overlay assays of Cry1A toxins to *Manduca sexta* brush border membrane proteins showed that loop 2 synthetic peptides competed binding of Cry1A toxins to cadherin-like Bt-R_1 receptor. These experiments identified loop 2 in domain II of as the cognate binding partner of Bt-R_1 ^{869}HITDTNNK^{876}. Finally, ten amino acids from α-6-loop 2 region of Cry1Ab toxin (^{363}SSTLYRRPFNI^{373}) showed hydropathic pattern complementarity to a ten amino acid region of Bt-R_1 (^{865}NITIHITDTNN^{875}) suggesting that binding of Cry1A toxins to Bt-R_1 is determined by hydropathic complementarity and that the binding epitope of Bt-R_1 may be larger than the one identified by amino acid sequence similarity to scFv73.

A cell–cell signaling peptide activates the PlcR virulence regulon in bacteria of the *Bacillus cereus* group

L. Slamti [1] and D. Lereclus [1, 2]

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PlcR is a pleiotropic regulator that activates the expression of genes encoding various virulence factors such as phospholipases C, proteases and hemolysins, in *Bacillus thuringiensis* and *Bacillus cereus*. We show that the activation mechanism is under the control of a small peptide: PapR. The *papR* gene belongs to the PlcR regulon and is located 70 bp downstream from *plcR*. It encodes a 48-amino acid peptide. Disruption of the *papR* gene abolished expression of the PlcR regulon, resulting in a large decrease in hemolysis and virulence in insect larvae. We demonstrated that the PapR polypeptide was secreted, then reimported *via* the oligopeptide permease Opp. Once inside the cell, a processed form of PapR, presumably a pentapeptide, activated the PlcR regulon by allowing PlcR to bind to its DNA target. This activating mechanism was found to be strain–specific, with this specificity determined by the first residue of the pentapeptide. **(STUDENT PAPER).**

The importance of N-terminal activation

A. Bravo[1], J. Sánchez[1], T. Kouskoura[2] and N. Crickmore[2]

[1] Insituto de Biotechnología, Universidad Nacional Autonoma de Mexico , Morelos, México and [2]School of Biological Sciences, University of Sussex, Brighton, UK

It is well known that activation of the Cry1 delta-endotoxins of *Bacillus thuringiensis* requires proteolytic removal of sequences from both the N- and C-termini. The C-terminal extension to the active toxin is not required for toxicity, but perhaps has a role in toxin crystallization or stabilization. Less is known about the function of the N-terminal peptide and whether its removal is an important step in the toxicity process. We will present data showing that removal of the N-terminal peptide is important, and that mutants in which this removal is prevented have significantly altered binding and pore-formation properties *in vitro* and reduced toxicity in bioassays.

Expression of cadherin-like receptors for Cry1Aa from silkworm in cultured mammalian cells

Y. Tsuda, K. Hashimoto, F. Nakatani, T. Fukada, K. Sugimoto And M. Himeno*

Department of Applied Biochemistry, College of Agriculture, Osaka Prefecture University, 1-1 Gakuen-cyo, Sakai, Osaka 599-8531, Japan (*preset address:Faculty of Home Economics, Kobe Women's University, Higashisuma, Suma, Kobe 654-8585,Japan)

The insecticidal crystalline protein bodies produced by *Bacillus thuringiensis* are dissolved in midgut after ingestion by susceptible insect larvae, and proteolytically processed into an active toxin (Cry toxin). Cry toxins bind to specific receptor in midgut epithelial cell and as a result, the cells swell and lyse. Two kinds of proteins in the silkworm midgut membrane, aminopeptidase N and cadherin-like protein (BtR175), were shown to bind to Cry1Aa toxin. We independently isolated cDNAs for three BtR175 variants, BtR175a, BtR175b, and BtR175c. The nucleotide sequences of these BtR175a, b, and c were different from BtR175 at 2, 31, and 28 positions, respectively, resulting in 1, 6, and 5 amino acids substitution. cDNAs of BtR175a, b, and c protein were joining to mammalian expression vectors, adding the

myc-epitope tag to the C-terminus of BtR175 genes and were introduced into non-susceptible cultured mammalian cells: Caco2, COS7, HEK293, and MDCK cells. Observation by immunofluorescence microscopy showed that the three BtR175 variants were transiently expressed in the transfected COS7 cells. All the BtR175 variants expressed in the mammalian cells localized in ER and plasma membrane, and had binding activity to Cry1Aa toxin. Immunoblot analysis showed that BtR175b was expressed in COS7 cells as a 200-kDa protein, slightly larger than the 180-kDa protein purified from silkworm. The tranformed HEK293 cells by *BtR175b* (clone HKb20) stably express BtR175b protein. ^{125}I-labeled activated Cry1Aa bound to the plasma membrane fraction prepared from clone HKb20 cells. BtR175 was detected as a 200 kDa protein on a western blot. These BtR175b-expressing cells swelled and died in the presence of activated Cry1Aa. It showed that BtR175b expression was sufficient to confer Cry1Aa-susuceptibility to mammalian cells.

Glycosyltransferases mediate Bt toxin action in *Caenorhabditis legans*

J. S. Griffitts, L.D. Marroquin, and R.V. Aroian

Division of Biology, University of California, San Diego, USA

Mutants resistant to *Bacillus thuringiensis* Cry toxins (called Bre for Bt toxin resistant) have been isolated in the worm *Caenorhabditis elegans*. These mutants identify five genes (*bre-1* through *bre-5*), each of which is required for *C. elegans* susceptibility to Cry5B, a member of the major family of insecticidal Cry proteins. Two of these genes, *bre-3* and *bre-5*, have been identified, and their predicted functions have been investigated. *bre-5* encodes a putative glycosyltransferase, belonging to a family which incudes beta-1,3 Gal- and beta-1,3 GlcNAc transferases. Members of this family can be found in nematodes, insects, and mammals. The predicted product of *bre-3* is remarkably similar (60% identical) to a Drosophila protein of demonstrated importance for insect development, but unknown biochemical activity. BRE-3 shares distant kinship with other beta-glycosyltransferases from bacteria. Given that BRE-3 and BRE-5 resemble glycosyltransferases, we have investigated the possibility that these genes are involved in the biosynthesis of a specific glycoconjugate. To demonstrate genetically that these enzymes act in a common pathway, we have quantified resistance in *bre-3* and *bre-5* loss-of-function single and double mutant strains. Early results from an assay based on growth inhibition indicate that dose response curves for the single mutants, as well as the double mutant, are virtually superimposable, suggesting that these genes control a single pathway. Analysis of genetic mosaics, along with ectopic expression and immunostaining experiments demonstrate that bre-3 and bre-5 are necessary and sufficient in the cells of the gut epithelium to mediate susceptibility, and that their protein products localize to cytoplasmic puncta which may be in the secretory pathway. These results are consistent with a role for BRE-3 and BRE-5 in the synthesis of a glycan structure required for the binding of toxin to the apical surface of gut epithelial cells. The evidence obtained so far for the inability of *bre-3* and *bre-5* mutant animals to bind toxin is based on the observation that these mutants are highly resistant to endocytosis of dye-labeled toxin into their gut cells, while normal animals exhibit rapid uptake of toxin under identical conditions. We have observed striking defects in the glycoconjugate profile of *bre-3* and *bre-5* mutants, have established an epistatic relationship between the genes, and are currently working to identify the precise substrates of BRE-3 and BRE-5 activity and their relationship to Bt toxin action. **(STUDENT PAPER).**

Charged Residues in Helix-4 of the *Bacillus thuringiensis* Cry4B Toxin are involved in Ion Channel Conductivity: Site Directed Mutagenesis and Molecular Dynamics Simulations Studies

I. Sramala[1], W. Fischer[2], M. Sansom[3], and S. Panyim[1], C. Angsuthanasombat[1]

[1]Laboratory of Molecular Biophysics, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom, 73170 Thailand [2]Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, UK [3]Laboratory of Molecular Biophysics, University of Oxford, Oxford, OX1 3QU, UK

We have previously demonstrated that Arg-158 in helix-4 of the 130-kDa *Bacillus thuringiensis* Cry4B toxin is important for mosquito-larvicidal activity, likely to be involved in ion channel formation. Mutant toxins with substitutions at Arg-158 and/or Glu-159 were further characterized by means of nanosecond (ns) molecular dynamics (MD) simulations and single channel properties. Unrestrained MD simulations were performed with a modelled pore consisting of 6 copies of the alpha-4-alpha-5 hairpin (residues Q_{140}-E_{198}) of Cry4B placed in an equilibrated POPC/water bilayer system with Arg-158 pointing inward to pore lumen. After modelled mutations at Arg-158 or Glu-159 was achieved by using the 2-ns coordinates as a template, MD simulations of the hexameric pore induced by Cry4B or mutants were conducted for 1 ns. Mutant channels showed alterations of electrostatic profiles and diameters of the channel cavity. Single channel properties of the 65-kDa activated Cry4B and mutant toxins, R158A and E159A, were studied via planar lipid bilayers. The results revealed that Cry4B and E159A formed cation selective channels with a similar maximum opening level of conductivity (ranging from 200-400 pS) while R158A showed a decrease in conductivity levels (ca 70 pS). The results suggest that R158A, which is a key residue in the toxin inducted channel cavity, is an important determinant for ion conductivity of the Cry4B toxin.

Early transcription of *Bacillus thuringiensis cry* genes in Lepidoptera-active strains

M. Porcar[1], C. Martínez[1], J. E. Ibarra[2], and V. Juárez-Pérez[1]

[1]Laboratoire des Bactéries et Champignons Entomopathogènes. Institut Pasteur, Paris, France; and [2] Departamento de Biotecnología y Bioquímica, CINVESTAV- I.P.N., Irapuato, Gto. México

Twenty-five strains of the entomopathogenic bacterium *Bacillus thuringiensis* highly toxic to the lepidopteran pests *Manduca sexta* and *Plutella xylostella* were analyzed. Firstly, *cry* genes of known lepidopteran activity were identified by a PCR screening. Secondly, six strains were selected according to their gene content

and insecticidal potency. These strains were later cultured under controlled conditions, and aliquots taken at different pre- and post-sporulation stages. Total RNA from these aliquots was used as template in RT-PCRs directed towards the identification of mRNAs of the *cry* genes identified previously by PCR. Results showed the transcription of genes *cry1A*, *cry1E*, *cry11*, and *cry2* not only after the onset of the sporulation, but also before this period. This is the first report of an early transcription of these *cry* genes. However, this early transcription show little expression levels of the corresponding Cry proteins, as deduced from SDS-PAGE profiles. The typical high expression levels of *cry* genes were observed from t5 to t11. Interestingly, transcripts of the *cry11* gene, a gene that has been reported as a cryptic gene in naturally occurring strains of *B. thuringiensis*, were detected during most of the analyzed period (including the early stage) and in all the strains; however, the expression of its corresponding protein was not detected. In addition, the *cry* gene content of each strain may have a delaying effect on the sporulation onset, due to the early transcription of *cry* genes. Strains with four different *cry* genes showed transcripts as early as t-2 and translation as early as t-1; while the strain with one *cry* gene showed transcripts from t5 onwards and translation from t9 onwards. Also, the transition phase between the growing period and the sporulation took longer in strains holding four different *cry* genes than that of the strain with one *cry* gene. Intermediate scores were observed in strains holding three *cry* genes. According to these results, we may speculate that the early transcription of *cry* genes probably compete with the transcription of sporulation genes, causing a delay in the sporulation process.

Addition of the *Bacillus sphaericus* binary toxin to *B. thuringiensis* subsp. *morrisoni* (PG-14) and *B. thuringiensis* subsp. *Jegathesan* markedly improves their toxicity

Hyun-Woo Park [1], D. K. Bideshi [1] and B. A. Federici [1, 2]

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

Several strains of *Bacillus sphaericus* such as 2362 produce small crystals of the binary protoxin (BIN toxin) that are composed of 42- and 51-kDa mosquiotcidal proteins highly toxic to certain species of mosquito larvae. In previous studies, we showed that high levels of the Bs2362 BIN protein could be produced in *B. thuringiensis* subsp. *israelensis* (Bti IPS-82) using *cyt1A* sporulation-dependent promoters combined with STAB-SD mRNA stabilizing sequence (*cyt1A*-p/STAB-SD). Recombinant Bti/Bs cells that produced both the normal complement of Bti toxins and the Bs BIN toxin were from eight to tenfold more toxic to *Culex* species than parental Bti or Bs cells. In the present study, markedly improved toxicity against 4th instar larvae of *Culex quinquefasciatus* was obtained by introducing binary toxin gene under the control of *cyt1A*-p/STAB-SD system into two other mosquitocidal strains of *B. thuringiensis*, *B. thuringiensis* subsp. *morrisoni* PG-14 (LC_{50} = 900 pg/ml) and subsp. *jegathesan*. In the Btm/Bs strain, the toxicity (LC_{50} = 1 ng/ml)) against 4th instars of *Cx. quinquefasciatus* was comparable to the Bti/Bs recombinant, whereas the toxicity (LC_{50} = 5.2 ng/ml) of the Btj/Bs recombinant was considerably lower, but nevertheless, more toxic than the parental strains. An advantage of the latter strain, even though not as toxic as the Bti/Bs strian is that its complement of mosquitocidal Cry proteins is greater than Bti's. Thus, this recombinant could be useful in managing the potential for resistance to Bs and Bti in mosquito populations. It must be stressed, however, that though high levels of resistance have been reported to Bs in field populations, no resistance has been reported to Bti after more than twenty years of use against many mosquito species.

Bacillus sphaericus resistance: *in vitro* binding of the binary toxin to *Culex quinquefasciatus* larvae selected with the strain IAB59

M. H. Silva-Filha[1], C. M. F. Oliveira[1], C. Nielsen-LeRoux[2] and L. Regis[1]

[1]Centro de Pesquisas Aggeu Magalhães- FIOCRUZ, Recife, Brazil and [2]Unité de Biochimie Microbienne, Institut Pasteur, Paris, France

Bacillus sphaericus (*Bs*) has proved its effectiveness against *Culex* larvae in vector control programs and it has also shown that populations submitted to strong selection pressure can develop resistance to *Bs*. Low and high resistance levels to strains 2362, 1593 and C3-41 were detected among *Culex* larvae. Colonies highly resistant to those strains, show reciprocal cross-resistance, while they display only a low cross-resistance towards the strain IAB59. The goal of this work was to investigate the ability of the Bin2 toxin, the major toxic factor found in some *Bs* strains (2362, 1593, C3-41), to bind the brush border membranes fraction (BBMF) from a *C. quinquefasciatus* colony maintained under selection with the strain IAB59, which express a Bin1 toxin. Previous assays showed that both Bin toxins bind in a similar manner to the BBMF from susceptible larvae. *In vitro* binding assays between the radiolabelled (^{125}I) Bin2 toxin and BBMF from the IAB59-selected larvae were carried out, at 3 different moments: before selection (parental generation), and during selection at generations 12 and 25. The Bin2 toxin showed specific and saturable binding to BBMF from the parental generation displaying a K_d and B_{max} of 9.8 ± 3.2 nM and 6.3 ± 1.2 pmol/mg of BBMF, respectively. At F12, the resistance ratio of the colony to IAB59 was 4.1-fold and the profile of Bin2 toxin binding detected, was similar to that observed for the parental. Resistance ratio to IAB59 at F25, corresponding to only 49.8-fold, was related to an absence of specific and saturable binding of the Bin2 toxin to the BBMF from these larvae. Previously, binding failure was only observed in colonies displaying high level resistance to *Bs* strains. However it may also be the case for this IAB59 low-level resistant colony, since it is highly resistant to both strain 2362 and Bin1 toxin. Data indicate that resistance is related to the binding failure of Bin toxins and it is likely that Bin2 and Bin1 use the same receptor. The moderate larval activity of strain IAB59 may be due to other toxic factors and further studies will be conducted to evaluate its mode of action and the potential of IAB59 to lead to high level resistance.

Monday, 14:00 - 15:15
Iguaçu I
CONTRIBUTED PAPERS - Nematodes 1
Chair: P. Grewal

Entomopathogenic nematodes and conservation biological control

C. Hoy, J. Lawrence and P. Grewal

Department of Entomology. Ohio Agricultural Research and Development Center. The Ohio State University. Wooster, Ohio 44691 USA

Use of entomopathogenic nematodes in crop protection has been characterized by inundative applications to achieve rapid control. This approach has been successful in many cases, particularly in soil or high-moisture environments. Extensive research has explored ways to increase the efficacy of inundative applications. The ecological understanding of entomopathogenic nematodes in natural systems, however, has accumulated more recently. Survey's demonstrate that entomopathogenic nematode species are quite common, suggesting that they can persist in varied environments. Their role in soil food webs is beginning to become known. A strategy of conserving entompathogenic nematodes to stabilize and suppress soil insect populations in agricultural systems could be built upon ecological knowledge of their role in natural systems and study of their population dynamics. Sensitivity analysis of simple population dynamics models can extend laboratory data on response to the abiotic environment and provide guidelines for field study of such ecological parameters as host species diversity, host distribution in time and space, nematode reproductive potential, development, survival, and dispersal capability. Potential means of manipulating these parameters to conserve nematode populations in crop systems can be evaluated as well.

Adaptation of entomopathogenic nematodes to insect food plant

M.E. Barbercheck[1], J. Wang[1] and C. Brownie[2]

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We conducted assays to determine if characteristics of entomopathogenic nematodes vary in response to continued exposure to a particular insect/host plant combination, and whether selection on an insect/host plant combination results in changed performance on other insect/host plant combinations. Three isolates of *Steinernema carpocapsae* (Agriotos, Mexican, and a Hybrid) were continuously cultured in corn-fed (*Zea mays*) or squash-fed (*Cucurbita pepo*) southern corn rootworm, *Diabrotica undecimpunctata howardi* , for 25 passages. The selected nematodes were also compared to the same isolates maintained on *Galleria mellonella* ("unselected"). The ability of nematodes selected on corn-fed rootworms to kill corn-fed rootworms compared to squash-fed rootworms improved significantly only in the Mexican isolate. The Agriotos isolate selected on corn-fed rootworms showed a non-significant tendency for an increase in ability kill corn-fed rootworms. Virulence of the Hybrid isolate did not change in response to selection on rootworms or host plant/rootworm combination. All nematode isolates selected on squash-fed rootworms declined in virulence and progeny production. In general, progeny production did not change with selection on corn-fed. Only the Mexican isolate showed a significant relationship between numbers of progeny produced from corn-fed rootworms and number of passages through rootworms. There were no effects of selection host or test host on length of IJ in selected or unselected nematode isolates, but IJ length was positively correlated with the area of stained lipids. The area of stained lipids, but not lipid density, was related to selection host. The Hybrid isolate had a greater area of stained lipids than the Agriotos or Mexican isolates. Infective juveniles from isolates selected on corn-fed rootworms had a greater area of stained lipids than IJ selected on squash-fed rootworms or maintained in *G. mellonella* and tested on rootworms. These results suggest that characteristics of entomopathogenic nematodes can respond to selection on insect/host plant combinations, but that changes are dependent on the nematode isolate and the particular host plant.

Endotoxin Activity of Lipopolysaccharide Produced by *Moraxella osloensis* against the Grey Garden Slug *Deroceras reticulatum*

L. Tan and P. S. Grewal

Department of Entomology, Ohio State University, Wooster, Ohio 44691, USA

Moraxella osloensis is a gram-negative aerobic bacterium associated with *Phasmarhabditis hermaphrodita*, a slug-parasitic nematode that has prospects for the biological control of mollusk pests, especially the grey garden slug *Deroceras reticulatum*. This bacteria-feeding nematode acts as a vector to transport *M. osloensis* into the shell cavity of the slug, and the bacteria then multiply and kill the slug within one to two weeks. We discovered that *M. osloensis* produces an endotoxin(s) to kill the slug when injected into the shell cavity. Injection of purified lipopolysaccharide (LPS) from *M. osloensis* cultures into the shell cavity caused slug death with an estimated LD_{50} of 48 µg per slug. No contact or oral toxicity of the LPS to the slug was detected. Isolated lipid A portion from the LPS was toxic to the slug after injection into the shell cavity, but the polysaccharide portion was not. Moreover, we semiquantitated the LPS as 6×10^7 endotoxin units per milligram and detected endotoxin activity in the lipid A portion but not in the polysaccharide portion by *limulus* amebocyte lysate assays. Furthermore, the analysis of the LPS by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by visualization with silver staining revealed that the LPS from *M. osloensis* is a rough-type LPS with an estimated molecular weight of 5,300. This appears to be the first report of an active biological toxin against molluscs. **(STUDENT PAPER).**

The Australian clinical isolates of *Photorhabdus* constitute a new taxon

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Photorhabdus spp. are usually found in symbiotic association with entomopathogenic nematodes of the family Heterorhabditidae. However, two groups of *Photorhabdus* have also been isolated from human clinical specimens. The first group, isolated from six patients from various parts of the USA, was shown to be significantly different from those *Photorhabdus* spp. associated with entomopathogenic nematodes and

assigned to a new species, *P. asymbiotica*. The second group was isolated from five patients from several eastern Australian states. PCR-RFLP analysis of the 16S rDNA gene indicated a very high level of homogeneity within each group but not between groups. A polyphasic study of the two groups, utilising *16S rDNA* and *gyrase B* gene sequencing, DNA/DNA hybridisation, and phenotypic characterisation, confirmed the taxonomic differentiation of the two groups of clinical *Photorhabdus*.

Endemic entomopathogenic nematodes in diverse vegetable landscapes

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Towards identifying suitable entomopathogenic nematode species/strains to manage pests reducing yields in diverse vegetable landscapes, a series of surveys and field evaluations were conducted in North East Ohio. Surveys involved sampling cropped areas as well as grassy banks and ditches adjacent to vegetable systems for nematode isolates. Field studies focused on identifying a strategy to improve nematode persistence through the manipulation of host interactions via crop sequences. Endemic nematode populations were isolated at >15% of all sites sampled through 2001, with a greater number of isolates being detected on grassy banks as compared with cropped areas. Isolates included strains of *Steinernema feltiae*, *Heterorhabditis bacteriophora* and *Heterorhabditis megidis*. In experimental research, higher levels of persistence were associated with a more continuous supply of hosts. **(STUDENT PAPER).**

Monday, 16:30 - 18:30
POSTER SESSION I

FP1 Transformation of *Beauveria bassiana* mediated by *Agrobacterium tumefaciens* using herbicide resistance gene as a selectable marker

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Beauveria bassiana has been developed for pest control and genetic engineering is a promising method to extend its usage. However, transformation of *Beauveria bassiana* had hitherto not been reported. Recently, *Agrobacterium tumefaciens*-mediated transformation has been proved successful in several filamentous fungi. We have developed a system for *B.bassiana* transformation mediated by this approach with a novel selectable marker. Although Hyromycin B resistance marker is commonly used in the transformation of plant, bacteria, and fungi, it is not suitable for transformation of *B.bassiana* that has great resistance to it (HygromycinB at 2mg ml^{-1}concentration cannot inhibit the growth of *B.bassiana*). Hence we introduced a herbicide resistance gene, *bar* encoding phosphinothricin acetlytransferase (PAT), as a selectable marker which was proved to be low cost and high efficiency for transformants selection, into an *A.tumefaciens* binary vector, pBANF-bar. T-DNA carried on pBANF-bar was transferred to *B.bassiana* mediated by *A.tumefaciens* with frequency of 80 transformants 10^{-5} conidia and 2-3 transformants 10^{-5} conidia in presence of 100μM AS (acetosynrigone) and in absence of As, respectively. Most of transformants contained one single copy of T-DNA. Phosphinothricin resistance and T-DNA integrated into genome were stable. Among twenty transformants analyzed, T_6 and T_{12} showed distinct differences in conidial yield, colony growth, virulence to insect and conidial germination behavior from Bb0062 (wild type strain). **(STUDENT POSTER).**

FP 2 Cloning and characterization of cuticle degrading enzyme CDEP-1 from *Beauveria bassiana*

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Beauveria bassiana extracellular subtilisin-like serine endoprotease is a potential virulence factor by virtue of its activity against insect cuticles. A cDNA library was constructed using mRNA from mycelia of *Beauveria bassiana* grown on cuticle/chitin cultures. A cDNA clone of the protease, designated CDEP-1, was isolated from cDNA library. CDEP-1 contained an 1134bp ORF that predicted a protein of 377amino acids with M_r=38616 and PI=8.302. The amino acid sequence of the gene shows 57.9%, 83.3% and 54.7% identity to *Metarhizium anisopliae* Pr1, *Beauveria bassiana* Pr1 and proteinase K, respectively. Southern analysis indicated that CDEP-1 was present as single copy in *Beauveria bassiana*. **(STUDENT POSTER).**

FP 3 Kinetics and mechanism of the amyloid-like interfacial self assembly of the hydrophobin Sc3

P. A. Stroud, J. S. Goodwin, C. L. McCormick, G. C. Cannon, and P. Butko

University of Southern Mississippi, Hattiesburg, MS 39406, USA

The amphipathic fungal proteins hydrophobins are able to self-assemble into highly stable insoluble supramolecular structures at polar/nonpolar interfaces. This property of hydrophobins is apparently utilized by parasitic fungi for attachment to the insect or plant hosts. Utilizing polyacrylamide gel electrophoresis, atomic-force microscopy, ultracentrifugation, fluorescence spectroscopy and light microscopy, we investigated kinetics and mechanism of hydrophobin self assembly and attempted to gain insight in the underlying protein conformational changes. Hydrophobin Sc3 from *Schizophyllum commune* was used as a model system. We found that two dyes that specifically interact with stacked beta sheets in amyloid fibrils - thioflavin T (ThT) and Congo red (CR) - also bind to Sc3 assemblies. This indicated that Sc3, an all-beta-protein, assembles into amyloid-like structures via beta-sheet stacking. However, unlike amyloid proteins, Sc3 assembly was not accelerated in a seeded reaction. The rate of assembly of Sc3 was found to be dependent on the concentration of the protein, the pH of the solution and the area of the air/water interface. Based on our results, we propose a novel three-state model for Sc3 self assembly.

FP 4 Entomopathogenic fungi for white grub control in south of Chile

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The wheat white grub, *Hylamorpha elegans* Burmeister and the Carmelito beetle prairie white grub, *Phytoloema hermanni* Germain, cause economic damage in pastures in central and south zones of Chile. Until now, control has been carried out mainly by the use of chemical products, with unsatisfactory results. Entomopathogenic fungi have proved abroad to be an effective control method for many species of white grub, and could be a viable solution for these two pests. Furthermore, INIA-Quilamapu has an entomopathogenic fungi native collection, which includes the genus *Metarhizium*, *Beauveria* and *Paecilomyces*. Therefore, 38 and 33 *Metarhizium* and *Beauveria* isolates from this collection were tested as dry conidia in third instar of *P. hermanni* and *H. elegans* larvae, respectively, to evaluated the effect on larval mortality and fungal sporulation at daily base. The results indicate significant differences among the isolates. The highest mortality were reached by isolates Qu-B294, Qu-M142 and Qu-M549 in *P. hermani* (71-85%), and Qu-M270 and Qu-M802 in *H. elegans* (100%). There were no record of mortality in the control treatments for both species. Besides, results shown great specificity, where the most effective isolates were those isolated from naturally infected white grubs. Further laboratory and greenhouse studies were accomplished to select the most virulent and effective strain, between two *M. anisopliae* isolates (Qu-M270 and Qu-M802) on *H. elegans* larvae. The first evaluation was carried out by immersion of third instar larvae on different conidial doses (0 to 10^8 conidia mL^{-1}) for each isolate. Comparisons were made by calculating the area under mortality progress curve. The results showed that Qu-M270 area was superior in 50% to Qu-M802 ($P=0.032$). Furthermore, the LC50 and LC90 for Qu-M270 were $10^{4.7}$ and $10^{7.5}$ conidia mL^{-1}, respectively. The greenhouse evaluation was performed in pots with pasteurized soil and 10 larvae of *H. elegans* on it. The treatments were four suspensions (0, 10^{12}, 10^{13} and 10^{14} conidia ha^{-1}) applied on the soil surface. After 30 days of inoculation, the doses of 10^{13} and 10^{14} conidia ha^{-1} did not show differences ($P<0.05$) between them, with 95 and 94% of mortality, respectively. The 10^{12} conidia ha^{-1} produced less mortality (74%) and was statistically inferior ($P=0.045$) to the previous doses. The control treatment (10%) was statistically different to all the treatments.

FP 5 Susceptibility of chilean target pests to native entomopathogenic nematodes

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Along Chile, 65 entomopathogenic nematode isolates have been collected in the last 6 years. The parasitic ability of these native entomopathogenic nematodes, to aerial and terrestrial larvae of insect pests such as *Aegorhinus superciliosus, Asynonychus cervinus, Hylamorpha elegans, Tipula apterogyne, Eumerus* sp., *Dalaca pallens, Delia antiqua, Cydia molesta* and *Cydia pomonella* was evaluated. Overall, inoculations were made by applying 50 infective juveniles on insect larvae. Symptoms and mortality of inoculated insects were evaluated daily. The most virulent nematode isolates were selected by assessment of lethal time for the different insect species. There were differences in pathogenicity among the nematode isolates, showing great specificity for some insect pests. Future evaluations will help us to determine the efficiency of these isolates in the field and their adaptations to different soils and temperatures.

FP 6 Selection of *Beauveria bassiana* (Bals.) Vuill. colonies resistant to pesticides

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Resistance to synthetic pesticides frequently applied in insect pests and diseases control is known in several organisms such as fungi, insects and mites. Entomopathogenic fungi, like other organisms, can be selected for resistance to pesticides through selection pressure by their frequent use, allowing the use of them for both chemical and biological control at the same time against pests and/or diseases. In this work, resistant *B. bassiana* colonies to fenpropathrin (Meothrin) and iprodione (Rovral) which show fungistatic properties on the pathogen, were selected after successive inoculations in medium containing these chemicals, and also evaluated the patterns of development of the fungus after elimination of the selection pressure. The fungus was inoculated several times in PDA medium containing the chemicals until no differences were detected with check in vegetative growth and sporulation, being then evaluated for germination (viability test) and pathogenicity on *Galleria melonella*. The parameters were evaluated after the selection of the resistant material with the fungus growing in medium without the addiction of the chemical product. Resistance of the fungus was obtained only for fenpropathrin after three successive inoculations. There was no change in the development parameters in the resistant material when inoculated in medium without the pesticide, indicating the possibility of fungus production in artificial medium after the selection for resistance to a pesticide, making possible their associated utilization.

FP 7 Characterization and mass production of *Paecilomyces tenuipes*, entomopathogenic fungus collected in Korea

Sung-Hee Nam[1], S. Lee[2], I-Yeon Jung, Sang-Duk Ji and Sae-Yun Cho

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About 300 species of entomopathogenic fungi have been published worldwide and their parmacololgically active have been widely recognized for a long time. Korean isolate of *Paecilomyces tenuipes*, anamorphic state of *Cordyceps takaomontana*, was inoculated into *Nosema bombysis* and successfully reproduced, of which products were proved to strengthen immune system, anti-fatigue

and anti-cancer activity. Therefore, this study was initiated to characterize Korean isolate of *Paecilomyces tenuipes* and find the optimal condition for cultivation and eventually to set up the mass production system. *Paecilomyces tenuipes* develops 1–5 synemata with a large amount of conidia in the upper part. Colonies on potato dextrose agar (PDA) show 32 mm diam. growth in 14 days at 25℃ and pale yellow with reverse yellowish white color. Conidia are ellipsoidal to ovoid in shape and 2.9–6.6 × 1.5–2.5μm in size. Phialides are grouped in 3–4 whorls, clavate and 4.0–6.6 × 2.0–2.2 μm in size. In the selecting test for medium, the growth rate was higher in all three types of silkworm larva agar (SLA-A, B, C) than in PDA. Among them SLA-A showed the highest growth, 61.8±1.25 mm in diameter which doubled that of PDA. In silkworm larva broth, the higher the spin speed produced the smaller diameter of hyphal clumps. Net weight was the highest in over 150 rpm and 12L/12D with 6 g of 6 mm bead as 1.322g. The production of conidia on brown rice agar was the greatest in pH 5.0 with temperature change from 24℃ to 20℃ as 4.3±0.35 $\times 10^8$ CFU/ml. Synemata was formed most excellent in the dark with an 12 hr-alternative 12℃ and 18℃ as 32.5±0.8 mm and 56.7 in count.

FP 8 Effects of temperature on the survival of propagules of the entomopathogenic Hyphomycete *Paecilomyces fumosoroseus* (Wize) Brown and Smith

A. L. Carmona, A. Asaff, O. Gómez and M. de la Torre

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The detrimental effect of high temperatures on the survival of propagules of the entomopathogenic Hyphomycete *Paecilomyces fumosoroseus* PfrD strain was studied by monitoring germinability and ability to form colonies (CFU) of propagules maintained at different temperatures and time intervals in an aqueous suspension (0.005 % Tween 80). All experiments were conducted in a water bath with a variation of temperature of ± 0.5°C. The fungus was grown in two different liquid media: a complex media for blastospores production and a defined media for conidiospore-like propagules (submerged conidia) production. Aerial conidia were obtained by culture on Sabouraud's dextrose agar (SDA). The rate of germination of propagules was estimated by determining the proportion of spores germinating on solid media maintained at 28°C at regular intervals. The criterion for germination was that propagule must form a germ tube of a length at least two times the diameter of propagule. Probit analysis was used to estimate time to 50% germination (GT_{50}) for each propagule. The values obtained of GT_{50} at 28°C were 6.3,14.0 and 11.0 h for blastospores, submerged conidia and aerial conidia respectively. Likewise, survival isothermals were obtained for several temperatures and for each one of propagules, which permitted to establish 3-D Gaussian mathematical models that will describe the viability of propagules as function of temperature and exposition time. Blastospores were the most sensible propagules to temperatures over 35°C. Both submerged an aerial conidia were more resistant to temperature than blastospores, although submerged conidia showed a little more resistance than aerial conidia. Each one of these propagules are employed as biocontrol agents and a good knowledge of it persistence and the environmental factors affecting it, such as temperature, are needed to estimate the probability of success as biopesticides on field crops. **(STUDENT POSTER).**

FP 9 Interactions between two species of mitosporic fungi, larvae of *Musca domestica* and *Stomoxys calcitrans*, and the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae)

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The stable fly (*S. calcitrans*) and the house fly (*M. domestica*) are major pests in animal husbandry, and both species are frequently found coexisting on farms. Stable flies are an increasing problem in Danish pig and cattle farms, and there is a need to develop biocontrol agents to control both fly species. *Spalangia came-roni* is the most common pupal parasitoid found in stable flies and house flies in stables in Denmark, and field releases of this natural enemy have shown considerable potential for suppression of filth fly populations. However, pupal parasitoids cannot be expected to control filth flies in all types of farms and at all times of year, unless supplemented with other (bio)control agents. Different species of mitosporic fungi have been tested against adults and larvae of house flies, while hardly any attention has been given to the stable fly. The objectives of this ongoing study therefore are to test 1) the susceptibility of stable fly larvae and house fly larvae to two species of mitosporic fungi and 2) the compatibility of these fungi with the parasitoid *Spalangia cameroni*. Dose-response experiments were conducted with L3 of *S. calcitrans* and *M. domestica* and with adult females of *S. cameroni*. Insects were inoculated with conidia of *M. anisopliae* or *P. fumosoroseus* in aqueous suspensions at concentrations ranging from $1x10^4$ to $1x10^8$ conidia/ml. *M. anisopliae* was the most virulent to stable fly larvae, causing 94% mortality at $1x10^8$ spores/ml. House fly larvae were most susceptible to *P. fumosoroseus* (66% mortality at 1 x 10^8 spores/ml), while *M. anisopliae* proved to be virulent to the parasitoids (mean mortality: 71% at 1 x 10^8 spores/ml). Further studies will focus on the effect of *M . anisopliae* on parasitoid oviposition behaviour, fecundity and development of progeny. Preliminary data from assays in breeding media indicate that very high inoculum levels may be needed to control fly larvae in the field. By targeting the aggregation sites of filth fly larvae fungus application however still may be a feasible control method, and although *S. cameroni* is also suceptible to infection it may be possible to integrate the fungus and the parasitoid in an optimal control strategy. **(STUDENT POSTER).**

FP 10 Mass cultivation of *Nomuraea rileyi* in bioreactors

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In India and other Far East countries the major part of the population's protein supply is covered by leguminous plants (pulses, peas). In recent years India's crop failures

for leguminous harvest has reached over 65%, half of it can be accounted to caterpillar grazing by *Helicoverpa armigera*. Due to the growing resistance of *H. armigera* towards most insecticides future pest control will be based on microbial agents with the fungus *Nomuraea rileyi* being one of the most potent candidates. Mass production of *N. rileyi* mycelium in liquid cultures was tested in shake flasks for media optimisation and in different bioreactors. For batch growth in Catroux medium a pH value of 5.0±0.2 is superior to higher or lower values. Glucose as carbon source supported mycelium growth far better than saccharose or starch. The same was true for yeast extract as N nitrogen source when compared with corn steep liquor. An optimised Catroux medium yielded 0.69 kg of dry biomass / kg substrate with a specific growth rate of 0.044 h^{-1}. Due to its versatility and worldwide availability the STR (stirred tank reactor) design was used in different configurations up to 14 l for mycelium cultivation. Low shear forces were crucial for mycelial growth. Impeller mixers at 80 – 100 rpm showed high biomass production rates compared to blade mixers and higher mixer speeds. Mycelium accumulated and dried out in insufficiently mixed reactor headspace leading to sporulation. Hence reactor configurations with height to diameter ratio of h:∅ > 4 were beneficial for biomass production. Fine pore aeration devices were prone to blockage by fungal mycelium, measurement of dissolved oxygen in the reactor broth is slowed down drastically due to membrane blockage by fungal proteins. With optimal configuration the STR can be used for high yield biomass production of *N. rileyi*.

FP 11 Effects of aphid-induced plant volatiles on intraguild interactions at the third trophic level

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The broad-bean plant *Vicia faba* (trophic level one), is attacked by the pea-aphid (trophic level two) which in turn is attacked by a guild of natural enemies (trophic level three). This guild includes the entomopathogenic fungus *Erynia neoaphidis*, the parasitoid *Aphidius ervi* (which both need the aphid to complete their lifecycles) and the predator *Coccinella septempunctata* (which uses the aphid as a food source). The broad-bean plant releases volatiles specific to the aphid species attacking it. These volatiles attract the parasitoid *Aphidius ervi*, which parasitises the aphid therefore protecting the plant. Potentially these volatiles could affect the interactions between the natural enemies of the aphid. For example, the volatiles may increase the rates at which *A. ervi* parasitise *E. neoaphidis*-infected aphids (intraguild competition) or *C. septempunctata* predate *E. neoaphidis*-infected aphids (intraguild predation). Results from the behavioural bioassays are presented and indicate that pea-aphid induced broad-bean plant volatiles have no effect on fungus-parasitoid and fungus-predator interactions. Implications for the use of multiple natural enemy species for the biological control of aphids are discussed.

FP 12 Molecular, morphological, and functional characterization of a peruvian isolate of *Metarhizium anisopliae* var. *acridum*

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The isolate of *Metarhizium anisopliae* var. *acridum*, CG 863, obtained from the grasshopper *Schistocerca interrita*, a serious pest in many crops in Peru, was characterized. Characterization was made by comparing this isolate to two other isolates of *M. anisopliae* var. *acridum*, from Brazil and Australia, and with an isolate of *M. anisopliae* var. *anisopliae*. The parameters used were conidial size, growing at 25 and 37°C, RAPD patterns and virulence against the grasshopper *Rhammatocerus shistocercoides*, from Mato Grosso, Brazil. The three *M. anisopliae* var. *acridum* isolates showed similar growth profile in agar plates in both temperatures, and identical RAPD patterns obtained with the analysis of three primers. However, regarding these parameters and conidial size, these isolates were very distinct from *M. anisopliae* var. *anisopliae* isolate. Peruvian and Brazilian isolates showed similar conidial size, which differed significantly ($P<0.1$) from the Australian isolate of *M. anisopliae* var. *acridum*. Preliminary bioassays indicated that the Peruvian isolate is as pathogenic as the Brazilian isolate against nymphs of *R. schistocercoides*.

FP 13 Attenuation of fungal infection in thermoregulating locusts, *Locusta migratoria*, is accompanied by changes in hemolymphal proteins

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The locust, *Locusta migratoria migratorioides* is susceptible to the deuteromycetous, insect pathogenic fungus, *Metarhizium anisopliae* var *acridum*. Field trials show promise for *M. anisopliae* as a biocontrol agent of grasshoppers and locusts. However, during exposure to conditions that allow thermoregulation, the infected locusts can elevate their temperature up to 46°C and locust survival increases. This phenomenon is termed behavioral fever and has also been observed in the fungus-infected grasshoppers. Using SDS-PAGE, we analyzed the hemolymph proteins of thermoregulating and non thermoregulating locusts, *L. migratoria migratorioides* infected with the fungus *M. anisopliae* var *acridum*. Under conditions that allowed locusts to thermoregulate, 2 proteins, ITB1 (ca. 18 kDa) and ITB2 (ca. 13 kDa) were induced 48 hr post inoculation. In contrast, under non thermoregulating conditions, only 1 band, INTB1 (ca. 18 kDa) was induced with similar molecular mass to ITB1. ITB1 and ITB2 were N-terminally sequenced but showed little homology to known proteins. The induction of hemolymphal proteins in infected, thermoregulating locusts and implication in insect immune defense will be discussed.

FP 14 Field testing of new biocontrol strategies to decrease the population density of *Melolontha hippocastani*, an important scarab species in Germany

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The economic importance of scarab species as pest insects in various crops is a growing item all over the world. In Germany, particularly in the Southern Federal States Hesse, Rhineland-Palatinate and Baden-Württemberg, a recent outbreak of the forest cockchafer, *Melolontha hippocastani*, starting in the eighties, reaches tremendous levels of damage now. Loss of trees of varying ages and in different habitats (total of approx. 7 500 hectares) are estimated here as damages to an extent of several millions. The main damage provoking cause, are the white grubs, that feed on the fine roots of nearly all tree species, during their four years lasting life in the soil. The application of conventional control strategies is impossible, neither against the white grubs, nor the beetles, due to the size of the forest area, which is too big to become treated with a non-selective insecticide, and generally due to the lack of a product registered for the control of *M. hippocastani*. As the state forest authorities from Hesse have decided to promote biocontrol methods, a combination of positive experiences made with other pest species, e.g. the use of *Beauveria brongniartii*-barley kernel products (MELOCONT® Pilzgerste, BEAUVERIA Schweizer® and Engerlingspilz®) against the white grubs of the field cockchafer, *M. melolontha* and the transfer of *B. bassiana* spores via pheromone traps in the case of bark beetle (*Ips typographus*)-control, have been applied against the forest cockchafer and were tested in the field in the surroundings of Darmstadt during the main flight period this year. The methods and first results of four different trials (two soil application methods, spray application of new formulations and combination pheromone trap/fungus) are presented and the significance of these new approaches for the practice are discussed.

FP 15 *Metarhizium anisopliae* and *Trichoderma viride* efficiently control colonies of *Atta cephalotes* in the field

E. Lopez and S. Orduz

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Leaf-cutting ants of the species *Atta cephalotes*, are an economically important agricultural pests in many countries from Central and South America. These ants use the material they cut to cultivate a fungus from which they feed. In this study three baits were applied to control *A. cephalotes* colonies in the field. The baits were based on the entomopathogenic fungus *Metarhizium anisopliae* strain M-137, strain T-26 of *Trichoderma viride*, an antagonist to the symbiont fungus of *A. cephalotes*, a combination of both fungi and the insecticide Pirimifos Metil (PM) applied with air pump. Five ant colonies per treatment were used including the control, and three applications of each treatment were performed at 5-weeek intervals. After the second application, 40% of the colonies treated with T-26 died, while 100% of the colonies died with the treatments with M-137 and the combined bait. On the other hand, only 20% of the colonies treated with PM died. The third application caused the death of 60% of the nests treated with PM. None of the control colonies died. After applications of PM the colonies presented inactivity during a few days, but after two or three weeks they recovered completely. On the other hand, in the nests treated with the fungi, the activity of the nests decreased slowly, but it was more lasting or definitive. This work shows the successful control of colonies of *A. cephalotes* in field with the entomopathogenic fungus *M. anisopliae* applied as bait.

FP 16 Advanced studies about biological control of Chagas disease vectors with entomopathogenic fungi in Central Brazil

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The Central Brazilian Cerrado is known for its immense biodiversity. Investigations about entomopathogenic fungi carried out since July 2000 in the State of Goiás revealed a high presence of hyphomycetic fungi in original areas of the Cerrado. Especially *Metarhizium anisopliae* and *Beauveria bassiana* were isolated from soil samples with selective Chase medium and an *in vivo* capture technique using *Triatoma infestans*. These fungi are known as potential candidates for biological control of Chagas disease vectors, and may be used to eliminate peridomestic vector populations and interrupt invasion of insect-free houses by vectors. Actually we dispose on 107 *M. anisopliae* and 41 *B. bassiana* isolates. Pathogenicity for *T. infestans* was confirmed for all new isolates at 25°C and RH close to saturation. First results about activity of *M. anisopliae* isolates at 75% RH showed a distinct reduction of virulence of most isolates tested in *T. infestans* third instar nymphs. Moreover, some of the *M. anisopliae* isolates showed to be highly active against larvae of mosquitoes. Further studies in different regions of Central Brazil using the same techniques to detect fungi as mentioned above showed that both, *M. anisopliae* and *B. bassiana*, can be found in peridomestic areas where *Triatoma sordida* and other vector species are common. However, these fungi could not yet be detected on alive or dead triatomine specimen captured in peridomestic habitats. First field tests during the rainy season of 2001/2002 were carried out in the rural proximity of São Luis de Montes Belos in central western Brazil. Conidia of the *B. bassiana* strain, CG14 (Embrapa), were suspended in 10% aqueous emulsifier added vegetable oil and sprayed in hen houses of four farms with peridomestic infestation of *T. sordida*. Only 9.4% of alive triatomines were found 25 days after application and 43.6% in the control populations compared to the number of insects counted before treatment. *B. bassiana* was observed on several dead insects in one of the treated ten houses. Results emphasize the importance to test entomopathogenic fungi under field conditions in order to promote biological control of peridomestic triatomine vectors.

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FP 17 Characterization of *Nomuraea* sp. isolated from long-horned grasshopper (Tettigoniidae)

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During a survey for natural enemies of soybean insect pests in Indonesia, mummified long-horned grasshoppers, were collected from a field in North Sulawesi in May, 1996. After incubation, an extensive mat of green conidia was produced that covered the entire body. In this paper, we describe phenotypic characteristics of this fungus (Ind GH-96) using scanning electron and light microscopy and examine the genotypic properties by internal transcribed spacer (ITS) sequence analysis. Initial diagnosis using light microscopy suggested that this pathogen was a *Nomuraea* sp.. Hyphal body cells appeared to be more elongate and cylindrical than those produced by the lepidopteran strains. Conidial production by Ind GH-96 was quite rapid at 27 °C (10 days on Sabouraud maltose agar with 2% yeast). Conidia were produced basipetally from a phialide ascending from conidiophores. The production of short chains of olive-green, elongate and usually distinctly curved conidia readily distinguished these isolates from known species of *Nomuraea*. High dosage bioassay tests of Ind GH-96 against long-horned grasshopper, short-horned, house cricket and a beet armyworm, *Spodoptera exigua*, showed that infection rates were higher in long-horned grasshopper than short-horned grasshopper. House crickets and beet armyworms were not infected. DNA was extracted using a modified CTAB protocol. ITS1-5.8s-ITS2 region of rDNA was amplified with a mixture of *Taq*DNA polymerase (Promega) and *PFU* polymerase (Stratagene), using the primers TW81 and AB28 for the ITS-5.8S. The sequencing of the ITS1-5.8s-ITS2 region of rDNA showed that Ind GH-96 is grouped within the *Nomuraea* clade. This appears to be the first report of infection by *Nomuraea* sp. in grasshoppers.

FP 18 Soil as an environment for winter survival of aphid-pathogenic Entomophthorales

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Fungus regularly causes epizootics among aphid pests suggesting great potential for utilizing *P. neoaphidis* for microbial control of aphids either by inoculation or by conservation of the environment. Regardless of the strategy, a better understanding of the epizootiology is, however, essential for success. So far, most attention has been given to the effects of *P. Pandora neoaphidis* is a well-known pathogen of many aphid species. The *neoaphidis* on aphid populations in economically important crops during the summer months, whereas knowledge concerning performance during the winter and initiation of infections in spring is limited. In the laboratory the winter survival of *Pandora neoaphidis* was studied for both discharged primary conidia and hyphal bodies inside aphid cadavers after storage on moist soil at different temperatures. The activity of the inoculum was quantified by the ability to produce replicate conidia as well as the ability to infect aphids. No effect of inoculum type was found, and both primary conidia and hyphal bodies retained the ability to initiate infections in aphids after storage for at least 14 days at 20°C, 32 days at 10°C and 64 days at 5°C. Morphological studies of the inoculum suggest that *P. neoaphidis* may survive unfavorable conditions as thick-walled conidia also known as loricoconidia. Furthermore, *P. neoaphidis* and *Conidiobolus obscurus* were documented for the first time in field-collected soil in early spring by baiting the soil with aphids. We hypothesize that germination of overwintering inoculum is stimulated by host-induced factors since inoculum apparently responded to the presence of aphids.

Viruses

VP 1 Polyhedral envelope protein mutants of *Rachiplusia ou* multi-nucleocapsid nucleopolyhedrovirus

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Rachiplusia ou multi-nucleocapsid nucleopolyhedrovirus (RoMNPV) has great potential as an environmentally benign control agent for certain lepidopteran (moth) pests. To increase the virulence of RoMNPV, we mutated the RoMNPV polyhedral envelope protein gene (*pep*) to prevent formation of the polyhedral envelope. The *pep* ORF was disrupted by insertion of *lacZ* to produce the recombinant virus RoPEPlacZ. Two additional *pep* mutant recombinant viruses were produced by site-directed mutagenesis that eliminated the *pep* initiation codon (RoPEPΔATG) or inserted a stop codon at position 13 of the *pep* ORF (RoPEPTAG). A fourth *pep* mutant virus, RoPEPΔATG-TAG, carried both the ablation of the *pep* initiation codon and the insertion of the stop codon. Western blot analysis revealed that PEP was still expressed during infection of Sf9 cells with RoPEPΔATG. A greatly reduced quantity of PEP was evident in Sf9 cells infected with RoPEPTAG or RoPEPΔATG-TAG, indicating that translation occurred past the inserted stop codon. No PEP was detected in Sf9 cells infected with RoPEPlacZ. Transmission, scanning, and immunogold electron microscopy indicated that the polyhedral envelope was absent from polyhedra of RoPEPTAG, RoPEPΔATG-TAG and RoPEPlacZ. Although immunogold electron microscopy suggested that a polyhedral envelope was still present on RoPEPΔATG polyhedra, scanning electron microscopy showed that polyhedra of this mutant has a pitted surface consistent with the absence of an envelope. In bioassays, *pep*-mutant viruses showed reduced potency against neonate larvae of a semi-permissive host, the European corn borer *Ostrinia nubilalis*. The activity of the mutant viruses was unaltered relative to wild-type RoMNPV against a permissive host, the cabbage looper *Trichoplusia ni*.

VP 2 Insecticidal activity and risk assessment of a recombinant baculovirus expressing a basement membrane-degrading protease

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Basement membranes surrounding the tissues of lepidopteran larvae are a potential barrier to baculovirus movement and establishment of systemic infection. One potential approach to improving the insecticidal activity of baculoviruses is to perforate or eliminate the basement membranes of their hosts. Towards this end, we constructed two recombinant clones of *Autographa californica* nucleopolyhedrovirus (AcMNPV) that expressed a basement membrane-degrading cathepsin L protease from the flesh fly, *Sarcophaga peregrina*. The recombinant viruses expressed *S. peregrina* cathepsin L from either the *ie-1* promoter (AcIE1TV3.ScathL) or the *p6.9* promoter (AcMLF9.ScathL). In survival time bioassays, AcMLF9.ScathL killed neonate *Heliothis virescens* 50% faster than wild-type virus and 30% faster than viruses expressing the scorpion toxins AaIT and LqhIT2 (AcMLF9.AaIT, AcMLF9.LqhIT2). Co-infections of larvae with AcMLF9.ScathL and a second insecticidal protein-expressing recombinant virus (AcMLF9.AaIT, AcMLF9.LqhIT2, AcJHE-SG, or AcJHE-KK) failed to produce additive or synergistic effects on host survival time. AcMLF9.ScathL caused premature cuticular melanization of 5th instar *Heliothis virescens*. Melanization of internal tissues suggests that *S. peregrina* cathepsin L expression was activating host prophenoloxidase. Infections of 5th instar *H. virescens* with AcMLF9.ScathL yielded fewer polyhedra than wild-type AcMNPV and AcMLF9.AaIT, indicating that the insecticidal properties of *S. peregrina* cathepsin L may be independent of any effect on virus movement or systemic infection. Risk assessment studies were performed to determine if AcMLF9.ScathL had detrimental effects on two species of nontarget insect predators, the ladybeetle *Coleomegilla maculata* and the green lacewing *Chrysoperla carnea*. The results suggest that the use of AcMLF9.ScathL in pest management would pose no greater risk to nontarget insect predators than would the use of the parental virus AcMNPV.

VP 3 Bioassays of balsam fir sawfly nucleopolyhedrovirus against its natural host and other insects

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A nucleopolyhedrovirus (NPV) isolated from larvae of the balsam fir sawfly, *Neodiprion abietis* (Diprionidae) (NeabNPV) from western Newfoundland is being developed as a biological control agent against this insect pest of balsam fir (*Abies balsamea*) forest. As part of the registration process, we have assayed NeabNPV against the natural host and other insects. Additional sawflies assayed were, *Acantholyda erythrocephala* (Pamphilidae), *Diprion similis*, *Gilpinia hercyniae* (Diprionidae) and *Pristiphora geniculata* (Tenthredinidae). Two bee species were challenged, *Apis mellifera* (Apidae) and *Megachile rotundata* (Megachilidae), and larvae of the following Lepidoptera, *Choristoneura fractivittana*, *Choristoneura rosaceana*, *Clepsis persicana* (Tortricidae), *Melanchra pulverulenta* and *Pyrrhia exprimens* (Noctuidae). NeabNPV did not cause mortalities, significantly beyond untreated control insects, in either bee species or any of the Lepidoptera. NeabNPV did, however, cause significant mortalities in larvae of all sawfly species tested. The latter results appear contrary to the general notion that sawfly NPVs are highly species specific. The aetiology of the mortality in these additional sawfly species is being investigated.

VP 4 Characterisation of *pif* (per os infectivity factor) from *Spodoptera littoralis* nucleopolyhedrovirus

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During plaque purification of *Spli*NPV in *S. littoralis* SL52 cell line, a deletion mutant virus was isolated. Analysis of the biological properties of this mutant virus revealed an absence of *per os* infectivity of the occluded virus. The infectivity by injection of the budded viruses is similar in the wild type and the deleted viruses. Restriction analysis of the mutant virus genome revealed a 4.5 kb deletion within the *Not* I D fragment. The observed phenotype was mapped to the deleted region. By co-transfecting the DNA of the deleted virus with plasmids derived from the wild -type virus it was possible to determine that the ORF 7 in this fragment is responsible for the observed phenotype. This ORF was called *pif* for *per os* infectivity factor. The product of this gene is an ODV envelope protein required only for the first steps of the larval infection as viruses being produced in cells expressing the gene, but not containing it in their genomes, are able to produce successful infections.

VP 5 Differences in the peritrophic membrane of susceptible and resistant *Anticarsia gemmatalis* larvae to the insect nucleopolyhedrovirus (AgMNPV)

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The velvetbean caterpillar, *A. gemmatalis* (Lepidoptera: Noctuidae), is the major soybean crop pest in Brazil. It has been controlled by a nucleopolyhedrovirus (AgMNPV) since 1983, with the area treated with this virus increasing substantially afterwards. Currently, the AgMNPV is applied on over 1,400,000 ha annually, that correspond to approximately 10% of the soybean cultivated area in the country. The constant use of this biological insecticide has raised concerns regarding the possibility of selection for resistance to the AgMNPV among field populations of the insect. Although this phenomenon has not been detected in the field yet, laboratory selection pressure experiments have led to a highly resistant *A. gemmatalis* population to the

AgMNPV (resistance ratio over 100.000 fold). This resistance may be related to the larval midgut, being the peritrophic membrane (PM) considered one of the most important gut barriers against viral infection. Our work aimed to study the PM along the midgut of susceptible (SL) and resistant (RL) *A. gemmatalis* larvae using fluorescence techniques. The midguts of SL and RL were fixed in Bouin's solution and embedded in paraffin. Histological sections were obtained along their length (proximal, medial and distal regions), washed in PBS (20mM phosphate buffer at pH 7.4, containing 0.15M NaCl), post-washed in PBS/0.2%Triton X-100 solution and incubated with FITC-WGA (fluorescein isothiocyanate-wheat germ agglutinin) in the presence of N-acetylglucosamine. The materials were examined in a Zeiss LSM 410 confocal microscope. The FITC-WGA labeling of the PM was different according to each midgut region. Both SL and RL showed an increase of the FITC-WGA reaction intensity from the proximal to the distal regions. The intensity of the PM reaction to the FITC-WGA in the RL, in all the regions, was greater when compared to respective regions in the SL. As the WGA is a lectin that binds specifically to chitin, the major component of the PM, our results showed that there are regional differences in the PM along the midgut of *A. gemmatalis* larvae. Furthermore, the RL presented a thicker PM all over the midgut that may be related with their resistance to the AgMNPV infection. **(STUDENT POSTER).**

VP 6 Establishment, growth kinetics, and susceptibility to AcNPV of heat tolerant lepidopteran cell-lines

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Adaptation of Sf9, High5 and TnMG1 cell-lines to 33°C and 35°C was carried out by shifting culture temperature between 28°C and 33°C or 28°C and 35°C. The cell lines with the temperature adaptation were designated as Sf9-ht33, Sf9-ht35, High5-ht33, High5-ht35, TnMG1-ht33 and TnMG1-ht35. The current passage numbers of these cell-lines are over 50 and their subculture is carried out twice a week. Cell sizes of ht cell-lines of TnMG1 are similar to those of controls, but the cell size of ht cell-lines of High5 is smaller than that of control cells and they have spindle shape with long fine projections. Cell size of ht cell-lines of Sf9 is bigger than the control. Growth curves for ht cell-lines of Sf9, TnMG1 and High5 show their faster growth in comparison to those of controls. However, the maximum cell densities of ht cell-line of Sf9 and TnMG1 cultures were 80% of the controls. When control and ht cell-lines were cultured for 4 days at 28°C, 33°C, 38°C, 40°C or 42°C, swelling and disintegration of cells were observed in the cultures of parental cell-lines at 38°C, 40°C and 42°C for TnMG1-ht33 and -ht35 cell-lines and at 40°C and 42°C for the other ht cell-lines. When the cells were infected with *Autographa californica* nucleopolyhedrovirus (AcNPV) at the same experimental conditions, ht33 cell-lines showed a slightly higher productivity of budded virus (BV) and occlusion body (OB) production in comparison with that of the controls at temperatures of 33°C, 38°C and 40°C, but ht35 cell-lines showed a productivity of BV and OB similar to that of the controls at all four temperatures. Production of recombinant secreted alkaline phosphatase and β-galactosidase for individual heat tolerant cell-lines is under investigation.

VP 7 The effects of an entomopoxvirus on the development of a pupal parasitoid, *Brachymeria lasus*, in its host *Homona magnanima* (Lepidoptera: Tortricidae)

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The effects of entomopoxvirus infection of *Homona magnanima* on the development of a pupal parasitoid, *Brachymeria lasus*, were investigated under laboratory conditions. When newly molted fifth-instar *H. magnanima* larvae were allowed to ingest 0.5-1 droplet containing 500 occlusion bodies of *H. magnanima* entomopoxvirus (HmEPV), more than 90% of the HmEPV-infected larvae pupated, but none of the pupae developed into adults. HmEPV-infected and non-infected pupae of different ages, *i.e.*, 1, 2, 3, 4, 5, and 6 days old, were exposed to parasitization by 3-day-old mated female *B. lasus*. Infection of *H. magnanima* pupae with HmEPV was detrimental and adversely affected the development of *B. lasus* when 1- to 4-day-old pupae were used as hosts. Significantly fewer parasitoids emerged from HmEPV-infected pupae, and these parasitoids spent an additional day in the HmEPV-infected pupae; the *B. lasus* that emerged from HmEPV-infected pupae were smaller than those from non-infected pupae. By contrast, significantly more parasitoids emerged from HmEPV-infected pupae when 5- and 6-day-old pupae were used as hosts. No parasitoids emerged from 6-day-old non-infected pupae, due to the advanced development of the host pupae. These data suggest that infection of *H. magnanima* pupae with HmEPV has both negative and positive effects on the survival of *B. lasus*.

VP 8 Comparative analysis of the genome and host range of *Chilo* iridescent virus and Cricket iridovirus isolate

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The iridovirus isolate termed cricket iridovirus has been isolated 1996 from *Gryllus campestris L.* and *Acheta domesticus L.* (both Orthoptera, Gryllidae). The cricket iridovirus isolate DNA shows distinct DNA restriction patterns different from those known for *Insect iridescent virus 6* (IIV-6). This observation led to the assumption that the cricket iridovirus isolate might be considered as a new species within the family *Iridoviridae*. The cricket iridovirus isolate can be transmitted perorally to orthopteran species causing specific fatal diseases. These species include *Gryllus bimaculatus* L. (Orthoptera, Gryllidae), the desert locust, *Schistocera gregaria*, and the African migratory locust, *Locusta migratoria migratorioides* (both Orthoptera, Acrididae), that represent two of the most important pest insects in

developing countries. Additionally, several species of cockroaches are infected, e. g. *Periplaneta americana*, *Blattella germanica*, and *Blatta orientalis* (all Orthoptera, Blattidae). The analysis of genomic and host range properties of this isolate was carried out in comparison to those known for IIV-6. The host range studies of the cricket iridovirus isolate and IIV-6 revealed that there were no differences in the peroral susceptibility in all insect species and developmental stages as far as tested. In order to compare the primary structure of the cricket iridovirus isolate and IIV-6 genome different gene loci of the IIV-6 genome were analyzed including the major capsid protein (274L), the thymidylate synthase (225R), an exonuclease (012L), the DNA-polymerase (037L), the ATPase (075L), DNA ligase (205R), and the open reading frame 339L homologous to the immediate early protein ICP-46 of Frog virus 3. The average identity of the selected viral genes and their gene products was found to be 95.98 % and 95.18 % at the nucleotide and amino acid level, respectively. These data led to the conclusion that cricket iridovirus isolate and IIV-6 are not different species within the *Iridoviridae* family and must be considered as a variant and/or novel strain of IIV-6.

VP 9 Biology, ecology and host-virus interactions of invertebrate iridescent viruses (*Iridoviridae*) in Diptera and Lepidoptera: recent advances

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Invertebrate iridescent viruses (IIVs) can cause two types of infection: one obvious (patent) and lethal, in which the host develops an iridescent lavender-blue hue and then dies, the other inapparent (covert) and non-lethal. (1) SUBLETHAL EFFECTS: We present evidence that covert infections affect longevity, fecundity and body size of *Aedes aegypti* resulting in a 50% reduction in the net reproductive rate of infected mosquitoes. (2) DETECTING COVERT INFECTIONS: Cell culture, insect bioassay and PCR techniques can be used for detecting the presence of IIV particles, but the insect bioassay was the most sensitive and required least preparative purification steps. The presence of covert infections in *G. mellonella* that had been injected with 1 - 10 particles of IIV-6, did not affect the reliability of the bioassay to an important degree. (3) PERSISTENCE: The half live of *Invertebrate iridescent virus 6* (IIV-6) at 25°C, was <24 h in dry soil (6.4% moisture), 4.9 d in non-sterile soil, 6.3 d in sterilized soil (17 and 37% moisture), and 12.9 d for purified virus suspension. When exposed to strong sunlight and high temperatures, virus suspensions lost ~4 logs of activity in 9 d. Persistence in water was improved when soil sediment was present. (4) TRANSMISSION: Insect bioassays indicated that infection by IIV-6 was transmitted vertically by covertly infected female *G. mellonella*, but none of the progeny developed patent infections. In contrast, an IIV isolated from *Spodoptera frugiperda* was efficiently transmitted by cannibalism; 83% of cannibals that consumed an infected conspecific themselves became infected. The presence of infected conspecifics also severely affected the survival of *S. frugiperda* larvae in laboratory microcosm experiments. All the braconid endoparasitoids that stung infected hosts transmitted the virus to healthy *S. frugiperda* larvae that were stung subsequently. However, the parasitoid could not generally complete its development in IIV infected hosts due to premature death of the host and/or direct infection of the parasitoid by the IIV. (5) SOLVENT SENSITIVITY: The sensitivity of these viruses to organic solvents, detergents and enzymes depends on the assay system: in certain cases *in vitro* cell culture assays indicated sensitivity whereas *in vivo* bioassays indicated no sensitivity.

VP 10 A baculovirus mutant with a host-specific defect in occlusion body formation in insect cells

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Baculovirus replication in insect cells results in the production of budded virus in the late phase of replication and occluded virus or polyhedra in the very late phase. Budded virus spreads infection within the insect host, while polyhedra enable transmission between insects. The transition from budded virus formation to occluded virus production is poorly understood. *Autographa californica* nucleopolyhedrovirus (AcMNPV) replicates in *Spodoptera frugiperda* and *Trichoplusia ni* cells. However, budded virus production in *T. ni* cells is about 10-fold lower than in *S. frugiperda* cells, suggesting that there are both virus and host-specific factors regulating the process. We have isolated an AcMNPV mutant (AcdefrT) which exhibits enhanced budded virus formation in *T. ni* cells, but is partially defective for polyhedra production in the same cells. Virus replication in AcdefrT-infected *T.ni* cells was also accompanied by apparent apoptosis, an observation inconsistent with enhanced budded virus production. However, no DNA laddering was recorded in these cells. The mutation within AcdefrT appears to be host-specific, since infection of *S. frugiperda* cells with this virus yielded normal budded virus and polyhedra production, with no signs of plasma membrane blebbing. **(STUDENT POSTER).**

VP 11 Effects of an optical brightener on the development of resistance to SfMNPV, the severity of sublethal effects and growth of *Spodoptera frugiperda*

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Certain optical brighteners can greatly enhance the insecticidal capacity of baculoviruses and these compounds have been patented for use in baculovirus formulations. We evaluated the effect of including the optical brightener Tinopal LPW in the inoculum (droplet feeding technique) on the rate at which a colony of *S. frugiperda* developed resistance to a homologous nucleopolyhedrovirus (SfMNPV). Second instars were exposed to LC_{50} concentrations of SfMNPV in the presence or absence of 0.1% Tinopal LPW over a period of 11 generations. At the end of the study, sublethal effects associated with virus and Tinopal exposure were evaluated. LC_{50} values were approximately 1 log higher

for insects that had been exposed to virus in each generation, compared to non-exposed controls. LC_{50} values fluctuated between generations 3 and 11 and the optical brightener did not affect the rate of development of resistance in a systematic manner. Exposure to Tinopal alone, virus alone and virus + Tinopal significantly affected the weight of pupae, adult fecundity and egg fertility. Studies on the effects of 0.05% Tinopal (incorporated into diet) on the development of immature *S. frugiperda* revealed significant reductions in the weight of larval and pupal stages and an increase in overall larva-pupa development time. We attribute these effects to the antifeedant properties of stillbene optical brighteners.

VP 12 High prevalence of *pif* deficient genotypes in a wild-type nucleopolyhedrovirus of *Spodoptera frugiperda*

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A wild-type nucleopolyhedrovirus (NPV) isolated from *Spodoptera frugiperda* in Nicaragua (Sf-NIC) has attracted interest as a potential bioinsecticide for small-scale maize growers in Latin-America. The DNA restriction profiles of this isolate show a number of submolar bands with different restriction enzymes indicating the genotypic heterogeneity of the viral population. Plaque purification using the Sf-9 ATTC cell line was used to purify several genotypic variants from the wild-type. Nine distinct genotypic variants were identified by the DNA restriction profiles produced after digestion with *Eco* RI, *Hind* III and *Pst* I. The Sf-NIC(B) variant was one of the most prevalent genotype (15%), and it was designated as the prototype Sf-NIC variant, while the rest of the variants [Sf-NIC(A), (C -I)] were present at a lower prevalence (ranging from 18% to 1%). The analysis of each variant, compared to the prototype Sf-NIC(B), showed that the variants A, F, G, H, and I, presented minor differences, whereas variants C, D, and E contained deletions of around 3.3 Kb located at the restriction fragment *Eco* RI-K. This fragment includes an essential gene required for penetration of the virus into the midgut cells named "*per os* infection factor" (*pif*, ORF-36 of SeMNPV) gene (López-Ferber, in press). Bioassays with the deletion variants (C, D, E) revealed an absence of *per os* infectivity of the occlusion bodies (OBs), whereas the virions derived from these OBs were infective by intrahaemocelic injection which resulted in the typical signs and symptoms produced during Sf-NIC infection in its homologous host. Further biological characterization of these deletion mutants is being performed in order to predict their possible effects on natural populations of this virus.

VP 13 Effects of Tinopal LPW on the infectivity and productivity of the *Spodoptera exigua* nucleopolyhedrovirus

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The beet armyworm, *Spodoptera exigua* (Lepidoptera, Noctuidae), is a polyphagous pest of many horticultural and ornamental crops throughout many temperate and subtropical areas of the world. This pest has developed resistance to many commonly used chemical insecticides, resulting in considerable interest in the development of entomopathogens as biological insecticides, particularly the *S. exigua* multiple nucleopolyhedrovirus (SeMNPV). In order to enhance the performance of this virus, particularly for the control of late instars, possible viral synergists are being evaluated. One of the most effective adjuvants has proved to be the stillbene derived optical brightener, Tinopal LPW. In the present work, we report the effects of Tinopal LPW on the biological performance of a purified genotypic variant of SeMNPV in the different host instars, in terms of median lethal dose LC_{50} and production of viral progeny. Tinopal LPW reduced the LC_{50} of SeMNPV against L_2, L_3, L_4, and L_5 *S. exigua* larvae by 2.6, 5.1, 70, and 580 fold, respectively. In later instars, the enhancing effect of Tinopal LPW becomes steadily stronger, and for the third and fourth instars the synergistic effect was greater at higher concentrations. We also assessed the influence of Tinopal LPW in the production of viral progeny when second and fourth instars were inoculated in the presence and absence of Tinopal LPW. The use of Tinopal LPW during the virus production process may significantly reduce the quantity of viral occlusion bodies required as inoculum for the large-scale production of this baculovirus.

VP 14 A novel vaccine delivery system using recombinant baculovirus occlusion bodies

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A system has been developed for the incorporation of foreign proteins into baculovirus occlusion bodies. This strategy involves expressing the foreign protein as a polyhedrin fusion protein, while maintaining a second native copy of the polyhedrin coding gene. Initial studies showed that assembly of occlusion bodies incorporating a foreign protein depends on an interaction between native polyhedrin and the polyhedrin fusion protein. This technology has been applied to the generation of three different recombinant baculoviruses that produce occlusion bodies incorporating influenza virus nucleoprotein, LCMV glycoprotein or Der-p1 antigen, respectively. SDS-PAGE analyses of the occlusion bodies of these recombinant viruses confirmed that they include not only polyhedrin fused antigens but also a large number of baculovirus proteins from the embedded virions. We are immunizing mice with a range of doses of recombinant occlusion bodies and comparing the immune response to that observed in animals inoculated with the same purified antigen delivered by a conventional route. Thus, these preliminary experiments will provide an initial indication of the utility of recombinant occlusion bodies for the delivery of subunit vaccines.

VP 15 Suppressive effects of *Xestia c-nigrum* granulovirus on nucleopolyhedrovirus infection in *Mamestra brassicae* and *Helicoverpa armigera*

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The ability of granuloviruses (GVs) to synergize or enhance nucleopolyhedrovirus (NPV) infection has been known for more than 40 years. More recently, molecular biological studies of the active proteins involved in this enhancement from GVs isolated from *Trichoplusia ni*, *Pseudaletia unipuncta*, *Helicoverpa armigera*, and *Xestia c-nigrum* have been performed. Enhancement of virus infection is an important consideration for the practical use of baculoviruses for insect pest control. In our previous study, a protein extract from *X. c-nigrum*GV (XcGV) capsules enhanced the infection of *X. c-nigrum*NPV, *Mamestra brassicae* NPV (MbNPV) and some other NPVs in larvae that were not only susceptible to XcGV (*X. c-nigrum* and *Mythimna separata*) but also non-susceptible or highly resistant to XcGV (*M. brassicae* and *H. armigera*). In the current study, the effect of mixed infection of intact XcGV capsules and polyhedra of MbNPV was investigated using larvae of *M. brassicae* and *H. armigera*. The enhancement of MbNPV infection by XcGV was not detected in the 3rd and 4th instar larvae of either species following inoculation by the droplet feeding method. Interestingly, in larvae of *M. brassicae*, coinfection of XcGV and MbNPV resulted in a lower rate of mortality and slower killing compared to infection with only MbNPV. This suggested that the XcGV capsules contain not only a virus enhancing factor but also some component that suppresses NPV infection in non-susceptible or highly resistant hosts of XcGV.

VP 16 ORF94 of HaSNPV encodes a novel major ODV envelope protein ODV-E43

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ORF94 of *Helicoverpa armigera* SNPV, a homologue of AcMNPV ORF109, appears to be conserved among baculoviruses. Sequence analysis indicated that the gene (*orf94*) is 1086 bp long and encodes a putative protein of 362 amino acids with a predicted molecular size of 41.5 kDa. A late baculoviral transcription initiation motif ATAAG was found 65 nt upstream of the putative translational start site and a polyadenylation signal AATAAA was identified 14 nt downstream of the TAA stop codon. To elucidate its function, *orf94* was expressed as a GST-fusion protein in *E. coli*. The expressed protein was purified and used to generate antibodies in rabbits. The transcription and expression of the putative gene were investigated in HzAM1 cells. Northern blot and RT-PCR results suggested *orf94* is a late gene. Western blot analysis of extracts of HaSNPV–infected HzAM1 cells revealed a specific protein of 43 kDa from 48 h to 96 h p.i.. To investigate whether ORF94 is a structural component of HaSNPV, Western blot analysis of proteins in budded viruses (BVs) and occlusion derived virions (ODVs) was conducted. The protein was detected in ODV but not in BV, suggesting that *orf94* encodes a structural component of ODVs. When ODVs were further fractionated into nucleocapsid and envelope components, the Western blot analysis indicated that the encoded protein was part of the envelope. In summary, the data show that *orf94* encodes a novel ODV envelope protein (ODV-E43) of HaSNPV.

VP 17 Characterization of a J domain gene of *Spodoptera litura* multicapsid nucleopolyhedrovirus

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Spodoptera litura multicapsid nucleopolyhedrovirus (SpltMNPV) ORF39 (designated as the *bjdp* gene) is the only J domain gene recorded in baculovirus genomes to date. Computer-assisted analysis revealed its deduced amino acids sequence possessed a coiled-coil region and a RNA recognition motif (RRM). *In vivo* the *bdjp* gene transcription was detected from 24 h post infection (p.i.) and abundant from 72 h to 96 h p.i., while *in vitro* it was 6 h p.i. and from 12 h to 24 h p.i., respectively. Time course of BJDP expression in SpltMNPV infected cells showed that the *bjdp* gene was expressed from 24 h through 96 h p.i. with the antiserum, which was prepared by using the 6×His tagged BJDP expressed in *Escherichia coli* as antigen. Western blot analysis indicated that BJDP could be found in both occlusion-derived virus (ODV) and budded virus. Furthermore, BJDP was shown to be present in both envelope and nucleocapsid fractions of ODV. These results suggested that BJDP might be a structural protein.

VP 18 Identification of a novel protein associated with envelope of occlusion-derived virus in *Spodoptera litura* multicapsid nucleopolyhedrovirus

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Spodoptera litura multicapsid nucleopolyhedrovirus (SpltMNPV) ORF137 (*Splt137*) is one of 29 unique SpltMNPV ORFs. *Splt137* has the potential to code for a polypeptide of 231 amino acid residues with predicted molecular weight of 27.5 kDa. Computer-assisted analysis of the predicted amino acid sequences of Splt137 protein showed 1 N-glycosylation site and 11 phosphorylation sites. For identification of *Splt137*, antibody was prepared by immunization of rabbits with purified Splt137 protein produced in *Escherichia coli*. This antibody was used to analyse of Splt137 protein using Western blot. A 36-kDa protein was found both in the infected cells and envelope fractions of occlusion-derived virus (ODV) but could not be detected in the budded virus (BV). Tunicamycin treatment of SpltMNPV infected cells suggested that the 36-kDa protein had undergone N-glycosylation. Our data suggested that Splt137 protein was a novel envelope protein of ODV and might exist as a more complex form of 79 kDa protein in intact ODV. Further transcriptional analysis with RT-PCR and 5′RACE analysis suggested that Splt137 might perform functions early and late in infection.

VP 19 A Comparison of Ascoviruses Isolates from Indonesia and the United States

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Two isolates obtained from noctuid larvae collected in Indonesia and three isolates from the United States were compared with respect to the general relatedness of their DNA, host range and morphology. Seven days after inoculation with INDO1, INDO2, SC1, SC2 and Sf82-126, the control larvae of *Spodoptera exigua* had all pupated, whereas the infected larvae lived more than 30 days after infection. All five isolates were tested against *Spodoptera exigua*, *Spodoptera frugiperda*, *Helicoverpa zea* and *Lymantria dispar*. *S. exigua* was susceptible to INDO1, SC1, SC2 and Sf82-126. INDO1 and SC1 caused the highest mortality and significantly higher than SC2 and Sf82-126. *S. frugiperda* was susceptible to all five isolates; however, INDO1 and INDO2 caused very low mortality. *H. zea* was susceptible to all isolates, whereas *L. dispar* was not infected by any of the isolates. *Trichoplusia ni* was tested against INDO1, SC1 and Sf82-126. *T. ni* was highly susceptible to Sf82-126, less susceptible to SC1, and was not infected by Sf82-126. *Anticarsia gematalis* was tested against INDO1, INDO2 and SC1 and was moderately susceptible to those isolates. The addition of fluorescent (optical) brightener to vesicles-containing hemolymph significantly enhanced the stability of ascoviruses against UV radiation. Without the brightener, ascoviruses lost their virulence after 10 minutes of radiation; with the brightener, ascoviruses were still virulent after up to 30 minutes of UV radiation. In all five isolates, the virions were found typically in spherical vesicles. The size of vesicles decreased and density of virions within the vesicle increased as the disease advanced. In both ultra thin section and negatively stained preparations, the virions of ascoviruses were essentially bacilliform in shape. The virions of INDO2 differed somewhat in shape from those of the four other isolates. Virions of INDO2 were wider than the other isolates. DNA restriction enzyme fragment profiles showed that both isolates from Indonesia were different from the isolates from the USA. INDO1 and INDO2 also differed from each other; SC2 was different from SC1 but not from Sf82-126. Partial DNA polymerase gene sequence analyses showed that all isolates were closely related to each other but distantly related to SfAV1 and HvAV3. However, partial DNA polymerase amino acid sequence analyses showed that all isolates were closely related to SfAV1.

VP 20 Activity of Selected Nucleopolyhedroviruses against Larvae of the Beet Armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae)

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Five isolates of *Spodoptera exigua* multicapsid nucleopolyhydrovirus (*Se*MNPV), and six other NPVs originating from other lepidopterous hosts were tested in the laboratory against beet armyworm (BAW) larvae. The *Se*MNPV isolates were two wild isolates from USA (*Se*MNPV-US1 and *Se*MNPV-US2), two wild isolates from Indonesia (*Se*MNPV-ID1 and *Se*MNPV-ID2), and one commercial isolate (Spod-X). The six other NPVs were wild isolates and consisted of *Spodoptera litura* NPV (*Sl*MNPV), *Spodoptera littoralis* NPV (*Sli*MNPV), *Spodoptera ornithogalli* NPV (*So*MNPV), *Helicoverpa armigera* NPV (*Ha*MNPV), *Rachiplusia ou* NPV (*Ro*MNPV), and *Plutella xylostella* NPV (*Px*MNPV). Bioassay tests for all isolates were conducted in the laboratory against 2nd instars of BAW by layering the polyhedral solutions on bean-based artificial diet. *Se*MNPV isolates, with the exception of *Se*MNPV-ID1, were more virulent than the NPVs that originated from other hosts. LD_{50} and LD_{90} of Spod-X were 765.6 and 5,064.8 PIBs/ larva, respectively. SeMNPV-US1 and *Se*MNPV-US2 were more virulent than Spod-X with the LD_{50} values of 218.0 and 24.9 PIBs/larva, respectively. LD_{90} values of *Se*MNPV-US1 and *Se*MNPV-US2 were 2,215.5 and 158.7 PIBs/larva, respectively. The virulence of *Se*MNPV-ID2 was about equal to that of Spod-X. Among the NPVs that originated from other hosts, *Sli*MNPV and *Ro*MNPV were more virulent than *Ha*MNPV, *Sl*MNPV, *So*MNPV and *Px*MNPV. The virulence of *Sli*MNPV and *Ro*MNPV against BAW larvae was about equal to that of Spod-X, while *Ha*MNPV was less virulent. *Sl*MNPV, *So*MNPV and *Px*MNPV showed low virulence against BAW larvae, with LD_{50} values 14-18 times higher than for Spod-X.

VP 21 Identification and characterization of Hz-2V structural protein genes

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Matrix-assisted lazer-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for tryptic peptides of SDS-PAGE purified Hz-2V structural proteins was used to identify four virus structural protein genes (p11.7, p15.1, p28.4 and p31.7) These genes have been mapped to open reading frames (orf) previously identified on the HZ-2V genome. None of the four predicted proteins coded for by these orfs showed homology with any proteins in GenBank. An analysis of the DNA sequence upstream of 2 of these orfs identified a conserved five base sequence AGTAT which is contained within a nine base sequence previously identified as containing a putative HZ-1V late promoter. The treatment of Hz-2V enveloped nucleocapsids with NP-40 removed the p11.7 protein from virus nucleocapsids. Consistent with this result, analysis of the gene coding sequence for this protein revealed the presence of two trans-membrane domains which flank potential glycosylation and phosphroylation sites in the protein.

Bacteria

BP 1 Quantification of K^+-dependant transmembrane potentials in *Manduca sexta* midgut brush border membranes by diS-C_3(5) assay induced by alkaline pH and Cry1Ab toxin

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We analyzed the *Manduca sexta* endogenous K^+ permeability of two different insect membrane preparations, by using a fluorometric assay sensitive to membrane potential. The use of this system was validated by analyzing the K^+ permeability induced by the K^+ ionophore valinomycin. The two different brush border membrane vesicles (BBMV) preparations were the following: a BBMV sample isolated from whole *M. sexta* midgut homogenate that presented intrinsic K^+ permeability susceptible to be partially blocked by different K^+ channel blockers, suggesting the existence of heterologous K^+ channels population in this BBMV preparation. The second BBMV sample, isolated from microvilli structures, showed higher enrichment of apical membrane markers, aminopeptidase and alkanine phosphatase and contained no K^+ channels. The effect of Cry1Ab toxin in both membrane preparations was analyzed. A quantifiable relationship between the concentration of Cry1Ab toxin and the resulting change in membrane potential was established. The alkaline pH has a moderate positive effect on Cry toxin activity.

BP 2 Characterization of *Bacillus thuringiensis aizawai* UNI498 and histopathology analysis of the toxic effect on midgut of the *Anticarsia gemmatalis*

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The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), is an important insect pest of soybeans in Brazil. Larvae of this insect cause serious damage by greatly reducing the leaf area and, consequently, lowering photosynthesis and productivity (Morales *et al.*, 1995). Control of *A. gemmatalis* with *Bt* products is an alternative because they have a limited and particular spectrum of toxicity to a specific range of insect species. This specificity is related to the presence of crystals containing different proteins . In this work we have characterized a *Bt* serovar *aizawai* named UNI 498 isolated from soil samples of south Brazil, determined the LC_{50} of their crystals proteins purified and analysed effects of these proteins in midgut ephitelial cells of *A. gemmatalis*. By eletron microscopy we observed that UNI 498 has crystals with cubic shape. Polymerase chain reaction (PCR) revealed the presence of *cry*1Aa, *cry*1Ab, *cry*1Ac, *cry*1C and *cry*1D genes. The presence of Cry1 proteins were confirmed by SDS-PAGE. Bioassays to determine the LC_{50} were performed with *Anticarsia gemmatalis* 3rd instar larvae using purified *Bt* crystals (protoxins) that were diluted in 100 mM sodium phosphate buffer (pH 7.4), homogenized and added to the surface of leaf discs of soybean placed in 30 mm plastic dishes. Leaf discs contained 4.2, 21, 106 and 530 ng/cm^2 of *Bt* UNI498 protoxin. In a control group, 20 µl of 100 mM sodium phosphate buffer (pH 7.4) was used. Results showed that protoxin from UNI498 strain was highly toxic against *Anticarsia gemmatalis* 3rd instar larvae with a LC_{50} of the 1.69 ng/cm^2. The results of histopathological analysis showed that changes were observed when larvae were treated with *Bt* UNI498 protoxin. On midgut from these larvae we observed disruption of microvilli and vacuolization of cytoplasm began 6h after ingestion. **(STUDENT POSTER).**

BP 3 Mutagenic Analysis of Conserved Residues in β17 within Domain III of the *Bacillus thuringiensis* Cry4B Toxin

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The function of the C-terminal domain III of *Bacillus thuringiensis* Cry δ-endotoxins is still in debate, although it has been reported that this domain could be involved in pore formation, receptor binding or structural integrity. In our studies, two highly conserved tyrosine residues, Tyr-537 and Tyr-543, located in the predicted β17 within domain III of the Cry4B mosquito-larvicidal protein were first substituted with alanine via PCR-based mutagenesis. Similar to the wild type, both mutant toxins were highly expressed as cytoplasmic inclusions in *Escherichia coli* upon IPTG induction. When *E. coli* cells expressing each of the mutant proteins were tested for toxicity against *Aedes aegypti* mosquito larvae, both mutant toxins still showed a high level of larvicidal activity comparable to the wild type, although a significant decrease in toxicity for Y543A mutation was observed. Interestingly, further substitutions of Tyr-543 with arginine, glutamate, glutamine or isoleucine had a drastic effect on larvicidal activity. These results suggest that the conserved tyrosine residue at position 543 within domain III of the Cry4B toxin play an important role in toxin function. **(STUDENT POSTER).**

BP 4 Mutations within the α4-α5 loop region of Cry4B affect membrane pore-forming properties

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The loop requirement for efficient membrane insertion of the α4-α5 hairpin within the pore-forming domain of the *Bacillus thuringiensis* -endotoxins has been recently demonstrated. In our studies, three residues located within the α4-α5 loop of Cry4B (Asn-166, Tyr-170 and Glu-171) were shown to play a crucial role in larvicidal activity against *Aedes aegypti* larvae, especially the polarlity at position 166 and aromaticity at position 170. Additionally, membrane pore-forming properties of wildtype Cry4B and its loop mutant toxins (N166C, N166D and N166I) were examined using receptor-free, artificial phospholipid bilayers. Similar to the wild type Cry4B toxin, N166D and N166C mutant toxins were still able to form a cation-selective channel with a conductance of approximately 500 pS. However, channel conductance observed for N166I was significantly reduced to be about 200 pS. In addition, membrane permeation was assessed by calcein release assay. The results showed that Cry4B and active mutant N166D were capable of releasing entrapped calcein from PC/PE/CH vesicles. However, N166I was found to be

less active than wild type. These results suggest that Asn-166 is involved in the passage of ions through the channel. In molecular dynamics simulations of hexameric α4-α5 of Cry4B in POPC/water box, a decrease in the extent of hydrogen-bonding at position 166 was found in agreement with the ion-channel conductance of the slightly active mutant in which asparagine was substituted with hydrophobic side chains. **(STUDENT POSTER).**

BP 5 Factors affecting crystallization of the Bt toxins

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We have previously shown that an accessory factor (Orf2) is required for the *in vivo* crystallization of Cry2Aa and have commented on similarities between Orf2 and the DNA molecules proposed to play a role in the crystallization of Cry1 toxins. We have investigated the possibility that Orf2 might be substituting for DNA in the Cry2Aa crystal, and have found that purified Cry2Aa crystals do not contain significant amounts of DNA. We also found no DNA in purified Cry1 crystals but were able to isolate DNA from impure crystals and acrystalliferous strains of Bt. It has also been reported that unlike Cry2Aa, Cry2Ac can form crystals in the absence of Orf2. Our attempts to confirm this revealed a requirement for Orf2 in the formation of Cry2Ac crystals.

BP 6 Investigation of parasporal inclusions from a mosquitocidal *Bacillus thuringiensis* serovar *sotto* strain

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The *Bacillus thuringiensis* serovar *sotto* strain 96-OK-85-24 produces parasporal inclusions with high larvicidal activity against mosquitoes (Ohgushi et al., 2001). When observed with an electron microscopy, parasporal inclusions of this strain were irregular-shaped bodies consisting of heterogeneous matrix surrounded by thick envelopes with low electron density. The hemagglutinating (HA) activity against sheep erythrocytes, associated with inclusion proteins, was specifically inhibited by D-mannose, but not by the seven other monosaccharides: D-galactose, L-fucose, D-glucose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine and *N*-acetylneuraminic acid. SDS-PAGE analysis showed that the inclusion proteins consisted of three major polypeptides of 73, 67 and 33 kDa. When digested with proteinase K, the 73- and 67-kDa proteins were degraded into several proteins ranging from 58 to 70 kDa. Trypsin and chymotrypsin treatments gave similar proteolytic profiles. Little immunological relationships existed between proteins of the strain 96-OK-85-24 and the two reference strains, *B. thuringiensis* serovar *israelensis* and *kyushuensis*. Also, no common antigens occurred between inclusion proteins from 96-OK-85-24 and the strain T84A1, a Lepidoptera-specific strain belonging to the serovar *sotto*. **(STUDENT POSTER).**

BP 7 Biological fitness of a *Culex quinquefasciatus* population its resistance to *Bacillus sphaericus*

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Biological fitness components of a field-collected colony of *Culex quinquefasciatus* say highly resistant to a *Bacillus sphaericus* strain 2362 (RR>163,000) after 46 generations of selection, were compared to those of a susceptible colony (CqSF) that had originated from the same parental cohort but which had not been exposed to *B. sphaericus*. The effect of *B. sphaericus* on the fitness of *Culex quinquefasciatus* was evaluated in terms of fecundity, fertility and development time. The resistant colony (CqRL) showed significantly lower fecundity and fertility, and slower pre-imaginal development than the susceptible colony. Development time from egg to adult emergence showed a 10-19% reduction in CqRL compared to CqSF. As a result of resistance evolution, the generation time increased from 21.6 days to 26 days for highly resistant generations of CqRL.

BP 8 Occurrence of *Bacillus thuringiensis* in feces of wildlife of Korea

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A total of 34 fecal samples, collected from 14 species of wild mammals in Korea, were examined for the occurrence of *Bacillus thuringiensis*. The organism was detected in 18 (52.9%) samples. Among 527 colonies of the *Bacillus cereus / B. thuringiensis* group examined, 43 (8.2%) were allocated to *B. thuringiensis* on the basis of the formation of parasporal inclusions. In general, feces of herbivorous animals contained *B. thuringiensis* at high frequencies. Examples included the roe deer, Korean water deer, Korean hare, and goat. Of 43 isolates, 13 were serologically allocated to the 9 H-antigen serogroups, 15 were untypable, and 15 were untestable. Insecticidal activity was associated with 23.3% of the fecal populations : 3 isolates were toxic to larvae of *Bombyx mori* and 7 isolates were toxic to *Aedes aegypti*. **(STUDENT POSTER).**

BP 9 Development of *Bacillus thuringiensis* formulation for phyto-sensitive crops in North-eastern Asia

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Bacillus thuringiensis formulations, at certain conditions, induced local burning of the leaves of the Chinese cabbage and other sensitive crops in Japan and Korea. There are several approaches to overcome the problem. One of the possibilities is to identify a *Bacillus*

thugringiensis culture that does not produce the metabolite that causes phytotoxicity. After the screening of hundreds of *Bacillus thuringiensis* isolates obtained from a screening program isolating *Bacillus* thuringienses from a world wide collection of soil sample, F810 was identified. This culture not only produces good potency yield in commercial substrate but also does not cause phytotoxicity in the leaves of Chinese cabbage. The culture was found to belong to *kurstaki* subspecies and produces Cry IAa, b, c plus CryII toxins. A patent application has been filed and a product, Tune Up based on F810 isolate is being marketed in Japan. Tune Up® WG formulation contains 100 BIU/Kg, one of the highest in the market.

BP 10 Cereolysin O: distribution, diversity and potential biological role in the *Bacillus cereus* group

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Bacillus cereus sensu lato comprises six closely related microorganisms displaying a broad range of virulence spectrum, from the food contaminant and opportunist *B. cereus* to the entomopathogenic *B. thuringiensis*. Beside the specific virulence factor (e.g. the crystal endotoxins of *B. thuringiensis*), these bacteria possess a large arsenal of others virulence factors (hemolysins, phospholipases, enterotoxins), whose role may be determinant in setting up and/or maintaining non-acute pathogenesis. The aim of this research was to focus on the contribution of one of these virulence factors, the sulfhydryl-activated cytolysin cereolysin O (CLO), to the pathogenic arsenal of *B. cereus s.l.* strains. More than 80 *B. cereus s.l.* strains originating both from reference collection and from natural sources (including 20 representative *B. thuringiensis* strains) have been analyzed. Using PCR, the *clo* gene was shown to be present in all strains, regardless of the bacterial species. The diversity of the *clo* genes, analyzed by RFLP, indicated a good conservation among the different strains. However, the most striking observation was certainly the presence, in at least 15 % of the strains, of more than one gene copy. Cloning and sequencing of a total of 13 *clo*-derived PCR products from 7 strains revealed a conservation varying between 90 to 99 % among themselves, as compared to the 75 % identity shared with the alveolysin gene of *Paenibacillus alvei*. The genes from the same strain also tended to be clustered. *Clo* knockout experiments, as well as cloning and expression of *clo* in *Bacillus subtilis* are currently under way to determine the relative contribution of this CLO toxin to the cytolytic activity of these bacteria towards various target cells (human and animal erythrocytes, Vero cells or insect cells) or living animals (insect larvae and arthropods). Analysis of the genetic context of *clo* has also been analyzed in characteristic strains of *B. cereus s.l.* and has revealed striking variations in the immediate vicinity of the genes.

BP 11 The *Bacillus thuringiensis* toxin specificity database

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Elucidating the molecular and biochemical basis of *Bacillus thuringiensis* delta-endotoxin specificity is a major research focus in laboratories around the world, with the ultimate goal of designing more effective insecticidal proteins. Data on insecticidal activity of many individual toxin proteins have been published since their genes were first cloned in the early-1980s. Interpretation of toxicity data is confounded by many factors that are known to affect toxicity, including the type of toxin gene, the choice of assay technique, the stage of the test insect, the form in which the toxin was tested (crystalline inclusions versus solubilized protoxins or activated toxins), and the degree of toxin purity. We designed a database that permits the user to take those factors into account. It is based on Microsoft Access 2000, and was first published on the internet in 1998. Newly published toxicity data are incorporated at least once or twice a year. The database was recently redesigned, using Cold Fusion software, and moved to a new server (http://www.glfc.cfs.nrcan.gc.ca/Bacillus/btsearch.cfm). It currently consists of 1038 records, obtained from 235 references pertaining to 120 insect species and 148 toxin genes. A brief description of how the database is set up and some examples of search outputs will be presented. We are maintaining the database as a service to the scientific community, industry, and regulatory agencies.

BP 12 Behavior of Brazilian *Bacillus thuringiensis* strains when submitted to Sodium Dodecyl Sulphate (SDS) as a plasmid curing agent

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Bacillus thuringiensis have been successfully used in biological control of agricultural pests and disease vectors, mainly for their high toxicity and specificity against Lepidoptera, Coleoptera and Diptera insects. This toxicity is due to delta-endotoxins produced during sporulation. These endotoxins are encoded by genes named *cry* and *cyt*, which are mainly located in the plasmidial DNA. The objective of this study was to submit ten Brazilian strains to low concentrations of Sodium Dodecyl Sulphate (SDS), a plasmid curing agent, observe their behavior (inhibition of protein synthesis) and compare treated strains to the non-cured ones. The preliminary results show that one strain was able to continue synthesizing endotoxins but became asporogenic, another one developed a larger parasporal crystal than in the native sample; and three became resistant to antibiotics to which they had previously been susceptible. Analyses of the Isoenzimatic, Cry-protein and plasmidial profiles are still under way.

BP 13 *In vitro* binding of *Bacillus thuringiensis* Cry11Bb and Cry11Aa toxins shows two different receptors in the midgut of mosquito larvae

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Cry11Aa and Cry11Bb are toxins with mosquitocidal activity, produced by *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *medellin*, respectively. Their specificity has been related to the presence of receptors in the insect midgut. In a previous work, the *in vivo* binding of the toxins Cry11Aa and Cry11Bb to the apical brush border membrane of mosquito midgut cells suggested that the midgut is the primary site of action of the Cry11Aa and Cry11Bb toxins. However, further studies are required to understand the mode of action of the Cry11 toxins. Cry11Bb and Cry11Aa were used for the *in vitro* binding analysis to midgut tissue sections from fourth instar *Aedes aegypti*, *Anopheles albimanus* and *Culex quinquefasciatus* mosquito larvae. The bound biotinylated Cry11Bb toxin was detected by avidin-peroxidase conjugate. In order to analyze the specificity of the interaction between Cry11Bb with the apical microvilli on midgut epithelial cells, homologous competition binding experiments were performed. Cry11Bb bound evenly to midgut microvilli displayed an equal pattern in all mosquito species evaluated. Binding of biotinylated Cry11Bb toxin was analyzed in the presence of 1, 10, and 100-fold excess of native Cry11Bb. 1 and 10-fold excess displace the binding of biotinylated Cry11Bb, the 100-fold excess of native Cry11Bb resulted in a complete loss of brush border staining. These data could suggest that Cry11Bb toxin binds in a specific manner to the epithelial cell microvilli. Heterologous competition experiments with Cry11Aa toxin were carried out incubating mosquito tissue sections with biotinylated Cry11Bb in the presence of a 100-fold excess of native Cry11Aa. The results indicate that the staining intensity was identical to the pattern obtained after incubation with biotinylated Cry11Bb alone. Therefore these data suggest that Cry11Bb and Cry11Aa toxins could have different receptors.

BP 14 The presence and number of *Bacillus thuringiensis* spores in Colombian soils are determined by the soil physicochemical characteristics

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One hundred and one Colombian soil samples were collected; for each of them, 14 physicochemical variables were evaluated, along with the presence and number of *Bacillus thuringiensis* spores, and a statistical study was performed. No simple regression between each of the variables and the presence of *B. thuringiensis* was found, what suggests that the relationship between *B. thuringiensis* and soil must be complex. By multiple regression, three models were found with several variables. Moreover, Logit regression analysis revealed another model that might allow to predict the presence of *B. thuringiensis* in a given soil sample. In all models, soil pH was the most important factor. It is known that pH has an influence on other variables and vice versa; therefore, it is suggested that the interaction of these variables is what may affect the presence of *B. thuringiensis*. The models found in the present study represent novel tools that could facilitate the understanding of the *B. thuringiensis* ecology.

BP 15 Effects of co-expression of Cry11Aa with Cyt1Aa and/or p20 in aquatic bacteria

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Bacillus thuringiensis serovar. *israelensis* (Bti) has some disadvantages when applied into the field to control mosquito larvae: toxins sink very quickly and are rapidly degraded by UV light. Alternative hosts for Bti toxins, such as prosthecated aquatic bacteria, can overcome this. They have a prostheca, what prevents them from sinking and the cytoplasmic located toxins are protected from UV radiation. Moreover, and the most important feature, the mosquito larvae feed on them. Previously, we presented results on a recombinant *Asticcacaulis excentricus* strain harbouring Cry11Aa. A low but detectable expression was obtained. This time, our aim was to increase the effectiveness of the recombinant strain by co-expressing other proteins that can enhance the activity of Cry11Aa. Plasmids carrying Cry11Aa plus Cyt1Aa, Cry11Aa plus p20 and Cry11Aa plus Cyt1Aa plus p20 were constructed. *A. excentricus* and *C. crescentus*, both prosthecated bacteria, were transformed with these plasmids separately. Toxin expression was evaluated and quantified by polyacrylamide gel electrophoresis and Western blot. The toxicity of transformed bacteria was also tested in first and third instar *Culex quinquefasciatus* (Diptera) larvae bioassays. Finally, we have attempted to electroporate native Colombian bacteria (*A. excentricus*, *C. crescentus* and *Prosthecobacter fusiformis*) isolated from water ponds.

BP 16 *Bacillus thuringiensis* – non target effect of a purified toxin and a commercial formulation

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The commercial formulation Dipel PM® containing spores and crystals of *Bacillus thuringiensis* var. *kurstaki* (17600 IU/mg) was applied against *Ageniaspis citricola*, a parasitoid of the citrus leafminer *Phyllocnistis citrella*. The study was performed using four treatments: distilled water as control (CT); Dipel® diluted in distilled water (10g/L) (CP); inactivated (120°C/20 min) solution of the CP treatment (BI); purified toxin cryIA(C) (0,0010 g/100mL of a 5% PBS buffer) (CRY). Each treatment was sprayed (50 mL) on eggs of *P. citrella* on shoots of lemon plantlets var. cravo. The shoots were randomized and then maintained caged in rearing rooms (28°C ± 1°C; RH 70% ± 10%, 12h photophase). Adults of *A. citricola* were then released in the cages (30 adults/cage). After 14 days development of immature parasitoid the leaves were examined under a stereomicroscope to check pupal chambers of *P. citrella* and to quantify the parasitism by *A. citricola*. Completely randomized statistical design was used to analyse the data, with four treatments and five replicates. Duncan test was applied to compare means. The results showed that CT, BI and CRY did not differ between themselves but they all differed significantly from CP treatment. These results indicated that the effect of the CP treatment can not be attributed to the toxin. Further studies are recommended to determine

if the interference in the parasitism could be attributed to the spores or to the ingredients of the formulation .

BP 17 Ion-channel Activity of the *Bacillus thuringiensis* Cry4B α1-α5 Pore-Forming Fragment

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Bacillus thuringiensis I -endotoxins are proposed to act by forming a lytic pore in the susceptible insect membrane. Tryptic activation of the 130-kDa Cry4B toxin produced protease-resistant products of ca. 47 kDa and ca. 21 kDa. The cloned 21-kDa fragment consisting of the N-terminal five-helix bundle (Γ1-Γ5) was previously shown to be capable of inducing liposome permeability. In this study, circular dichroism spectroscopy indicated that this pore-forming (PF) fragment exists as an Γ-helical structure in Tris buffer, pH 8.8. The ability of the PF fragment to form channels was investigated in a receptor-free, planar lipid bilayer system. The channel properties of the PF protein appeared to be voltage independent with conductance levels higher than that of the 65-kDa full-length activated toxin. In addition, the channels formed by the PF fragment, which always remain at an opening state, displayed cation-selectivity with a reverse potential similar to that of the activated toxin. These results clearly demonstrated that this five-helix bundle is a component responsible for ion channel formation of the active toxin.

BP 18 Implications for the sustainability of transgenic Bt maize Monitoring the susceptibility of European Corn Borer in Germany

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Transgenic maize cultivars expressing *Bacillus thuringiensis* (Bt) toxin need resistance management of the target pest species European corn borer (ECB, *Ostrinia nubilalis* Huebner). For this reason we measured the baseline susceptibility of different ECB populations in Germany. We performed tests by treating first instar larvae with different Bt-toxin concentrations incorporated into the nutrition medium or applied onto the medium (surface treatment). LC50 values range from 1.02 – 1.63 µg/ml for untruncated Cry1Ab to 0.12 – 0.24 µg/ml for truncated Cry1Ab. No significant differences were detected for populations from different regions of Germany. Our monitoring approach includes the definition of geographical distinct ECB populations by help of molecular fingerprinting techniques such as AFLP and RAPD-PCR. So far we could not differentiate between the examined populations or the different strains. The target pest species European Corn Borer is common in Germany as E- and Z-strain, but only the Z-strain normally causes damage in corn. Estimation of gene flow between the two strains is important for refuge strategies. Additional effort is spent into the methodology on how to detect susceptibility change through regular monitoring, including harmonization of different approaches in various research groups of EU countries.

BP 19 Effects of Bt-corn growing on the epigeic and herbaceous layer fauna of different trophic levels

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The research project focuses on potential side effects of the cultivation of genetic modified Bt maize on the entomo biocoenosis of different trophic levels. We compare arthropod communities found in three maize cultivation treatments: (i) isogenic varietiy without insecticide (control), (ii) the isogenic variatiy with chemical insecticide and (iii) Bt-maize expressing recombinant Bt-toxins. The field trails are conducted at two fields of 6 ha total in a period of three years. Fields are divided in plots of 0.25 ha with 8 replication for each treatment. Sites are described by pedological, phytosociological and climatic parameters. The research program is divided in 4 moduls: (i) effects on non target organisms in the herbaceous layer, (ii) effects on flower visitors, (iii) effects on predatory arthropods of the epigaeon and (iv) effects of pollen deposition on arthropods living on associated plants. The project started in 2001. On our Bonn test site we observed a high infestion with European corn borer in the control plots. The insecticide treatment reduced European corn borer infestation, but sufficient protection was only observed in the Bt-maize plots. Diversity and abundance of selected arthropod groups was surveyed by standard methods with special regard on carabids, spiders, thrips, aphids and lepidopteran larvae and their antagonists. First results will be presented.

Microsporidia

MP 1 Epizootic and enzootic features of microporidia in *Simulium pertinax* (Diptera: Simuliidae) larvae in the state of Rio de Janeiro, Brazil

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The search for natural pathogens of Brazilian species of simuliids is necessary to discover effective, alternative methods of control with reduced costs. The objectives of this study were to isolate microsporidia from *Simulium pertinax* larvae with potential as biological control agents for these insects, as well as to establish the natural epizootic and enzootic features to enhance IPM programs for the control of simuliids. Larvae were collected from the Soberbo river in Guapimirim and in Andorinhas river in Santo Aleixo, both located in the area of the Serra dos Órgãos in the state of Rio de Janeiro. These sites were

chosen because black flies were present at high densities and they are serious pests of man and animals. Larvae were collected and maintained in breeding site water with aeration to assure their survival during transport to and while held in the laboratory. Larvae must be alive to observe the external symptomology of infection and therefore larvae were maintained in the laboratory during screening to detect those infected with microsporidia. Infected larvae were dissected in distilled water and saline solution making separate smears of the intestinal tract and fat body. Smears were fixed in methanol and stained with Giemsa and examined for the presence of vegetative stages and spores of microsporidia. The microsporidia found thus far were identified as *Amblyospora* sp., *Polydispyrenia simulii*., *Microsporidium* sp., *Caudospora* sp. and *Polydispyrenia* sp. with this latter species the most abundant.

* Partial support PIBIC/CNPq / IOC/FIOCRUZ

MP 2 Changes in reproductive life history patterns of the gerrid, *Aquarius remigis*, alter trypanosomatid prevalence

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Local fluctuations in season length and other environmental factors, such as temperature and food abundance, can affect the number of generations a temperate, non-migrating insect species completes in one season, i.e. voltinism. Changes in voltinism patterns (e.g. from one generation to two per year) alter opportunities for contact between potential insect hosts and the parasites that rely on host interactive behavior for transmission. As a result, prevalence patterns of insect parasites may fluctuate. Using field observations from four streams over a two-year period, we investigated the prevalence of trypanosomatid parasites in the water strider host, *Aquarius remigis*. Prevalence peaked in May for adults and in the fall for nymphs in both years. Prevalence, as well as gerrid density, was generally higher in 2002. We observed two distinct mating periods in 2002, indicative of a bivoltine life cycle, while in 1999 only one mating period was detected. We argue that climatic differences between the two years (low rainfall in 1999) induced different gerrid life histories that influenced trypanosomatid prevalence and gerrid density patterns. **(STUDENT PAPER).**

Nematodes

NP 1 Susceptibility of chilean target pests to native entomopathogenic nematodes

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Along Chile, 65 entomopathogenic nematode isolates have been collected in the last 6 years. The parasitic ability of these native entomopathogenic nematodes, to aerial and terrestrial larvae of insect pests such as *Aegorhinus superciliosus, Asynonychus cervinus, Hylamorpha elegans, Tipula apterogyne, Eumerus* sp., *Dalaca pallens, Delia antiqua, Cydia molesta* and *Cydia pomonella* was evaluated. Overall, inoculations were made by applying 50 infective juveniles on insect larvae. Symptoms and mortality of inoculated insects were evaluated daily. The most virulent nematode isolates were selected by assessment of lethal time for the different insect species. There were differences in pathogenicity among the nematode isolates, showing great specificity for some insect pests. Future evaluations will help us to determine the efficiency of these isolates in the field and their adaptations to different soils and temperatures.

NP 2 Evolution of the oxygen consumption of *Steinernema feltiae* and *Xenorhbadus nematophilus* in axenic and monoxenic cultures

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Development of processes for mass production of entomopathogenic nematodes requires establishing diverse parameters as the oxygen demand. In this work the oxygen consumption of *Steinernema feltiae* (Mexican) and *Xenorhabdus nematophilus* in submerged axenic and monoxenic culture was investigated.Axenic cultures of *Xenorhabdus nematophilus* had a maximal oxygen demand of 1 $gO_2 L^{-1} h^{-1}$, after five hours of cultivation in SDm medium (yeast extract, egg yolk, corn oil, and NaCl) or in a medium with trypticase soy broth plus yeast extract and cholesterol. *Steinernema feltiae* in axenic culture had a maximal oxygen demand of 0.13 $gO_2 L^{-1} h^{-1}$ at day 7. At this time the population was formed by 8% adults and 90% J2. However, a maximal specific oxygen demand of 2.3 x 10^{-6} gO_2 nematode^{-1} h^{-1} was reached at day 6 when 90% adults formed the population. In the monoxenic culture, 90% adults were reached at day 3, at this time the volumetric oxygen demand was 0.3 gO_2 L^{-1} h^{-1}, equivalent to 1.7 x 10^{-4} gO_2 nematode^{-1} h^{-1}. A maximal volumetric oxygen demand of 0.9 $gO_2 L^{-1} h^{-1}$ was reached at day 5 when the population was 2% adults and 96% J2. The maximal volumetric oxygen demand in monoxenic culture was 7 times higher than axenic culture, but the specific oxygen demand for adult nematodes in monoxenic culture was 74 times higher than in the axenic. Besides, multiplication factor was 80 for the monoxenic culture and only 14 for the axenic. This fact can explain the differences between oxygen consumption in monoxenic and axenic cultures. It is clearly evident that the presence of the symbiotic bacteria is very important to obtain a high productivity of nematodes and consequently increases the specific oxygen demand of nematodes. **(STUDENT POSTER).**

NP 3 Comparative efficacy of different species of entomopathogenic nematodes for the control of guava weevil *Conotrachelus psidii* (Coleoptera: Curculionidae)

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The family Curculionidae contains many important pest species responsable for losses in agricultural production worldwide. The guava weevil *Conotrachelus psidii* is no exception. It attacks guava orchards in the State of Rio de Janeiro, seriously damaging the quality of the fruits. The biology of this insect is unusual, spending the larval period (1st-3rd instar) in the fruit. When the fruit falls to the ground, the last larval instar (4th) migrates to the soil where it stays for up to 4 months, until becoming adult. This biology indicates that entomopathogenic nematodes maybe useful for the biological control of *C. psidii*. Therefore we tested four different species for pathogenicity against 4th instar guava weevils: *Steinernema feltiae* Gvoulot, *S. glaseri, S. carpocapsae* All, and *Heterorhabditis bacteriophora* Brecon. One hundred infective juveniles suspended in 0.5 ml of sterile distiled water were distributed evenly onto a 5.5 cm-diameter filter paper in the bottom of a 6.0 cm Petri dish, before placing larvae in the dishes. A total of eight insect larvae were used for each nematode species tested. The percentage mortality was 100%, 87.5%, 62.5 % and 62.5, respectively. *S. glaseri* was found to cause rapid mortality (24 to 96 hours after infection), the second fastest was *S. feltiae* (48-216 hours), followed by *S. carpocapsae* (up to 240 hours) and *H. bacteriophora* (>336 hours). *H. bacteriophora* took longer to kill the larvae, but the insects displayed symptoms of infection (loss of colour and lethagia) 24 hours following infection. Our preliminary studies show that *S. feltiae* maybe a candiate for the biological control of the guava weevil. However more studies are being carried out to confirm these results and to evaluate if this species will adapt to the crop conditions.

NP 4 Survey of native populations of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and their symbiotic bacteria from Costa Rica

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A survey for entomopathogenic nematodes (EPN) were conducted at the Area de Conservación Guanacaste (ACG), Costa Rica, Central America. Located in northwestern Costa Rica, ACG has been selected as a focal point for this survey due to its designation as "hotspot" for conservation, and its research facilities. A total of 57 soil samples from 6 different areas ranging from coastal to valley between volcanoes were collected and examined for the occurrence of nematodes. EPN were recovered from the dry forest associated to the coastal area, and rainforest located between volcanoes and the Caribbean zone. One isolate was identified as *Heterorhabditis indica* Poinar, Karunakar and David, a species that has previously been reported in other tropical areas in Central America (Puerto Rico), SE Asia (India) and North America (Hawaii). Based on molecular evidence and preliminary morphological examination (scanning electron microscopy), the two remaining isolates were diagnosed as new species of *Steinernema*. Morphological characterization and formal species description of these isolates is currently being conducted. Three bacterial strains were cultivated from the EPN isolated, which were identified as two *Xenorhabdus sp.* and one *Photorhabdus sp.*. Although conventional phenotypic criteria, relate these isolates to *Xenorhabdus nematophilus* and *Photorhabdus luminiscens*, it is well known that in both genera, identification of new isolates or species is difficult because most strains are phenotypically very similar and fail to give positive results in many classical test for identification and because of a lack of sufficient members per taxon. As a result, a molecular approach must be undertaken to complete the specific level identification of theses isolates.

NP 5 Pathogenicity of *Heterorhabditis* spp. and *Steinernema* spp. against the citrus root weevil, *Naupactus* sp.

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Serious damages to citrus plant have been observed as result of attacks of the citrus root weevils, *Naupactus* spp. and *Pantomorus* spp., in São Paulo State, Brazil. The adults eat the leaf and lay the eggs on the fruit chalice. The eggs hatch 7 to 10 days later and the larvae drop on soil in order to attack the plant root. Due the larval behavior, the control by chemical insecticides is very difficult. The aim of this study was to evaluate the pathogenicity of two strains of *Heterorhabditis* (strain CB-n5 and strain CCA) and six of *Steinernema* (*S. carpocapsae*, *S. anomali*, *S. glaseri*, *Steinernema* sp. - strain Cb-n6, *Steinernema* sp. - strain Cb-n7 and *Steinernema* sp. strain Cb-n9) against *Naupactus* sp. larvae in laboratory conditions. Replications per treatment were 5, represented by 10 larvae of the first instar inside Petri dishes, over a filter paper. Each replication received 2 mL suspension containing 2000 infectives juveniles of the nematodes. The Petri dishes were sealed and kept in chamber at 24 ± 2°C, under darkness. Larval mortality was registered 2, 4, 6, 8 and 10 days after inoculation. *S. anomali* was the most pathogenic species, with 62% of mortality at 10th day evaluation, followed by *Heterorhabditis* sp. - strain CB-n5 and *S. carpocapsae*, both with 34% mortality, *S. glaseri* (17%), *Steinernema* sp. – strain CB-n6 (20%), *Heterorhabditis* sp. strain CCA (22%), *Steinernema* sp. strain CB-n9 (23%). This result suggests the study should be continued to evaluate the three best strains against older larvae in sand column.

NP 6 Pathogenicity of *Heterorhabditis* sp. and *Steinernema* spp. (Nemata: Rhabditida), in different dosages, against the citrus root weevil

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Species of *Naupactus/Pantomorus* complex are important pests of citrus crop in São Paulo State, Brazil. The larvae attack the plant root and the adults eat the leaf, decreasing the crop yield. Living in the soil, the larvae become

difficult target for the implementation of control measures, except for the use of entomopathogenic nematodes. This research aimed evaluating the pathogenicity of *Heterorhabditis* sp. (strain CB-n5), *S. anomali* and *S. carpocapsae*, in three different dosages, against *Naupactus* spp./*Pantomorus* spp. larvae in laboratory conditions. Replications per treatment were 5, with each replication represented by 8 larvae of 3 th to 5th instar kept inside a plastic dish containing 300 mL of soil. The inocule were 2 mL suspension containing 40, 80 and 160 infective juveniles /insect, applied over the soil. The dishes containing the larvae and nematodes were sealed and incubated at 24°C under the darkness. Evaluations were done with 4 and 8 days. Dead insects were transferred to other dishes and incubated in wet conditions, under 24°C. *Heterohabidtis* sp. was the most pathogenic specie, with mortality varying from 62% at the lower dosage to 75% at the higher one, followed by *S. anomali*, with mortality from 45% to 68%, and *S. carpocapsae*, with mortality from 37% to 62%. *Heterorhabditis* sp. is a native strain, isolated from soil of citrus crop located at South São Paulo State, Brazil.

NP 7 Biological Control of *Tecia solanivora* using *Steinernema feltiae* Colombia Strain in potatoes in Cundinamarca Colombia.

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Tecia solanivora (Lepidoptera: Gelechiidae), an introduced pest from Central America, is currently the most limiting factor for potato production and marketing in the Andean region of Colombia. Although attempts have been made to biologically control the pest using parasitoids, bacteria, and fungi, field results have not been very promising. Naturally occurring populations of *Steinernema feltiae* attacking *T. solanivora* suggest the possibility of using this natural enemy for biological control of the pest. We evaluated the ability of *S. feltiae* to attack soil inhabiting larvae of *T. solanivora* in two crop cycles at the Colombian National University experimental station, in Mosquera, Cundinamarca. Four doses of infective juveniles (J3) were tested: 1.5 x 10^4, 3.0 x 10^4, 6.0 x 10^4 and 1.2 x 10^5 J3/m^2. Nematodes were applied once to the base of potato plants using a knap-sac sprayer calibrated at 20 psi at 35 or 75 days after planting. Experimental areas of 2,430 m^2 were divided into 30 plots consisting of 9 rows (10 m long) and 25 plants per row in a completely randomized blocks design with 3 replicates. An absolute control (no application) and a commercial control using pesticides were also included. Adult *T. solanivora* were monitored weekly using sex pheromone traps. At harvest time, total yields (Kg/ha) and percent damage to tubers were estimated. Damage levels were reduced to 78% and 91% for the first and second cycles respectively by the presence of *S. feltiae*. All doses of J3 were equally capable of controlling larvae of *T. solanivora*, however damage to tubers was lower when the nematodes were applied at 75 than at 35 days after planting. Absolute and commercial controls showed 92% and 45% damage to tubers respectively. These results suggest that S. *feltiae* is a promising agent for biological control of T. *solanivora* in field potatoes in the region.

NP 8 Geographical distribution of *Steinernema feltiae* in Cundinamarca and Boyacá, Colombia

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Soil samples were collected in 16 municipalities in Cundinamarca and 3 in Boyacá, Colombia ranging in altitudes from 2,430 to 3,610 meters above sea level and soil textures ranging from sandy loam to clay loam. Out of 770 samples, 53% were positive for populations of *Steinernema feltiae* (Rhabditida: Steinernematidae). Samples were stored in black plastic bags the laboratory at 22°C and 60% H.R. Nematodes were recovered after 2 and 12 months of storage using last instar larvae of *Galleria mellonella*, *Achroia grisella* (Lepidoptera: Pyralidae) and *Tecia solanivora* (Lepidoptera: Gelechiidae). Samples retained 95% and 10% moisture after 2 and 12 months of storage respectively. Most populations of *S. feltiae* (69.9%) were isolated from natural andean soils (cloud forests), pH 4.9 and average soil temperature of 14°C. The rest (30.1%) of the populations were isolated from areas cultivated with potatoes (sampled during flowering and at harvest time). Eighteen populations from natural cloud forests were collected between 2,751 and 2,854 m.a.s.l. and 19 populations were found at altitudes between 3,000 and 3,610 m.a.s.l. Five populations from cultivated areas were collected between 2,430 and 2,670 m.a.s.l., and 11 between 2,750 and 3,150 m.a.s.l. Natural hosts for *S. feltiae* in potato crops included larvae of *T. solanivora*, *Pthorimaea operculella* (Lepidoptera: Gelechiidae) and *Premnotrypes vorax* (Coleoptera: Curculionidae) all of economic importance for potatoes in the region. This is the first record for this species of entomopathogenic nematode in Colombia and the first record of *S. feltiae* naturally occurring on these host species.

Microbial Control

MC 1 Bioinsecticide formulations using microencapsulation process

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The aim of this paper was to develop microparticulate formulations containing encapsulated spores and δ-endotoxins of *Bacillus thuringiensis var.kurstaki* (Btk). The development of appropriate formulations which are economical and which can deliver a viable organism and an intact protein are critical for successful biocontrol products used against plant pathogens and mosquitoes. These formulations should provide desirable characteristics for the microbial insecticide, such as long shelf life and appropriate survival in soil and water as well as sufficient cell density and activity. Calcium alginate and chitosan-alginate microparticles containing spore and toxin are formed by atomising dispersions of the sodium alginate solution and Btk suspensions into an aqueous solutions of calcium chloride. When chitosan-alginate microparticles were prepared, calcium alginate gel microparticles were first formed, followed by a chitosan-alginate membrane forming. The gelled microparticles were dried under vacuum, at room temperature. The granulometric distribution and the morphology of the microparticles were determined and

the cell viability was measured after Btk survival control tests. The spores counts showed that around 97% of the spores were incorporated within the microparticles. The encapsulation of suspensions of Btk containing spore and toxin in alginate and chitosan-alginate microparticles did not decrease the larvicidal activity of this bioinsecticide against lepidopteran pests.

MC 2 Distribution of fungal conidia in the canopy of chrysanthemum using hydraulic and electrostatic sprayers

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Droplet size, number of microbial propagules in droplets and the number of droplets per unit area of plant are important indices and critical for effective biological pest control. For optimization of the spray technology we estimated the efficiency of two sprayers, including electrostatic and hydraulic types. This work was conducted with the industrial formulation BotaniGard™ WP (*Beauveria bassiana)* on chrysanthemum plants. The carrier was water with Tween 80 (0.05%). All spray parameters were same for both sprayers (full cone nozzle, pressure 40, time 3 sec). The number of droplets per unit area, size of droplets and the number of spore in each droplet were estimated in different zones of the plant canopy. Droplets and conidia calculation were made by placing cover slips in the top, middle and lower parts of the canopy. Scotch type was used to remove conidia from leaf surfaces after spraying. The number of conidia per 1 mm^2 using the hydraulic sprayer was 17±1 for the top of the plants, 5±1 for the middle and 18±2 for lower canopy; for the electrostatic sprayer these indexes were 72±3, 16±1 and 12±1 for upper, middle and lower respectively. Distribution of droplets according to size was for group 1 (fine aerosol droplets, 25μm ∅) – electrostatic19.2%, hydraulic - 12.9%,; group 2 (coarse aerosol droplets, 26-50μm ∅) – electrostatic 30.8%, hydraulic – 30.5%; group 3 (mist droplets, 51-100μm ∅) - electrostatic 34.6%, hydraulic – 37.9%; group 4 (fine spray droplets, 101-200μm ∅) - electrostatic 15.4%, hydraulic –18.5%. It is well documented that electrostatic sprayers produce large amounts of fine aerosol droplets. We did not find any advantage to using electrostatic sprayers in comparison with hydraulic sprayers. Fine aerosol droplets have a strong tendency to drift and they do not reach leaf surface of chrysanthemum. The number of fungal conidia in droplets was for electrostatic sprayer - group 1 - 15±2.1, group 2 - 32±2.9, group 3 – 116 ±11.4 and group 5 - 374±66.6; for hydraulic sprayer – 8.7±0.9, 19.3±1.6, 84±6.7 and 168±30.7 respectively. In all cases droplets from electrostatic sprayer contained significantly more conidia. It is possible the most fine and partly coarse aerosol electrostatic droplets do not include conidia and these very small droplets do not fall on the leaf surface. As a result the larger droplets have more conidia.

MC 3 Cloning and expression of *Bacillus thuringiensis cry2Aa* gene from Bt66 strain

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A toxin gene from *Bacillus thuringiensis* strain, Bt66, was cloned and construction of a recombinant Bt strain was carried out. First, HindIII digested fragments from strain Bt66 plasmid DNA were hybridized with *cry2Aa* DNA probe. The result of southern blotting showed that one positive band of 5.0-kb. The 5.0-kb fragment was inserted into the HindIII site of cloning vector pBluescriptSK(+), and then transformed into *E.coli* JM107. PCR and enzyme digestion analysis demonstrated that the positive transformant clones contained the 5.0-kb fragment of the gene. By subcloning, a 4.0-kb fragment was obtained. Nucleotide acid sequence as well as deduced amino acid analysis suggested that this gene was composed of 1902-bps and one of the bases had been changed compared with known *cry2Aa* genes (from A to G) and subsequently resulted in one amino acid change (from Ser to Gly). 633 amino acid were deduced from its nucleotide sequence and its MW was 70.821kDa. Also, this gene was registered in Genbank (Accession number: 252262) and named as a new gene called *cry2Aa8*. Expression vector pGW1105 was constructed by *cry2Aa8* inserted into pGM1105. This *Bacillus thuringiensis* genetic engineering strain BiotII176 was constructed from introduction of pGW1105 into natural isolate UV17 by electroporation. The expression of Cry2Aa crystal protein in BiotII176 was characterized by SDS-PAGE and optical microscope. The results, an intense protein band of Cry2Aa8 70kDa in SDS-PAGE and formation of cubic crystal, indicated that *cry2Aa8* gene could express normally and stably in UV17. The expression was due to ORF2 protein contained in pGW1105 that was known to promote Cry2Aa crystal formation during sporulation. Insecticidal activity of engineering strain BiotII176 holding two distinct *cry-type* genes was assayed against *Plutella xylostella* larvae. Natural strain UV17 was used as positive control in this study. Bioassay demonstrated that the toxicity of BiotII176 was enforced. It implicated that both Cry2Aa and Cry1Ba promoted each other in their toxic activities against insects. **(STUDENT POSTER)**

MC 4 Optimized batch production of *Bacillus thuringiensis* subsp. *Israelensis* based on activity against *Aedes aegypti* larvae

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Bacillus thuringiensis subsp. *israelensis* (Bti) is one of the most effective larvicidal agents used in mosquito control programs. For Bti production, batch, fed-batch and continuos cultures have been studied, although batch culture renders the highest crystal:spore rate, which increases insecticidal activity. The optimized batch production of Bti strain 225 was made in a 5 l total volume stirred Bioflo III microfermentor using a previously selected medium, the temperature and pH were controlled to 30°C and 7.0 respectively, and operational conditions ranging between 100-500 rpm and 0.2-1 vvm agitation and aeration rates. These conditions were established according to Box Rotational Matrix design, including five combinations with three replications to complete 15 runs; at the end of

fermentation, the larvicidal extracts were recovered using lactose and acetone method and preliminary evaluation against *Aedes aegypti* larvae at 0.05 mg/l, later, the LC_{50} was determined through the procedure reported by de Barjac and Larget-Thiéry (1984). The bioinsecticide yields obtained were from 9.1 to 15.7 g/l, the total fermentation times ranging between 18 to 26.3 h, the dissolved oxygen became to descend below to 20% in several runs, whereas the toxicity showed by the different extracts was variable; the most toxic extracts were obtained when were used conditions of 300 rpm-1 vvm and 500 rpm-0.6 vvm combinations, with mortality percentages on *Aedes aegypti* of 47.2 and 59.7 respectively; in the preliminary test; the subsequent evaluation for LC_{50} were found values of 0.2675 and 0.0685 mg/l, respectively, both values compared by a *t* test at 0.05 level resulted no significantly different, however, the insecticidal extracts obtained using 300 rpm-1 vvm resulted with higher variations in the toxic activity than the extracts obtained with 500 rpm-0.6 vvm. We not found correlation between k_La and toxic activity.

MC 5 Field evaluation of polymer-based granular formulations of *Bacillus thuringiensis* subsp. *israelensis* H-14

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Efforts are being made to improve the effectiveness of the *Bacillus thuringiensis* subsp. *israelensis* (Bti) formulations by prolonging its activity, as well as delivery by targeting the active ingredient in the larval feeding zone. These improvements are primarily based through the use of biopolymers, such as sodium alginate, gelatin, lignin and others. The spore-crystal complex of Bti strain 225 was produced in a Bioflo III microfermentor, using an earlier described medium; the larvicidal extracts were recovered using lactose and acetone and evaluated by the procedure described by de Barjac and Larget-Thiéry (1984), as well as the International Standard IPS-82 for titration of our extract obtained against laboratory-reared *Aedes aegypti* larvae. This Bti extract was formulated with one of three polymers (gelatin, acacia gum and paraffin) and a solar protectant (malachite green) for increase residual activity in the field. The prepared formulations were tested for efficacy using 200 l-plastic containers, colonized previously with mosquito larvae, in a random square design with 4 treatments, three formulations and an untreated control applied at dose 13.7 mg/l (Two replications/treatment). Larval population densities were assessed each 3-4 days after treatment, taking 10 dipper samples from each treatment, and a test for residual activity was done at days 21 and 33 posttreatment against laboratory-reared 4th instar *Aedes aegypti* larvae. The data showed that the three polymer-Bti formulations, which arbitrary potency was 196.8 ITU/mg, were equally effective at 4 and 7 days posttreatment against *Ae. aegypti* and *Culex* sp total larvae, whereas the average total pupae was significantly higher in control than treatments until day 7 after application, but after day 11 no differences were detected. After 21 and 33 days posttreatment, was found residual activity inducing 85-59% and 69-54% larval mortality respectively, on laboratory-reared 4th instar *Ae. aegypti* larvae in water samples obtained from treated containers.

MC 6 Production of active ingredient (conidia or blastospores) of *Beauveria bassiana* (Bals.) Vuillemin (Deuteromycotina: Hyphomycetes) in different liquid media

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Beauveria bassiana is an entomopathogenic fungus that attacks a broad range of insects in tropical and temperate climates and is one of the most promising fungi for insect biological control. At present, our studies with *B. bassiana* spore production in liquid media have had a morphological focus. The production of fungal spores in liquid media has potential to reduce production costs. In this study, we evaluated *B. bassiana* spore production and spore desiccation tolerance in liquid media containing various sources of nitrogen. *B. bassiana* precultures were inoculated with conidia at a concentration of $1x\ 10^6$ spores/ml and incubated at 300 RPM and 25°C for three days. *B. bassiana* spore production cultures were inoculated with $1x10^7$ blastospores/ml and were grown for three days under above described conditions. For desiccation tolerance studies, liquid culture produced spores were mixed with diatomaceous earth, dewatered and air dried at room temperature to moisture levels below 4%. Dried B. bassiana spore preparations were stored in plastic bags at 4°C and 26°C. Our results demonstrated that media containing corn steep liquor or Casamino acids produced very high spore concentrations, $7.56x10^9$ and $2.25x10^9$ spores/ml, respectively. These yields were significantly higher than those obtained with peptone-supplemented media ($1.5x10^9$ spores/ml). Evaluation of spore morphology indicated that media containing corn steep liquor produced predominately submerged conidia while Casamino acid supplemented media or peptone produced blastospores. Our desiccation tolerance and storage studies demonstrated that spore viability and stability are affected by the nitrogen source used. The impact of nitrogen source on actual spore yield after drying and storage will be discussed. **(STUDENT POSTER).**

MC 7 Biological control of the phytophagous mite *Tetranychus urticae* (Acari: Tetranychidae) using *Beauveria bassiana*

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Phytophagous mites are responsible for huge losses in agricultural production worldwide and are capable of rapidly developing resistance to conventional pesticides. Alternative approaches to control are therefore urgently required. Natural pathogens of certain phytophagous mite species have been investigated and at least one microbial acaricide (Mycar® based on *Hirsutella thompsonii*) has been produced for the control of the citrus rust mite. The possibility of using fungi which are not natural pathogens of phytophagous mites, has to date not been well

investigated. In this preliminary study we tested 3 isolates of *Beauveria bassiana* against an important mite pest of papaya, *Tetranychus urticae* (Acari: Tetranychidae). Conidial suspensions in water + Triton X (1×10^6 conidia ml^{-1}) were applied to papaya leaf discs previously infested with female mites using a Potter tower. Discs were maintained at 25°C and ~70% RH. Mites were transferred to fresh leaf discs every 24 hours and mortality was observed at this time. For all three isolates tested, mortality of *T. urticae* was approximately 70% within 5 days of applying the fungal inoculum. Control mortality was 6%. However, differences were seen between isolates when considering sporulation on the mite integument. Isolate UL 100 caused the highest rate of sporulation. The results show that isolates of *B. bassiana* should be considered as potential agents for the control of mites. We are currently investigating the resistance of isolates to the agricultural products used on a regular basis in Brazil. **(STUDENT POSTER)**

MC 8 A *Bacillus thuringiensis* autoagglutinating strain highly toxic to their source of isolation, *Simulium pertinax* larvae

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Application of formulations containing the entomopathogenic *Bacillus thuringiensis* serovar *israelensis* strain IPS-82 for mosquito control is widespread around the world. The strain IPS-82 used in vector control programmes is highly active against *Aedes aegypti* when compared with other well-known vectors such as Culex *quinquefasciatus* and *Simulium spp.* larvae. Among 18 strains of *B. thuringiensis* isolated from *Simulium pertinax* larvae naturally occurring in rivers of Southeast Brazil, one showed special toxic features. Simulated field tests against *Simulium pertinax* larvae showed that the native Brazilian *B. thuringiensis* (LFB-FIOCRUZ 1035) has a LC_{50} at least 20 times lower than the standard strain IPS-82. The same bacteria preparation was also tested against *Ae. aegypti* larvae in laboratory trials and the LC_{50} values obtained with LFB-FIOCRUZ 1035 were at least six times lower than for the strain IPS 82. The results indicate that the strain is more toxic than the standard one, *B. thuringiensis* serovar *israelensis* (H14), in the two species tested. It is noteworthy that differences between LC_{50} values were more evident in *Simulium pertinax* larvae, the source of isolation.

MC 9 Evaluation of three differentes formulation based on *Bacillus thuringiensis* sorovar. *Israelensis* to the control of *Culex Quinquefasciatus* and *Aedes egypti*

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Culex quinquefasciatus and *Aedes aegypti*, are among the main species of domiciled Culicidae responsible for transmission of pathogen agents which affect the quality of life in Brazil, such as the great dengue epidemic registered in the country in current year. This work evaluates the action of Vectobac – AS 1,200 ITU/mg - lot 69-149N9, Teknar® 3,000 AAU/mg - lot NF099065 and Aquabac® XT 1,200 ITU/mg, lot F295, liquid formulated products from the *Bacillus thuringiensis* sorovar. *israelensis*, on these two species of Culicidae. Bioassays were carried out under room temperature, with at least 5 repetitions, in different days, using 25 from 4th instars, with 5 days of life, kept in pots with 150 mL of distilled water. Data collected after 24 hours of product inoculation were analyzed by Probit. The following results in ppm were obtained for *C. quinquefasciatus* under an average room temperature of 27±3.62°C: Vectobac (LC_{50} = 0.22±0.66; LC_{95} = 0.44±0.30); Aquabac (LC_{50} =0.31±0.12; LC_{95} = 0.56±0.26) and Teknar (LC_{50} = 0.27±0.06; LC_{95} = 0.47±0.09). As for the *Ae. aegypti*, under an average temperature of 26.4±3.34°C, the following results were obtained: Vectobac (LC_{50} = 0.29±0.03; LC_{95} = 0.52±0.11); Aquabac (LC_{50} = 0.55±0.11; LC_{95} = 1.04±0.28) and Teknar (LC_{50} = 0.28±0.05; LC_{95} = 0.52±0.15 ppm). These results show that the three products are efficient in controlling the two species of Culicidae under the tested conditions.

MC 10 Different substrates in local *Bacillus thuringiensis* var. *israelensis* production

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Bacillus thuringiensis var. *israelensis* (Bti) has been used as a alternative to chemical insecticides. The feasibility of *B. thuringiensis* production depends necessarily (i) on the efficiency of toxin production by the medium used in fermentation; (ii) on the cost of the production process. In order to test local Bti productions using cheaper media than those already reported for *Culex* biological control, a pilot-project was implemented in Londrina/PR-Brazil. The microorganism used was Bti, serotype H14, characterized at Unité des Bactéries et Champignons Entomopathogènes/Pasteur Institute and supplied by EMCAPA/ES-Brazil. Three liquid media, corn meal, boiled potato water and soybean meal, were tested for Bti production. All the media were supplied with 0.3% glucose after autoclaving. The media were placed in fermentation flasks and the system was supplied with filtered air using membrane GV, 0.22µm, Millipore. The fermentation media were inoculated with 5% by volume of the seed culture and incubated at 30°C. The products were examined by microscopic phase-contrast, spore counting was performed by colony forming units on agar plates and samples were used for bioassays against third-instar larvae of *Culex quinquefaciatus*. Microscopic monitoring showed that complete sporulation is more rapid in soybean medium than in the other fermentation media. Complete sporulation occurred with 3 days. All the three media resulted in the same spores yields, about $10^9 - 10^{10}$ spores ml^{-1}. The bioassays showed that bioinsecticide produced in soybean medium is more efficient for *Culex* larvae control. The LC_{50} was 4.04±0.76 ppm, 4.01 fold more efficient than potato or corn meal media. This Bti toxin production controlled *Culex* on sewage effluents using 100mL per 25 m^2.

MC 11 Binding sites of *Bacillus thuringiensis* Insecticidal Crystal Proteins on the midgut of *Anticarsia gemmatalis* (Lepidoptera, Noctuidae) larvae

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Velvetbean caterpillar is a very important insect pest of soybean culture in Brazil, this larvae cause a lot of damage, determining great reductions of leaf areas and, consequently, lowering photosynthesis and productivity. In this work, the presence of specific receptors for *Bacillus thuringiensis* (*Bt*) delta-endotoxins on the brush-border membrane, obtained from *A. gemmatalis* larvae midgut cells, was demonstrated by an *in vitro* binding site assay. The insects were obtained from soybean fields in the "Rio Grande do Sul", Brazil. The larvae were reared with Greene diet and maintained at 25°C, 70% relative humidity and 12 h of photoperiod. The delta-endotoxins Cry1Aa, Cry1Ac and Cry1Ba were obtained from *B. thuringiensis* subsp. *dendrolimus* HD 37, *B. thuringiensis* subsp. *kurstaki* HD 73 and *B. thuringiensis* subsp. *thuringiensis* 4412, respectively. These strains contain only a single ICP and were supplied from Pasteur Institut (IEBC-Paris, France) and Pant Genetics Systems (PGS-Ghent, Belgium). Binding of these ICPs to midgut sections of the *A. gemmatalis* larvae was studied using streptavidin-mediated detection. Negative controls were performed by omission of biotinylated delta-endotoxins or enzyme-conjugated streptavidin. Staining was not observed when individual steps were omitted. The observed staining patterns showed that Cry1Aa, Cry1Ac and Cry1Ba bound to the brush border throughout the whole length of the midgut microvilli in contrast with the control. These binding data has been confirmed by *in vivo* bioassay analysis to show a correlation between binding and toxicity of the tested ICPs in this target pest.

MC 12 Pathogenic effect of *Bacillus thuringiensis* isolate from south of Brazil against *Oryzophagus oryzae* (Coleoptera, Curculionidae)

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The *Oryzophagus oryzae* is the most important pests of paddy fields in south Brazil. Until now, the control of this pest has been ensured by use of chemical pesticides. Regarding the effect of chemical products on the environment, alternative methods of pest control are being considered such as biological control and the development of resistant varieties of rice. The *Bacillus thuringiensis* (*Bt*) is an alternative to control this pest, due to its efficiency against coleopteran larvae and safety for the environmental. In this work, a new bioassay method were determinate and the pathogenic effect of *Bt* 2014-2 isolate were tested against 2nd and 3rd instar of *O. oryzae* larvae, obtained from corn and irrigated rice's fields in south of Brazil. Spore-crystal suspensions (8.10^{10} cells/mL) were applied on the flask containing 8 ml of water and rice plants. The control group was treated with sterile distilled water. Each test was repeated 3 times using 20 larvae by treatment. The bioassay was carried out in B.O.D chamber maintained at 25°C, with 70% relative humidity and a 12h photoperiod. The mortality of *O. oryzae* larvae was assessed 7 days after treatment applications and the mortality were corrected according to Abbott (1925). The new Bt isolate (*Bt* 2014-2) exhibited a moderate toxicity against the target pest, promoting more than 50% mortality. PCR was used to amplify DNA fragments related to known *cry*3 genes and the data reveled a expected PCR products of between 589 and 604 bp. This Bt isolates has been evaluated by SDS-PAGE according to protein profile and the toxicity of their ICPs.

MC 13 Histopathological study of the fall armyworm parasitated by *Campoletis flavicincta* and infected with *Bacillus thuringiensis aizawai*

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Spodoptera frugiperda (Lepidoptera, Noctuidae) is one of the most important pests that damage gramineous crops in Brazil, being feasible its control with *Bacillus thuringiensis* (*Bt*) products. In terms of parasitism, *Campoletis flavicincta* (Hymenoptera, Ichneumonidae) is one of the main *S. frugiperda* parasitoids. In this work, it was evaluated the parasitoid-bacteria interaction by histopathological analysis of *S. frugiperda* larvaes. Second instar larvaes were used to form four groups: the first was exposed to *C. flavicincta* parasitism; the second one was infected by *Bt aizawai* (Xen Tari/Abbott Laboratórios do Brasil Ltda) at 0,5 mg ml^{-1} (LC_{50} determinated in laboratory); the third group, parasitated and infected, and the fourth with no parasitism-infection (control). The four groups resultant larvaes were fixed in *Bouin Hollande Sublimé*, at intervals of 6, 12 and 24 hours, 7 and 10 days, after the infection. Paraffin blocks were cutted at 6 µm thickness. The insect tissues were stained with hematoxilin and eosin. In the following step, 162 individuals were observed in optical microscope. Regarding the treatments applied, the parasitoids' eggs and larvaes were found out in the parasitated and parasitated-infected *S. frugiperda*'s larvaes. The *C. flavicincta*'s eggs were found out in host larvaes fixed up to 24 hours after *Bt* infection, and the parasitoid's larvaes in host larvaes fixed after 7 and 11 days. This study brings strong evidences that the parasitoid's development, in larvaes submited to Xen Tari treatment, is not affected. Therefore, the two control methods must be compatibles to *S. frugiperda* control.

Cross Division

CDP 1 Culture of insect cells in airlift reactors: study of the influence of geometrical characteristics and gas flow rate on culture behaviour

G. A. Visnovsky[1], J. D. Claus[2] and J. C. Merchuk[3]

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Large-scale cultivation is an essential step towards the feasible production of Baculovirus in insect cell cultures. Airlift reactors (ALRs) appear to offer considerable advantages over other insect cell culture systems. In order to evaluate the impact of reactor design on the behaviour of insect cell cultures, the IPLB-Sf-21 cell line was cultivated in three different concentric tube airlift reactors (ALR 1, 2 and 3) that differ in their geometrical parameters. The ratio of downcomer to riser cross sectional areas, the shape of the bottom and the ratio of height to diameter of the reactor proved to be important since they produce significant differences on cell growth behaviour. The cellular growth rate could be improved from 0.016/h (ALR 1) to 0.031/h (ALR 3) by modifications of the reactor design, while the maximum viable cell density could be increased from $9x10^5$ to $2.4x10^6$ cells/ml. Glucose was the main carbon source for IPLB-Sf-21 cells. The reactor design also influences the efficiency of carbon utilization for cell synthesis, probably due to better oxygenation transfer to the culture, improving yield coefficient values from 1.72×10^8 (ALR 1) to 2.81×10^8 (ALR 3) cells/mmol glucose. Once selected a reactor configuration, the influence of gas flow rate was determined, finding an optimal value of superficial gas velocity that renders sufficient oxygenation without affecting significantly the cellular viability. In addition, the influence of the reactor design on fluid circulation in the reactor was tested.

CDP 2 Elimination of *Wolbachia* from *Urolepis rufipes* (Ashmead) (Hymenoptera: Pteromalidae) with heat and antibiotic treatments: implications for host reproduction

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Wolbachia are a group of cytoplasmically inherited bacteria that induce a variety of reproductive changes in their arthropod hosts. Such changes may include cytoplasmic incompatibility, parthenogenesis, male-killing and feminization. A PCR-based method was used to detect *Wolbachia* in laboratory colonies and field populations of *Urolepis rufipes* (Hymenoptera: Pteromalidae) from southern Alberta. This wasp harbours *Wolbachia* type-A with respect to *Wolbachia ftsZ* A and B genes. Cytoplasmic incompatibility is expressed through embryonic abortion, a male-biased sex ratio and a low hatchability. We eliminated infections *Wolbachia* from *U. rufipes* using both antibiotic and heat treatments. We then compared the effect of *Wolbachia* on the fitness of *U. rufipes* using experimental crosses for all possible combinations of infected and cured wasps. We discuss the effect of infections on sex ratios, number of progeny and embryonic abortion. We also discussed the prevalence and distribution of *Wolbachia* infection in field populations of *U. rufipes* and other filth fly parasitoids in southern Alberta, Canada.

CDP 3 Insect digestive biochemistry: A genomics and proteomics perspective

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The first complex eukaryotic organism to have its entire genome sequenced was the model Dipteran system *Drosophila melanogaster*. We have selected *Mamestra configurata* (Bertha armyworm) as a model system since it is an economically significant pest, it is large and easily reared and a midgut epithelial cell culture system is available. We aim to use Genomics and Proteomics tools to comprehensively survey the proteins expressed by the midgut epithelium as well as those associated with the peritrophic matrix (PM). The PM is involved in a variety of digestive functions including the regulation and compartmentalization of digestive enzymes, ion and water exchange and resistance to pathogens. Unfortunately, limited information is available regarding the composition or function of the numerous and diverse array of proteins resident within this vital structure. Our approach has been to couple one and two-dimensional gel resolution of PM proteins with MALDI-mass spectrometry to generate peptide mass fingerprinting data as well as sequence information for specific peptides. In parallel, ca. 1000 ESTs have now been generated from a midgut-specific cDNA library yielding a database of genes expressed in the midgut. Bioinformatics tools are now being used to link the data sets (peptide and cDNA sequencing) leading to the identification of genes for corresponding PM proteins, digestive enzymes, nutrient transporters, toxin receptors, and others. This information will ultimately lead to the identification of proteins that can serve as targets for "designer" insect control molecules, as well as to further our understanding of insect midgut physiology and digestive biochemistry.

CDP 4 Formulation of biopesticides for application to soil

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While many biocontrol agents can be shown to work in the laboratory, achieving success in the field is often more difficult. Formulation technology can be used to improve microbial stability, maintain product quality through distribution and storage and allow application methods consistent with agricultural practices. During the development of microbial biopesticides for control of soil dwelling pests, we have developed several thermostable biopolymer formulations that allow microbial storage and distribution without significant loss of viability. The stabilised microbes can be incorporated into suspensions, powders or granules, which provide different options for delivery. Microbes in granules can be applied directly to the soil using conventional seed drills. Incorporation of microbes into granules also allows the release rate to be manipulated to meet the needs of different plant production systems. The bacterium *Serratia entomophila* has been incorporated into granule formulations to improve distribution and application to pasture soils for biological control for the New Zealand grass grub, *Costelytra zealandica*. Laboratory experiments comparing the survival of formulated and unformulated *Serratia* in soil have shown significant improvements in viability of *Serratia* in both dry and moist soils. Bacteria were released at different rates in the various soil moistures. Release of *Serratia* from granules in soil cores subjected to various levels of irrigation showed the importance of soil water in distribution of bacterial inoculum in the soil profile.

Following field application of the granules, the population of *Serratia* was maintained at approximately 10^4 colony forming units per g soil, the level required to allow establishment of disease in the grass grub population. The techniques developed for formulation of *Serratia* which is a non-sporeforming bacterium, sensitive to uv light and desiccation, can be readily applied to other insect pathogens. Fungal insect pathogens, (*Beauveria* spp. and *Metarhizium anisopliae*), have also been incorporated into biopolymer based formulation systems. Formulation of microbes in biopesticides plays a major role in product adoption by providing a consistent product for use in the field.

CDP 5 Origins of *Yersinia pestis* pathogenesis and investigation of the potential of *Yersinia pseudotuberculosis* as an insect pathogen

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Phylogenetic analysis shows that *Yersinia pestis* is a typical strain of *Y. pseudotuberculosis* that diverged 2000-20000 years ago. Although there are now obvious phenotypic differences in pathogenicity between the enteric pathogen *Y. pseudotuberculosis* and the vector-borne systemic pathogen *Y. pestis*, the key steps in this differentiation are not elucidated. An increased ability of *Y. pestis* to live as a commensal in the insect vector and its reported resistance to insect antimicrobial peptides might both have contributed to its evolution. Nevertheless various genes are detected in *Y. pseudotuberculosis* which appear to relate to an insect-associated life-style. The differences between the two organisms cannot be explained solely by the acquisition of the pestis-specific plasmids (pFra and pPst). The chromosomal *hms* gene required for blockage of the flea midgut by *Y. pestis* to maximise its onward transmission is also present in *Y. pseudotuberculosis*. Homologues of the *tca* insecticidal toxin operon from *Photorhabdus luminescens* which are found in the *Y. pestis* genome are also present in certain *Y. pseudotuberculosis* strains. We are investigating the basis for these phenotypic differences by studying the interactions of *Y. pseudotuberculosis* with insect cells *in vitro*. Adhesion, invasion and cytotoxicity assays suggest that *Y. pseudotuberculosis* can adhere, invade and kill insect cells. The causes of this cytotoxicity are being studied using *Y. pseudotuberculosis* mutants defective in the main virulence-related genes. This approach includes an investigation of the bacterial changes in transcription and expression upon infection as well as an investigation of the host response by RAP-PCR, microarray and 2-D gel electrophoresis.

CDP 6 Mucoid secretion is a virulence factor for QPX, a protist pathogenic for the clam *Mercenaria mercenaria*

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Quahog Parasite Unknown (QPX) is a thraustochytrid responsible for mass mortalities of the hardshell clam from Atlantic Canada southward along the US east coast to New Jersey. In vivo and in laboratory cultures of QPX are characterized by the presence of mucoid secretion that surround the cells. Clam plasma contains naturally-occurring factors that modulate the growth of mucus-free, or denuded QPX (dQPX). Dilute plasma concentrations (<10 ug protein/ml) promote dQPX growth, while higher contentrations show dose-dependent inhibition of growth. The inhibitory factor(s) show thermal stability after 10 min at 65 degrees C. and act by inhibiting growth, not by killing the QPX. The factor(s) is apparently quite specific, with high activity vs QPX but with low activity against C9G, a thrautochytrid very closely related to QPX. If dQPX is preincubated for various time periods prior to exposure to plasma, a time-dependent protection from the inhibitory activity is seen. This protective effect was correlated with the development of the mucioid coat. This is in line with the observation that clams injected with dQPX fail to develop infections or disease.

Tuesday, 8:00 - 10:00
Iguaçu I
CONTRIBUTED PAPERS - Microbial Control 1
Chair: C. Nielsen

The main features of microbial plant protection in Siberia

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Siberia as a great part of Russia is characterized by continental climate and very short vegetation period. These circumstances demand strong necessity to provide the optimal plant protection based on ecological approaches. The most important problems are connected with the protection of vegetable crops and first of all the cabbage as principal crop for different regions of Siberia. The vegetable crops are main objects of the microbial insect control. The cabbage and others cruciferous vegetables are infested by cabbage moth - *Mamestra brassicae* L., diamondback moth - *Plutella xylostella* L. and white butterfly - *Pieris brassicae* L. Commercial formulations based on *Bacillus thuringiensis (Bt)* are rather effective against two latter larvae and the most harmful *M. brassicae* is more resistant to it. Therefore it is necessary to use the mixture of *Bt* formulations with *M. brassicae* nucleopolyhedrosis virus in suitable proportion. On the one hand short seasonal period allows applying microbial insecticides not more than two times. On the other hand sharp variation of temperature and rather strong UV-radiation during season requires using some ecologically safe additives in order to enhance microbial control efficacy. Our experiments show what the cabbage is grown without artificial irrigation one microbial treatment provides the crop protection. In greenhouses some species of aphids, whitefly *Trialeurodes vaporariorum* Westw., and onion thrips *Thrips tabaci* Lind. are common pests. In Siberia climate the microbial formulations play greater role than entomophagous invertebrates. Fungal formulations based on *Verticillium lecanii*, *Beauveria bassiana* and entomophthoralean fungus *Conidiobolus tromboides* are used for microbial control of mentioned pests and of spider mite partly. It has been shown that microbial control is suitable for very harmful insect such as beet webworm *Pyrausta sticticalis* L., damaging many agricultural crops in Siberia. For prompt treatment of huge agricultural areas is used special aerosol equipment.

Field-scale studies on spatio-temporal relationships between aphids and natural enemies

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Habitat diversification schemes, such as the planting of field margins to increase farmland biodiversity, are currently being encouraged in the United Kingdom. Since 2000, studies have been carried out at Rothamsted on the potential use of field margins as refugia for aphid-pathogenic fungi, especially *Erynia neoaphidis*, for conservation biocontrol of pest aphid species. The spatial and temporal occurrences of aphids, parasitoids, predators and entomopathogenic fungi are being monitored in an experimental grass/wildflower margin in a field that is continuously cropped with wheat. In addition, the population dynamics of the large nettle aphid, *Microlophium carnosum*, are being studied in the entire perimeter of the field. The blackberry aphid, *Sitobion fragariae*, was the dominant aphid species in both the field margin and wheat crop during sampling carried out between June to September, 2001. The dispersion of counts was analysed using the Spatial Analysis by Distance IndicEs (SADIE) software. Counts for living aphids were significantly aggregated on three sample dates in the margin and crop. Spatial arrangements of parasitised mummies formed statistically significant clusters on two sample dates and the dispersion of infected cadavers was significantly aggregated on one date. The large nettle aphid is an early-season host of *E. neoaphidis*. Repeated sampling of nettle patches revealed that infection by *E. neoaphidis*, and other entomophthoralean fungi, started in the western perimeter of the field and progressed towards the southeastern boundary. The use of molecular techniques to track *E. neoaphidis* isolates between nettles, field margins and cereal crops will be discussed.

Lab-scale mass production and field trials with mycoinsecticides for the biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in pulses

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Pulses (lentils, chickpea/gram and others) are the major protein source for the nutrition of the population in India. In recent years the agricultural production of this staple food repeatedly suffered from average yield losses of 67%, half of which were caused by the caterpillar *Helicoverpa armigera* due to high levels of insecticide resistance. Within the Indo-Swiss Collaboration in Biotechnology (ISCB), a research collaboration is aimed at the development of a biocontrol agent against this pest insect based on mitosporic fungi. More than 50 entomopathogenic fungi, belonging to the genera *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomurea rileyi* and *Paecilomyces sp.*, have been isolated from soil samples or infected *Spodoptera littoralis* larve in Pune. The entomopathogenic hyphomycete *M. anisopliae* (B6M), which was isolated from soil in Pune (India) showed the highest efficacy against *Helicoverpa armigera* larvae compared to other isolates from India. Improvements of mass production techniques of *M. anisopliae* aerial conidia are presented. Improvements for the mass production of conidia were achieved through substrate optimization. Furthermore, novel plastic bags for biomass production and usage of the Mycoharvester (CABI Bioscience) facilitated handling and harvesting. Mass-production experiments indicated that peeled hard wheat (*Triticum durum*) is most suitable for growth and conidiation of *Metarhizium anisopliae* in comparison with other cereal substrates. The spore yield reached more than $1x10^9$ spores/g solid substrate after 8 d incubation at 25°C. The viability of the aerial conidia in different carriers was investigated, and the highest germination rate was observed in an emulsion containing Diesel/Sunflower oil, Emeleo R® and Tween 80 (0.1%). Additionally to the laboratory experiment, two field trials were done each in pigeon pea and check pea in Pune (India). 3 isolates of *M. anisopliae*, *B. bassiana* and *N. rileyi* were applied twice at a dose of $5x10^{12}$ conidia/ha (sprayed in 3 l/ha). Endosulfan and HaNVP were applied as positive control treatments. The *M. anisopliae* isolate caused significant better control ($P<0.05$) of the larval population of *H. armigera* in pigeon pea and chick pea as well as a reduction in pod damage compared to the Endosulfan.

Efficacy of blastospores of *Beauveria bassiana* compared to Mycotrol® and Meta-sin® spores efficacy against colding moth *Cydia pomonella* L

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Colding moth (CM) *Cydia pomonella* L. is an important plague of apple orchard in Durango, Mexico. At the present time the CM control is carry out using chemical insecticides, however the biological control using *Beauveria bassiana* in a Management Integrate Program is now investigated. Strain code BbP1 was produced in liquid medium contained molasses as carbon source to produce blastospores, and their toxicity was compared to Mycotrol® (*B. bassiana* GHA) and Meta-Sin® (*Metarhizium anisopliae*) spore toxicity. A topic bioassay was applied using larvae of 1-5 days old reared in laboratory (25°C and 75%HR), using artificial diet; a serial dilution was prepared to rate $1.92x10^6$-$1.2x10^9$ blastospores/ml from 1% fungus solution, each larvae was infected with 100µl per dilution. LC_{50} was calculated using a POLO PC Program. To field level the effectiveness of BbP1 strain to concentration of $1x10^9$ blastospores/ml was compared to Mycotrol®($2.1x10^{13}$ spores/l) and Meta-Sin®($1.2x10^{12}$ spores/l), to dose of 1l/ha and azinphos-methyl 1kg/ha against a natural colding moth infestation. The damages caused by *C. pomonella* larvae on one hundred apples (three replicates/treatment) were evaluated in an orchard apple in Canatlán, Durango during summer 2001. The *C. pomonella* larvae were susceptible to BbP1 strain with

86% of mortality while Mycotrol® and Meta-Sin® showed mortality of 85.3-84%, respectively. The mortality values showed more insect susceptibility to BbP1 blastospores than Mycotrol® and Meta-Sin® spores, while LC_{50} value to BbP1 strain was $2.4x10^7$, and to Mycotrol® and Meta-Sin® were $3.2x10^7$ and $1.2x10^8$ blastospores/ml, respectively, so BbP1 was 1.3 and 5 times more toxic than Mycotrol® and Meta-Sin®. The insect infection using BbP1 strain and Mycotrol® began to 24h, and the total sporulation time was 72h after infection, while *M. anisopliae* had a sporulation time of 5 days. To field level there was statistic differences between treatments ($F=3.31$, $p<0.032$). The azinphos-methyl was the best insecticide to CM control (0.95%) in relation to Mycotrol® (2.05%), BbP1 (2.38%), and *M. anisopliae* (4%) of fruits damages, so it was found that the BbP1 strain and Mycotrol®, as well as Meta-sin® were also effective to natural infestation of pest control.

Biocompatibility of *Beauveria bassiana* (BotaniGard® 22WP and ES) and the parasitoid *Eretmocerus eremicus* in a silverleaf whitefly control strategy on poinsettia

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Several biological control agents may be required to regulate silverleaf whitefly (SLWF), *Bemisia argentifolii*, in commercial poinsettia production. Information on interactions between biocontrol agents, though, is limited. The goal of the current study was to assess interactions between commercial formulations of the fungal entomopathogen *Beauveria bassiana* and the parasitoid *Eretmocerus eremicus* (Hymenoptera: Aphelinidae). In greenhouse experiments, caged patches of immature whiteflies on poinsettia were exposed to *E. eremicus* alone, *B. bassiana* (BotaniGard 22 WP and BotaniGard ES) alone, and three parasitoid-fungus combination treatments using different spray and release strategies. Formulation blanks were also included to assess effects of formulation ingredients on parasitoid activity. In the first and second spray and release experiments, SLWF were treated with fungi followed by an introduction of *E. eremicus* i. on the same day; and ii. seven days later. Parasitism rates were low when either the ES blank or BotaniGard ES spray preceded *E. eremicus* release on the same day (1-6%). Parasitism rates were higher following an application of BotaniGard 22 WP and a combined treatment reduced whitefly survival to 3 and 10% in two replicate trials. When SLWF were sprayed with BotaniGard ES and *E. eremicus* released 7 d later, high rates of infection were observed (87-95%) but parasitism rates were low (<1%). When sprayed with BotaniGard 22WP, 74-85% of the whiteflies were infected and successful parasitism occurred in the remaining, uninfected population. In the third spray and release experiment, whiteflies were sprayed with BotaniGard three days after parasitoid release. While the data are statistically inconclusive, in two of the three replicate experiments, parasitoid emergence was low (2%) when whiteflies were sprayed with BotaniGard ES.
Overall, the WP formulation and *E. eremicus* appear to be compatible when used concurrently, but the paraffinic oil used in the ES appears to have a negative effect on *E. eremicus* parasitism (repellent effect) and survival. Additional trials are now needed to optimize spray/release rates and strategies to obtain maximum efficacy in the most cost-effective manner.

Field efficacy of emulsifiable suspensions of *Beauveria bassiana* conidia for control of *Myzus persicae* population on Chinese cabbage

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The powder of *Beauveria bassiana* SG8702 conidia produced using a diphasic fermentation technology was formulated into emulsifiable suspentions (ES) I containing $>10^{10}$ conidia/ml and II, which was a mixture of ES-I with 1% (w/v) of 10% imidacloprid WP (a chemical insecticide biologically compatible to *B. bassiana* conidia). Three dilutions (10^7, 10^6, and 10^5 conidia/ml) of both ES-I and ES-II were sprayed onto cabbage plants for control of *Myzus persicae* population in field plots in Kunming, Yunnan during July 2001. Each dilution and a water spray as control were replicated three times. During a 28-day period of sampling at 3- or 4-day intervals, the spray of ES-II at the concentration of 10^7 conidia/ml (1000-fold dilution) effectively controlled increase of the aphid population by >90% consistently from day 7 after spray. The efficacy for spraying of ES-I at the same concentration reached 85% on day 7 after spray, exceeded 70% in the following two weeks, then declined to 64.4% on day 24 and 52.6% on day 28. At the lower concentrations sprayed, both suspensions resulted in significant control of the aphid pest but ES-II containing 1% of 10% imidacloprid WP consistently had a better control than ES-I. The summer weather of Kunming with mild temperature and frequent rainy days was favorable to the use of the emulsifiable suspensions for aphid control.

Biological control of weevils in Danish greenery production

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The insect family Curculionidae contains some of the economically most important pest insect species in greenery production for Christmas decorations in Denmark. In particular, the two species *Strophosoma melanogrammum* and *S. capitatum* are important pest species. At present no chemical treatment of these weevils is allowed in Denmark. Biological control including the use of insect pathogenic fungi from the class Hyphomycetes may thus provide a potential to minimise the damage caused by these weevils. We studied the laboratory mortality and field effects of selected isolates of the insect pathogenic fungi *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces* spp. In the laboratory, all tested isolates were able to infect and cause mycosis in both adults and larvae of *Strophosoma melanogrammum* and *S. capitatum*. Based on the results from the laboratory bioassays, isolates were chosen for test under field conditions in an *Abies procera* plantation. Four application strategies were used: 1) Soil application against ovipositing females during spring; 2) Soil

application against larvae dropping to the soil during summer; 3) Soil application in autumn against the new generations of adults; 4) Application of tree trunks with fungal inoculated lanes in spring and autumn against weevils seeking to the canopy to feed. The population development of adult weevils was monitored by weekly sampling from emergence traps. Adults were collected, counted and determined to species level and incubated individually in plastic cups to estimate the prevalence of the applied fungus as well as the prevalence of natural infections. Preliminary results proved that we were able to initiate infections in the treated plots and that populations of weevils were reduced significantly.

Development of the Entomopathogenic Fungus *Beauveria bassiana* (Balsamo) for the Control of *Lygus lineolaris* (Hemiptera : Miridae) on Wild Host Plants in the Mississippi Delta of the Unites States

J. E. Leland,

USDA-ARS, SINRU, USA

New approaches for managing tarnished plant bugs, *Lygus lineolaris* (Hemiptera: Miridae) in the cotton growing regions of the Midsouthern United States, have focused on reducing populations in wild host plants before they move into cotton. Microbial biopesticides may be particularly well suited to this management approach for the following reasons: 1) chemical insecticides are not labeled for *L. lineolaris* control on wild host plants 2) early season chemical control could increase development of chemical resistance; 3) a narrow-spectrum-microbial biopesticides may allow for the preservation of beneficial insects; and 4) the slower mode of action of microbial insecticides relative to chemical pesticides would be acceptable in non-crop areas, since crops would not be damaged prior to insect mortality. Of the potential microbial biopesticides, fungi are the most promising for controlling *L. lineolaris* because their piercing sucking mouthparts limit the use pathogens with peroral modes of action. Commercially available strains of *Beauveria bassiana*, non-commercial strains with demonstrated high virulence to *L. lineolaris*, and new isolates obtained from *L. lineolaris* and *L. hesperus* are being evaluated for virulence to *L. lineolaris* over a range of temperatures and relative humidities. Strategies for protecting spores from environmental stress (solar radiation, heat, and water balance) are being evaluated. Initial experiments are investigating formulations of spores coated with water-soluble and water-insoluble lignins for protection from solar radiation. Survival of spores following exposure to simulated sunlight and virulence to *L. lineolaris* over a range of relative humidities and temperatures are being tested for coated spores that are either suspended in water, water emulsions, or oil carriers.

Efficiency of *Bt* formulation for genetically distinct lines of potato tubeworm

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Efficiency of the bacterial formulation "Lepidocid" based on *Bt subsp. kurstaki* was studied for potato tubeworm with different genetic structure. Superoxide dismutase allozymes were used as genetic markers, and each of the variants contained four insect lines distinct in *Sod* genotype composition. Three lines in each variant were single-genotype (FF, FS and SS), and one mixed line contained all the three genotypes in the ratio 1:1: 1. Each of the homozygous line proceeded from progeny obtained from not less than 50 individual crosses. Heterozygous lines eggs were obtained from 50 crosses of males and females proceeding from different homozygous lines. Each of the line had fifteen replications. Two-way analysis of variance for viability in control and experimental potato tube worm lines distinct in genetic composition at *Sod* locus showed significance of all the three values of *F* criterion (*Bt* formulation: F=99; Genetics: F=29.0; Interaction: F=17,8; all $P<0.001$). These data testify that factors, formulation and genotype compositions significantly influenced egg-to-adult viability of potato tubeworm. Besides that interaction was also significant. Within the control variant the lines distinct in genetic composition did not significantly distinguish from each other in egg-to-adult viability. Within the experimental variant the *FF*-line showed lower viability and it was also lower that in the control FF-line (t=9.6; $P<0.001$). Other experimental lines also differ significantly from corresponding control strains in viability excluding only mixed lines, containing all the three genotypes (for *FS*-strains t=5.2; $P<0.001$; for *SS*-lines t=6.9; $P<0.001$). The data obtaining show that genetically distinct populations are distinguished in their replay to application of bacterial formulation. At the same time, the formulation had lowest efficiency in mixed lines. Thus, genetic heterogeneity, which has been highest in mixed lines, marks resistance of the potato tubeworm population to the pathogen. This means that efficiency of the pathogen may depend on the degree of intro-population competition for a food, which seems to be lowest in genetically mixed strains of the insect.

Tuesday, 8:00 - 10:00
Cataratas I
CONTRIBUTED PAPERS - Bacteria 2
Chair: P. Butko

Plasma membrane microdomains integrity is essential for pore formation activity of *Bacillus thuringiensis* Cry1Ab toxin

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Lipid rafts are conceived as spatially differentiated microdomains in cell Membranes that are enriched in glicosphingolipids and cholesterol. Several pore forming bacterial toxins interact with glycosylphosphatidylinositol (GPI)-anchored proteins and it has been proposed that association of their receptors with lipid rafts is a crucial step in their oligomerization and insertion into membrane. Previously, it was demonstrated that the integrity of lipid raft is important on the mode of action of Cry1A toxins (Zhuang et al., J.Biol.Chem, inp ress). In this work lipid rafts were isolated from brush border membranes vesicles isolated from purified microvilli structures rather than from whole midgut homogenate (Bravo et al. Biochem.Biophys.Acta, inpress). The specific enrichment of apical membrane enzyme markers aminopeptidase and alkaline phosphatase were 35 and 22

fold, respectively to the whole midgut cell homogenate. Ligand-blot and Western-blot experiments showed that Cry1A specific receptors were enriched. GPI-anchored Aminopeptidase-N Cry1A receptor was located in lipid rafts that were isolated by their insolubility in Triton-X100 at low temperature and their flotation on sucrose density gradients. Finally, the disruption of lipid rafts integrity by incubation with the cholesterol binding agent methyl cyclodextrin (-MC) greatly inhibited Cry1Ab induced K^+ permeability. The effect with -MC was not due to disruption of the vesicles since the K^+ permeability induced by valinomycin was not affected by this treatment. Our results confirm that lipid rafts are important elements in the mode of action of Cry1Ab toxin.

Cyt toxins - pore formers or detergents?

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The prevalent hypothesis on the mechanism of action of the Cyt toxins is that of colloid-osmotic lysis. The hypothesis is based on the notion of channels or pores formed by the Cyt toxins in the lipid membrane. In this contribution we examine the evidence for and against the Cyt pores and formulate an alternative hypothesis which is based on the results of in vitro experiments using the toxin Cyt1Aa1 and lipid vesicles. Fluorescence spectroscopy, Fourier-transform infrared spectroscopy and differential scanning calorimetry were used as tools. First, we found that hundreds of toxin molecules need to bind to a lipid vesicle before the latter starts to leak its contents. This is in contrast with only a few protein molecules required to form an oligomeric pore. The leakage is an all-or-none process, i.e., there are no vesicles that would only partially release their contents. Second, the membrane-bound toxin seems to be in the molten-globule state, i.e., it does not have any appreciable tertiary structure, unlike other membrane-penetrating proteins. The toxin appears to be splayed on the membrane surface, with all the peptide-bond amides exposed to the solvent. Third and most significant, the cytolytic action of the Cyt toxin in osmotically equilibrated conditions is accompanied by the appearance of smaller lipid/ protein aggregates, which is not expected in the pore-forming model. We interpret our data as evidence for a detergent-like action of the Cyt toxins.

Distribution of Cry1A toxins and receptors in membrane fractions Isolated from lepidoptera midgut

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Heliothis virescens, an important agricultural pest in the United States, is highly susceptible to Cry1A toxins. These toxins bind epithelial cell membrane receptors in the midgut of susceptible insects. To further understand the mechanism of this association we have determined the distribution of toxins membrane fragments isolated from the lepidopteran midgut. One membrane component, are lipid rafts, which are characterized by their insolubility in non-ionic detergents such as Triton X-100 at 4°C. In order to understand the possible role of lipid rafts in the lepidopteran insect midgut and in Cry toxin action, we isolated and analyzed the protein and lipid components of lipid raft microdomains from *Manduca sexta* and *Heliothis virescens* midgut epithelial membrane. We showed several putative *Bacillus thuringiensis* Cry1A receptors from *H. virescens* and *M. sexta* preferentially partitioned into lipid rafts, and that lipid raft integrity was essential for *in vitro* Cry1Ab pore-forming activity. We also demonstrated that Cry1A toxins are associated with lipid rafts. The significance of the distribution of the Cry1A toxins and their receptors and toxin will be discussed.

Molecular Basis of Membrane Pore-Formation by the *Bacillus huringiensis* Cry4B Mosquito-Larvicidal Protein

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Upon tryptic activation, the *Bacillus thuringiensis* Cry4B mosquito-larvicidal protein was processed to be protease-resistant products of ca. 47 kDa and ca. 21 kDa. The cloned 21-kDa fragment (α1-α5) was capable of inducing liposome permeability and cation-selective channel activity, constituting the region responsible for ionic pore formation within the toxin molecule. Single proline substitutions revealed that α4 and α5 within the Cry4B pore-forming domain are important for the toxin activity against *Aedes aegypti* larvae. Further analysis by charged-to alanine mutagenesis in helix 4 revealed a crucial role in both larvicidal and ion-channel activities for the positively charged side chain of arginine-158, conceivably in the passage of ions through the pore. In addition, directed mutations within the loop linking α4 and α5 revealed that Asn-166, Tyr-170 and Glu-171 play a crucial role in larvicidal activity of the Cry4B toxin, especially polarity at position 166 and aromaticity at position 170. The relevance of a hexameric pore model comprising the Cry4B α4-α5 hairpin in solvated lipid membrane bilayers (POPC/water) will be discussed.

The high dose - Refugia management resistance strategy for Bt transgenic crops: the influence of refugia

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Through the use of a spatially and temporally explicit simulation model, the present paper explores the use of refugia in the high dose refugia resistance management strategy for *Bacillus thuringiensis* (*Bt*) transgenic crops. The model represents a patchy population of an insect

pest in which recessive resistance is conferred by a single locus with two alleles. A function for tracking the frequency of the resistance allele between generations in the insect population was based on the selection intensity, the fitness cost, the percentage of refugia, the percentage mortality in refugia due to insecticides, the initial frequency of the resistance allele and the rate of migration. The simulation was run using three different spatial patterns of refugia inside the fields crop, border, central and equidistant, four crop rotation patterns, five different sizes of refugia (4% - 25%) and three different levels of non-*Bt* insecticide control in the refugia. It was found that: a) the greater the size of refugia the lower the rate of spread of resistance, b) positioning refugia in a border resulted in a lower rate of spread of resistance compared with the other patterns tested, c) the frequency of the resistance allele increased markedly when the refugia was less than 10%, d) use of temporal refugia (crop rotation) led to a decline in the frequency of the resistance allele, e) higher rates of migration increased the rate at which resistance spread and f) the frequency of the resistance allele increased when the refugia was sprayed with insecticide. Possible mechanisms to optimise the use of refugia on resistance management are discussed. **(STUDENT PAPER).**

Bacillus thuringiensis resistance and associated fitness costs in *Trichoplusia ni* populations

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Continued use of the microbial insecticide, *Bacillus thuringiensis (Bt)*, ultimately depends on our ability to prevent resistance from developing in pest populations. Currently, only one species, *Plutella xylostella*, has been reported to have developed significant resistance to *Bt* outside of the laboratory. Surveys of *Trichoplusia ni* populations present in commercial vegetable greenhouses in British Columbia, Canada, demonstrate that *T. ni* populations are developing resistance to *Bacillus thuringiensis kurstaki* (Dipel, Abbott). Resistance levels assayed in the laboratory correlate directly to the amount of *Bt* applied by growers. Furthermore, pupal weights of resistant *T. ni* populations are significantly smaller than susceptible populations. Within one greenhouse population, *Bt* resistance levels were observed to increase to levels 40-fold higher than a susceptible laboratory colony and associated pupal weights decreased by 19%. The observed decrease in pupal weights is presumably due to negative fitness effects associated with resistance to *Bt* and may explain the rapid decline of resistance in collected populations maintained in the laboratory. These results indicate that it may be possible to reverse the buildup of resistance in greenhouse *T. ni* populations through the withdrawal of *Bt* use. **(STUDENT PAPER).**

Genetic variability for resistance to *Bacillus thuringiensis* toxins: a case study with the diamondback moth

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The potential of insects to evolve resistance to insecticides is well known. Many insect pest have developed, under laboratory conditions, resistance to *B. thuringiensis* products, and the diamondback moth has even done it in the field several times independently. Many cases of resistance to *B. thuringiensis* commercial products imply that the insects have become resistant to more than one Cry protein, since the active component of the formulate consists of crystals with more than one Cry protein. In these cases, the question is if the insects have been selected for a single gene conferring resistance to multiple toxins or if they have been selected for various genes. We have been working with a diamondback colony for 9 years and, in spite to its expected loss of genetic variability due to its confinement, we have found that the initial sample collected in the Philippines in 1993 carried at least 4 different alleles (either from the same locus or different loci) that confer resistance to Cry1A toxins. Initially, when insects were brought to the laboratory, the colony showed a unique phenotype, in which the insects were resistant to Cry1Ab but not to Cry1Aa or Cry1Ac. After selection with a chimaeric Cry1Ac/Cry1Ab protoxin, the insects become resistant to the three Cry1A toxins. A gene conferring resistance to Cry1Aa could be shown to segregate independently from another gene conferring resistance to Cry1Ab. Further selection of the colony with either crystalline Cry1Aa or activated Cry1Ab resulted in two selection lines with new resistant phenotypes. The inheritance of resistance was different in the two selection lines, as well as their cross-resistance pattern and the binding site alteration. In summary, this colony has been shown to carry genes conferring resistance to: 1) just Cry1Ab, 2) Cry1Aa, 3) Cry1Ab and Cry1Ac, but not to Cry1F, with loss of Cry1Ab binding but not of Cry1Ac binding, and 4) Cry1Ab, Cry1Ac and Cry1F, with loss of binding of both Cry1Ab and Cry1Ac.

BT-r, a novel resistant gene against BT-toxin, Cry1Ab, was mapped on the Molecular map based on RFLP of EST-cDNA clones in the silkworm, Bombyx mori

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DNA markers are considered to be convenient and powerful tools to analyze the polygenic characters like ETL and QTL. In the silkworm, Bombyx mori, a genetic map of EST-cDNA clones based on RFLP analysis has been constructed and the methodology for linkage analysis and mapping by using ackcross (BF1) individuals has been improved. In the stocks of our institute, resistant strains against the Cry1Ab toxin were found by using a leaf test bioassay and the resistance of one Chinese strain was analyzed. 401, a strain showing strong resistance, was crossed to 606, a sensitive tropical strain, and their F1 hybrid of them was sensitive, indicating that this resistance was recessive. After confirming that the hybrid was killed by a discriminating dose of BT-toxin, residuals of the same batch was reared. The females were individually crossed to 401-males for linkage analysis and the males were crossed to 401-females for recombination mapping of this resistant gene. Half of every BF1 population survived after eating BT-toxin, indicating that a single recessive gene controls this

resistance. Individual DNAs were prepared from controls and surviving BF1 larvae after feeding toxin. On genomic Southern of BF1 segregants all survivors in the cross designed for linkage analysis were homozygous for the clone
m44, which is on the 4th chromosome but homozygous and heterozygous bands were detected from non-treated individuals used as a control. All clones on the other 27 chromosomes gave homozygous and heterozygous patterns for the survivors. These results indicated that this resistant gene, BT-r, was linked to m44 on 4th chromosome. Both clones m44 and m75 on the fourth chromosome showed effective patterns for mapping. From the Southern blot analysis of 84 BF1 segregants treated with BT-toxin, BT-r was mapped in between m44 and 75, 2.3 cM from the m44 and 17 cM from m75.

Tuesday, 10:30 - 12:30
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CONTRIBUTED PAPERS - Microsporidia 1
Chair: T. Andreadis

Comparative Phylogenetic Analysis of *Amblyospora* and related species

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Amblyospora species and their relatives show the most complex life cycles of any Microsporidia thus far examined. Their definitive hosts, the hosts in which the sexual cycle takes place, are mosquitoes and their alternate hosts are copepod Crustaceans. The *Amblyospora* species are very specific for their definitive, mosquito host and thus there is only one *Amblyospora* species for each mosquito species examined. The copepods however may be host to several *Amblyospora* species and more than one copepod may host a single species of *Amblyospora*. As a result of their definitive host specificity, the *Amblyospora* form a definitive clade which include species such as *Edhazardia aedis* and *Culicospora magna* which have secondarily lost the intermediate host. In addition it is apparent that several species which have been classified as *Amblyospora* based on general spore shape but which do not infect mosquitoes, such as *Amblyospora bracteata* (a parasite of a blackfly), do not fall into this clade are therefore are not "true" *Amblyospora* species. Most importantly our data reveal a correlation between the *Amblyospora* species and their mosquito hosts. We find a group infecting the *Aedes/Ochlerotatus* mosquito species, a group infecting the *Culex* mosquito species and an *Anopheles* infecting group. We also present information on the relative positions of other aquatic Microsporidia such as *Hazardia milleri*, *Marssoniella elegans* and *Berwaldia schaefernai*.

Efficacy of Neem seed extract and an entomopathogenic microsporidian on *Haritalodes derogata* (F.) and *Spodoptera litura* (F.) larvae

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Efficacy of neem seed extract (NSE) and the microsporidian pathogen, *Vairimorpha* sp. on two important leaf feeding vegetable pests, *Haritalodes derogata* (F.) and *Spodoptera litura* (F.) larvae was examined. *H. derogata* and *S. litura* larvae are the dominant foliage feeding insect pests of okra plants *Abelmoschus esculentus* (L.) grown in the southern part of Sri Lanka. However, their different feeding habits may reduce their competition for food and the leaf roll made by *H. derogata* larvae protect it further from the natural enemies and contact insecticides. *Vairimorpha* sp. was initially isolated from dead *S. litura* larvae collected from vegetable fields in southern part of Sri Lanka. Estimation of *Vairimorpha* sp. population in the dead larvae ranged from $10.4x10^7$ to 46.3×10^7 spores/ ml. All the larval instars of experimental insects had lower body weight and reduced growth rate when they were fed on the okra leaves treated with neem seed extract. Different instars of larvae were fed on the okra leaves treated with the serial dilutions of *Vairimorpha* sp. spores and higher mortality was observed in early instars of *H. derogata* and *S. litura* larvae than that of the later instars.
Combinations of sub lethal concentration of NSE with sub lethal concentrations of *Vairimorpha* sp. on both larvae shown significantly higher mortality than the individual treatments. Present study indicate that the use of neem seed extract together with *Vairimorpha* sp can be effectively use in IPM for two major lepidopteran pests of vegetables.

A remnant mitochondrion in the microsporidian *Trachipleistophora hominis*

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Microsporidia are important obligate intracellular parasites of a variety of eukaryotes. They have a highly complex and unique infection apparatus but otherwise are thought to be structurally simple. In particular, typical eukaryotic organelles such as mitochondria and peroxisomes are absent. This was widely interpreted as support for the Archezoa hypothesis[1] that suggested that these peculiar eukaryotes diverged prior to the mitochondrial endosymbiosis, making microsporidia potentially one of the earliest offshoots in eukaryotic evolution. Over the last five years the view of microsporidia evolutionary biology has changed dramatically. Firstly recent gene trees and more sophisticated phylogenetic analyses have generally recovered microsporidia as the relatives of fungi, rather than as basal eukaryotes[2-4]. Secondly, several genes which appear to have originated in the mitochondrial genome have been cloned suggesting the presence of a reduced or secondarily absent mitochondrion[5-7]. However, no mitochondrion-like organelle has ever been identified in microsporidia despite extensive ultrastructural studies. In order to investigate whether these mitochondrion-related genes produced proteins which were targeted to a remnant mitochondrion, we developed a highly specific antibody to a

Trachipleistophora hominis mitochondrial Hsp70 orthologue (mtHsp70). Using western blotting we have been able to demonstrate for the first time the expression of a mitochondrially associated protein in spore and meronts stages of parasites. Furthermore we have used this antibody to identify the localisation of the mitochondrial protein in *T. hominis* cells. This revealed the presence of numerous small discrete compartments within the parasite. These data provide the first direct link between a protein of endosymbiotic origin to a microsporidian organelle of putative mitochondrial ancestry, and show that microsporidia have retained a small mitochondrion-derived organelle the role of which remains to be elucidated.

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Mattesia oryzaephili (Sporozoea; Neogregarinida) host range and virulence

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The neogregarine, *Mattesia oryzaephili*, has previously only been reported from the sawtoothed grain beetle, *Oryzaephilus surinamensis*. It has been the cause of collapses in colonies of the rusty grain beetle, *Cryptolestes ferrugineus*, in our laboratory. Tests of physiological host range indicate an affinity for stored-product insects across orders with *C. ferrugineus* being the most susceptible species tested. In addition to the grain beetles, it is infective for larvae of the lesser grain borer, *Rhyzopertha dominica*, the Indianmeal moth, *Plodia interpunctella*, the Mediterranean flour moth, *Ephestia kuehniella*, and adults of a parasitoid wasp of grain beetles, *Cephalonomia tarsalis*. For larvae of *C. ferrugineus* and *O. surinamensis*, the LC_{50} values at 14 days post-treatment are on the order of 10^3 and 10^5 oocysts/g of diet, respectively, while infection and mortality of adults of those species did not exceed 50% after 21 days post-exposure to 10^6 oocysts/g of diet. For *E. kuehniella*, the LC_{50} value at 21 days was ca. 10^6 oocysts/g of diet. Less then 10% of *Ostrinia nubilalis* and *Tribolium castaneum* larvae were infected when exposed to the high dose of 10^6 oocysts/g of diet. *Aedes aegypti*, *Manduca sexta*, *Helicoverpa zea*, *Tenebrio molitor*, *Zophobas atratus*, *Trogoderma variable*, and *Liposcelis bostrichophila* were not infected in laboratory exposures. *Mattesia oryzaephili* oocysts germinated in the guts of both susceptible and resistant insects. Adult females of the wasp, *C. tarsalis*, become infected by feeding on infected *O. surinamensis* larvae and survive from 9 to 29 days from exposure. The male wasps do not feed on beetle larvae and do not become infected. There is no transmission from infected wasps to their progeny or to host beetles.

Histopathological observations and bionomical effects of microsporidian infections of the gypsy moth, *Lymantria dispar* L.

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During an outbreak from 1995 to 1997, two microsporidian isolates were recovered from gypsy moth (*Lymantria dispar* L.; GM) populations in the vicinities of Veslec and Levishte in northern Bulgaria. We studied the histopathology of the infection and the influence of both isolates on the development and performance of the host in laboratory bioassays. Microsporidian spores were fed to GM third instar larvae at 5 different dosages ranging from $2x10^2$ to $5x10^4$ spores/µl. Larvae were reared individually on standard artificial diet and the following parameters were recorded every second day: larval mortality, larval stage and weight, day of pupation, pupal weight, and day of adult eclosion. For transmission experiments, infected larvae were reared together with susceptible larvae in cages of different volumes. For histopathology, tissue samples from infected larvae were inspected at a daily basis, beginning with day 3 post infection. Infection levels in all larvae were verified by dissection and microscopic examination of tissues. Histological studies have confirmed that both isolates are morphologically very similiar and belong to the genus *Nosema*. However, the isolate from Veslec caused higher mortality than the isolate from Levishte (89-98 % vs. 79-93 %). Dosage did not have a significant influence on the mortality rates of either isolates. The Veslec isolate acted faster and killed infected individuals as larval instars, while the Levishte isolate caused mortality during the prepupal and pupal stages. These results were compared to the spread of the infection from the midgut tissue to the fat body and the timing of sporulation by histological observations. The isolate from Veslec did not have an effect on the development time, however, the time from infection to pupation of male larvae infected with the isolate from Levishte was significantly prolonged by 2-3 days. Neither isolate had an effect on the weights of female pupae, but infected male pupae were significantly heavier than uninfected male pupae. The *Nosema* isolate from Veslec was very effectively transmitted horizontally. Independent from rearing densities, over 95% of the susceptible larvae were infected after contact with infected individuals. The isolate from Levishte had a lower transmission efficiency; only between 40% and 48% of all susceptible hosts were infected. The infections were most effectively transmitted from day 13 to 19 post

infection ("window of transmission"). Results on the vertical transmission of both isolates will be presented.

A genomic sequence survey of *Nosema locustae*: microsporidian origins, metabolism and variation in genomic structure

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The 5.4 Mbp genome of the microsporidian grasshopper parasite, *Nosema locustae*, has been the focus of an ongoing sequence survey. Clones have been randomly end-sequenced, and several large, multi-gene contigs have been created, which allow for descriptions of gene order and genomic structure (lengths of intergenic spacers etc.). In addition, the genes possessed by *N. locustae* give insight into the core metabolism of microsporidia and provide examples of metabolic pathways such as particular steps in pyruvate metabolism that are, so far, unique to microsporidia. Results of the genomic survey also shed light on microsporidian origins. Genes identified in the survey that possess significant phylogenetic signal of their fungal ancestry can be sought from a broad diversity of fungi, and analyzed phylogenetically to identify where within the fungal radiation the microsporidia arose. Other evolutionary events, such as lateral gene transfer, have also molded microsporidian genomes. A single metabolic pathway can be composed of enzymes with different histories; the majority arise by vertical descent and are related to their fungal homologs, whereas another enzyme's phylogenetic affinity suggests a closer relationship with animals, raising the possibility that perhaps the gene was transferred to the parasite from its host. The recent completion of the genome sequence of *Encephalitozoon cuniculi* provides a source for comparison, as the two genomes differ substantially in size. The genome of *N. locustae* is just shy of twice the size of that of *E. cuniculi*, allowing for comparisons of compaction such as the lengths of intergenic spaces and the genes themselves, in addition to comparisons of gene order between the two microsporidian species.

Impact of a new species of microsporidia on two weevils, *Neochetina eichhorniae* and *N. bruchi*, biological control agents of water hyacinth (*Eichhornia crassipes*)

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Water hyacinth (*Eichhornia crassipes*) is an exotic aquatic weed that clogs natural waterways, displaces native vegetation and degrades wildlife habitat. Two closely related South American weevils, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache were first introduced into Florida in 1972 and 1974, respectively, and from there worldwide to other countries to control this plant. The success of these introductions has been variable with the reasons for poor performance or failure to establish being unclear. We suspected that entomopathogenic organisms, particularly a new microsporidian species found in Florida, might be at fault. We therefore evaluated this microsporidia to determine it's impact on these biological control agents. Infections in *N. eichhorniae* were typically found to be systemic with the mid-gut, Malpighian tubules, and fat body heavily infected. Infections in *N. bruchi* are typically restricted to the mid-gut. Development of the microsporidium is completely haplokaryotic producing large sporogonial plasmodia and eventually sporophorous vesicles containing up to 50 uninucleate spores. Morphological and molecular studies are in progress to determine the taxonomic placement of this species. Field and laboratory studies to determine the natural infection levels of this new microsporidium and the impact of microsporidiosis on the survival and reproductive capacity of the *Neochetina* spp. were conducted with adults collected from 5 sites in South Florida over a 2-year period (2000-2001). *N. eichhorniae* adults had higher levels of microsporidia infection than *N. bruchi* both on a yearly basis (average of 9.3% *vs.* 4.4%; n=1300) and a seasonal basis (winter: 10.4% *vs.* 3.6%; spring: 9.2% *vs.* 4%; summer: 9.6% *vs.* 6.4%, and fall: 8% *vs.* 3.6%). The sublethal effects associated with this pathogen when compared to healthy adults (n=25 in each group) were expressed by 1) lower fertility rates where *N. eichhorniae* produced an average of 51.0±48.1 eggs when infected vs. 135.2±18.5 when healthy, while infected *N. bruchi* produced an average of 44.6±20.4 eggs *vs.*

Defense response of *Lymantria dispar* larvae to microsporidian species in the genera *Nosema* and *Vairimorpha*

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We compared the course of infections by several microsporidian species in *Lymantria dispar* larvae and monitored host defense reactions against the pathogens. *L. dispar* is a permissive host for *Nosema portugal* and a *Vairimorpha* sp., both of which naturally occur in this species and cause heavy infections in larval hosts. *L. dispar* is only semi-permissive for *Vairimorpha necatrix* and another *Vairimorpha* sp., originally isolated from *Pseudaletia unipuncta* and *Hyphantria cunea*, respectively. To evaluate host defense reactions against these infections, we counted the number of hemocytes, measured the activity of phenoloxidase in the hemolymph of infected *L. dispar* larvae, and followed the course of the microsporidioses under the light microscope. *Vairimorpha* from *L. dispar* induced a highly significant increase in hemolymph phenoloxidase activity as well as in numbers of circulating hemocytes. Larvae infected with *N. portugal* showed increased phenoloxidase activity throughout the course of infection. *V. necatrix* infection did not lead to increased numbers of hemocytes. Phenoloxidase activity, however, was higher than in controls in the early phase of infection when the target tissues were invaded, but decreased to control levels later. *Vairimorpha* from *H. cunea* likewise triggered an increase in phenoloxidase activity when the invasion of the host fat body began. Pseudoparasitism of *L. dispar* larvae, as achieved by oviposition by *Glyptapanteles liparidis* (Hym., Braconidae) females that had been irradiated, supported a simultaneous infection with

Vairimorpha from *L. dispar*. Pseudoparasitized, and thus immune-compromised, infected larvae died earlier; furthermore, such hosts produced more microsporidian spores per unit fresh weight. The effects of pseudoparasitism on infections with *V. necatrix* and *Vairimorpha* from *H. cunea* were evaluated; preliminary data will be presented in this paper.

Two new early-branching microsporidians from crustaceans distinguished using DNA

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Microsporidia parasitizing marine and freshwater crustaceans in southwestern British Columbia, Canada have not been adequately examined. We looked for ultrastructural and DNA evidence to better distinguish between microsporidians causing cotton shrimp disease in panaeid shrimp (Decapoda, Pandalidae) and other marine crustaceans. We also found a new microsporidian in a common freshwater cyclopoid copepod while surveying microcrustaceans from ditch water. Two new species were described using light and electron microscopy and ribosomal and protein-coding DNA. The first was an octosporous species similar to *Thelohania butleri* described by Johnson *et al.* (1978) from musculature of pink shrimp, *Panadalus jordani*. Fresh spores measured 4.5 µm long x 3.3 µm wide and were seen in TEM to be uninucleate, in groups of 8 per sporophorous vesicle, filled with a striated looking matrix. Spores contained 7 or 8 double coiled isofilar polar filaments. The second species from copepods has pear-shaped spores resembling those of *Larssonia obtusa* from *Daphnia* sp. in Lithuania. Fresh spores were 3.8 µm long x 2.3 µm wide. Ribosomal small and large subunit DNA differed by 11.7 and 24.3 %, respectively, between this species in British Columbia and *L. obtusa* from Europe, suggesting these are not very close sister-species. Phylogenetic analysis placed these two species as expected based on morphology: the first allied with the early-branching *Thelohania* group and the second alongside the also early-branching *Amblyospora* group. Our examination of these two new microsporidians helps to flesh-out the poorly understood history of host-parasite relationships among these curious members of the fungi, and raises the question of whether the first host was a crustacean. Also, past studies of related microsporidians suggests these pathogens may have an important impact on host population size. **(STUDENT PAPER).**

Tuesday, 10:30 - 12:15
Cataratas I
CONTRIBUTED PAPERS - Bacteria 3
Chair: R. Akhurst

Interactions of *B. thuringiensis* subsp. *israelensis* insecticidal proteins withthe bloodworm *Chironomus tepperi* and their potential in creating Bloodworm-resistant transgenic rice

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The bloodworm *Chironomus tepperi* is the single major insect pest of Australian rice. This insect, which is capable of causing significant seedling damage, is currently controlled by the application of insecticides into the irrigation water at planting. Although Bti kills bloodworm in laboratory bioassays, field application is not economically viable because of the application rates required to deliver suitable dosages into the sediment where the bloodworm feed. An alternative to traditional application methods is to express the toxin in transgenic plants. With this approach, the toxin is delivered to the insects where they feed in the sediment. Consequently, only those insects that eat rice are directly affected and there are no pesticides residues in the irrigation water. Each of the *cry* genes of Bti were cloned and expressed in an acrystalliferous Bt strain to assess the toxicity of their products for *C. tepperi*. The toxicity of each individual protein and its interactions with the other δ-endotoxins was determined by bioassay. The *cry11A* gene, which was identified as potentially useful in transgenic rice, was transformed into rice. Plants expressing Cry11A have been regenerated and tested for resistance to *C. tepperi*.

The research on *cry1Ie1* gene from *Bacillus thuringiensis*

F. Song[1,2] J. Zhang[1] Y. Wu[1] C. Li[1] L. Han[1] D. Huang[2]

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A novel silent insecticidal gene from *Bacillus thuringiensis* isolate Btc007 which encoded a protein with MW 81kDa and composed of 719 amino acids (aa) was cloned. This gene was named *cry1Ie1*, a holotype gene. The *cry1Ie1* was inserted into *Bt-E. coli* shuttle vector pSXY422 which contained the STAB-SD sequence and *cry3Aa7* promoter from Bt isolate Bt22 and highly expressed in Bt acrystalliferous mutant strain 4Q7. Its expressed product was toxic to *Leguminivora glycinivorella* larvae besides diamondback moth (*Plutella xylostella*) and Asian corn borer (*Ostrinia furnacalis*). We studied the minimum active region of Cry1Ie1 protein against *Ostrinia furnacalis* larvae. Removal of 62 and 59 amino acids from the C terminus had little effect on toxicity. And then we had obtained several proteins IE638 with deletion of 80 aa from C terminus , IE648 with deletion of 70 aa from C terminus, IE045-656 with deletion of 62 aa from C terminus and 44 aa from N terminus, and IE099-656 with deletion of 62 aa from C terminus and 98 aa from N terminus. Activities of these proteins are being detected. Efforts are made to find the minimum toxic fragment of the Cry1Ie1 proteins. **(STUDENT PAPER).**

Cloning and Expression of Bt *cry* Genes from BtLy30 Strain with High Toxicity and wider anti-insect spectrum

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A novel strain of *Bt* (named as Ly30) was isolated from the ant *Componotus japonicu Mayr* found in the forest of Yuntai Mountains, in Lianyungang city, Jiangsu Province, in 1992. It was the first time to get a Bt isolate from *Hymenopteran* pest in China. Based on the results of identification in morphology, biochemical reaction and serology, it belongs to *Bacillus thuringiensis* subsp. *aizawai*, serotype H_7. The results of bioassay proved that Ly30 isolate had higher toxicity and wider anti-insect spectrum. Using the CAPS (Cleaved Amplified Polymorphic Sequences) identification system of cry1/7/9, cry2, and cry1I-type genes, five ICPs genes (*cry1Aa*, *cry1Ac*, *cry1Ia*, *cry 2Aa* and *cry 2Ab*) were found in Ly30 strain. These genes have been cloned, subcloned and sequenced. The sequences of these genes had also been registered in GenBank and named by International Nomenclature Committee. The expression products of these genes in *E.coli* have high insecticidal activity against several pests, eg. Cry1Aa12 against *Plutella xylostella, Dendrolimus latipennis, Spodopera exigua;* Cry1Ac against *Helicoverpa armigera;* Cry2Aa10 against *Oedaleus infernalis de Saussure*. These genes were induced into a *Pseudomonas fluorescens* strain P303 and an acrystalliferous mutant obtained by a shuttle vector pHT315 separately. The engineering bacteria also showed highly toxic to the pests above.

Assessing non-target effects of *Bacillus thuringiensis* and neem using *Folsomia candida* (Collembola)

M. Brownbridge[1], H. Kato[1] and M. Broza[2]

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As we strive to reduce chemical inputs in all sectors of agriculture and promote the use of microbial and biorational pesticides, the impact of large-scale applications on the soil ecosystem must be considered. Collembola are abundant in robust and productive soils, and play a vital role in the removal and breakdown of microbes and crop residues. The purpose of the current project, therefore, was to evaluate acute and chronic side-effects of selected *Bacillus thuringiensis* (Bt) products and the botanical insecticide neem on *Folsomia candida*. Three Bt products, Xentari (*aizawai*), Dipel (*kurstaki*) and MVP II (contains 20% Cry1Ac Bt protoxin expressed by and encapsulated in *Pseudomonas fluorescens*) were presented to mature *F. candida* using a novel feeding assay. The Collembola were fed on treated diet exclusively for 4 weeks, and were then fed on a yeast-agar diet for an additional 4 weeks. An aqueous neem seed extract was also prepared and administered using the same methodology. Effects on longevity and fecundity were monitored over the 8 week test period. Administered at 0.5 - 4 times the recommended field application rate, none of the Bt products affected egg production and longevity, compared with the non-treated controls. In contrast, egg production was significantly impaired in populations presented with the neem-contaminated diet after only 2 weeks exposure; egg production ceased altogether by week 4. Effects were sustained even when the insects were fed on the yeast-agar diet. Fewer Collembola survived the neem treatments than the untreated control. It is unclear whether effects were due to neem's repellant effect, which appeared to impair feeding on the diet blocks, or as a result of a physiological response. Although data cannot be directly extrapolated to predict effects under field conditions, these assays allow us to begin to quantify potential ecological impacts of microbial control agents and botanical insecticides in a rapid and reproducible manner.

Evaluation of non-target insect populations on conventional cotton, and transgenic Bollgard® cotton and Bollgard® II cotton, under two insecticide management regimes

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Non-target insect populations were evaluated in field studies conducted in Mississippi and Arizona in 1999 with transgenic Bollgard® II cotton (containing two lepidopteran active Bt proteins: Cry1Ac and Cry2Ab), transgenic DP50B cotton (original Bollgard® containing Cry1Ac) and the non-transgenic control line DP50, with and without insecticide applications. Field design of these studies was complete randomized blocks with 4 replicates, and each plot containing approximately 3000 plants (12 rows by 60 feet each). Pitfall traps and sweep-net samples were used throughout the season to monitor insect populations. All captured insects were counted and identified to order and family. Data were analyzed separately for each order or family using a general linear model including the effect of line and insecticide application and their interaction with dates of the counting during the season as repeated measurements. As expected, the numbers of insects observed changed greatly with the season but the transgenic Bollgard® II cotton and transgenic DP50B cotton, in general, did not show negative impact on non-target insect populations when compared with the non-transgenic control cotton. The application of the insecticide, however, did show some effect on the non-target organism.

Characterization of newly isolated Cry1A-binding proteins of *B. mori* larva.

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Cry1Aa, 1Ab and 1Ac show about 85-90% amino acid sequence homology to one another. However, their toxicity to *Bombyx mori* larva varies significantly. For example, Cry1Aa and 1Ab are highly toxic to *B. mori*, but Cry1Ac show only very least toxicity. To understand the relationship between the toxicity of the respective Cry1A toxins and their binding specificities, we attempted to characterize the Cry1A-binding proteins in the brush border membrane vesicles (BBMV) of *B. mori*. The ligand blot analysis and Cry1Aa-affinity column chromatography revealed the presence of seven Cry1Aa-binding proteins. Among them, the proteins with

molecular sizes 120 (P120), 110 (P110) and 100-kDa (P100) were homologs of 120-kDa aminopeptidase N (APN) that has already been reported as Cry1Aa receptor, and were detected by both ligand blotting and affinity column chromatography. Interestingly, two binding proteins, 95 (P95) and 86-kDa (P86) were detected only by affinity column. In contrast the remaining other two proteins 245 (P245) and 185-kDa (P185) were detected only by ligand blot analysis. These suggested the diversities in the interactions between Cry1Aa and their binding proteins. On the other hand, characterization of *B. mori* APNs showed a novel Cry1Ac specific binding protein with 90-kDa size (P90) that showed APN activity, however, interestingly, they were not recognized by anti-120-kDa APN antiserum. We also investigated the pore forming abilities of Cry1A preliminary by the calcein release assay using PS/PC vesicles. The assay exhibited the relatively high pore-forming ability of Cry1Ab compared to that of Cry1Aa and 1Ac. Thus, our results suggested that various factors in addition to receptor binding, contributes to the toxicity of Cry1A proteins.

Identification of a Mexican strain of *Serratia* spp. phatogenic against larvae of *Phyllophaga* spp (Coleoptera:Scarabaeidae)

M. E. Nuñez-Valdez[1], M. A. Calderon[2], E. Aranda[2], L. Hernández[3], R. M. Ramirez-Gama[3] and F. J. Villalobos[1]

[1]Centro de Desarrollo e Investigación Agropecuaria, FaCAgr., [2]Centro de Investigación en Biotecnología, Universidad Autonóma del Estado de Morelos, [3]Facultad de Química, Universidad Nacional Autónoma de México, México.Larvae of scarab beetles of the *Pyllophaga* genus are important soil dwelling pests causing damage to several crops in Mexico. During a general program on the search for pathogenic bacteria to control larvae pests of *Phyllophaga* spp (Coleoptera:Scarabaeidae), a native *Serratia* spp (strain Mor4.1), was isolated from a dead 3rd instar larvae of *Phyllophaga blanchardi* collected from the field. We report that oral bioassays done with healthy larvae of *P. blanchardi* and *P. trichodes* fed with small pieces of carrot coated with the Mor4.1 isolate, showed that this strain was able to cause anti-feeding effect (AFE) and larvae loss of weight. These disease symptoms were alike to those reported for amber disease caused by *Serratia entomophila* and *Serratia proteamaculans* to larvae of *Costelytra zealandica*, although some differences were observed. Phenotypic and biochemical tests used for *Serratia* spp. taxonomy showed that the Mor4.1 isolate is very close to the *S. entomophila* species. Amber disease caused by *S. entomophila* (strain A1MO2) to *C. zealandica* has been partially characterized. Genetic evidence has suggested that the products of five genes named AnfA, AnfB, SepA, SepB y SepC are involved in the disease. It was also suggested that extracellular toxin proteins might be involved in the disease, since concentrated culture supernatants (CCS) were able to cause amber disease symptoms to *C. zealandica* larvae. We have hypothesized that the strain Mor4.1 might produce extracellular proteins similar to those produced by *S. entomophila* A1MO2. Oral bioassays were done with the CCS from the Mor4.1 isolate, fed to *Phyllophaga* spp larvae to search for disease symptoms. Preliminary results have shown that Mor4.1 CCS produce AFE and mortality to 1rs instar larvae of *P. ravida*. SDS-PAGE analysis of the CCS from the Mor4.1 strain and from *S. entomophila* A1MO2, has shown the presence of several proteins that might be associated with the pathogenicity symptoms to the scarab larvae.

Field efficacy of formulation based on β-exotoxin *Bt* for Uzbekistan population of Colorado potato beetle

E. N. Abdullaev

Alisher Navoi Samarkand State University, 15 University Avenue, Samarkand,Uzbekistan, 703004

During 1999-2001 years we conducted field experiments for efficacy estimation of Bitoxibacillin-202 formulation (Russia) based on β-exotoxin *Bacillus thuringiensis subsp.thuringiensis* for Colorado potato beetle – *Leptinotarsa decemlineata*. Formulation Bitoxibacillin-202 contains 45 billions of spores, same number of endotoxin crystals and 0.6-0.8% β-exotoxin. All experiments were conducted in Zerafshan valley. For these field experiments were used the formulation with amount of 2 and 4 kg/ha and high-volume sprays (around 400 l/ha) with concentration of formulation - 1.0% and 2.0%. The chemical insecticide-acaricide formulation – Phosalone was as a etalon. The biological formulation was used in different variants. In the first variant spray was single, in the second variant – double and in the third – triple. In case of double and third spraying we used only 2 kg microbial formulation per ha. The efficacy of formulation was estimated after 3, 5, 7, 10 and 15 days after spraying. The experimental variants with different amount of formulation (2 and 4 kg/ha) show same effectiveness on 7 day after spraying. The level of insect mortality was 69.1% for first dosage and 70.4 for second dosage. After 15 days the mortality was 73.4% and 74.4% respectively. The double and third spray allows receiving the insect mortality level from 88.5% to 92.5% after 10 days. Our experiments show that microbial formulation – Bitoxibacillin can be used for control of 1-2 instars larvae Colorado potato beetle. Rational amount of formulation is 2 kg/ha. Application multiplicity can be double or triple.

Laboratory efficacy of formulation based on β-exotoxin *Bt* for Uzbekistan population of Colorado potato beetle

E. N. Abdullaev

Alisher Navoi Samarkand State University 15 University Avenue, Samarkand, Uzbekistan, 703004

During 1999-2001 years we conducted laboratory experiments for estimation of "Bitoxibacillin-202" formulation (Russia) based on β-exotoxin *Bacillus thuringiensis subsp.thuringiensis* for Colorado potato beetle – *Leptinotarsa decemlineata*. "Bitoxibacillin-202" contains 45 billions spores, same number endotoxin crystals and 0.6-0.8% β-exotoxin. For these experiments were used different age of potato beetle larvae and adult insects. In our experiments potato leaves were sprayed as a field high-volume spray (around 400 l/ha) with concentration of formulation - 0.25%, 0.5%, 1.0% and 2.0%. Each variant includes 100 insects, number of repetitions – 4. Laboratory efficacy of formulation was realized on young larvae (1-2 instars), third and fourth instars larvae and adult insects. It was established that young larvae have the highest sensitiveness to tested *Bt*

formulation. Level of mortality for 1-2 instars larvae was 99.2% for formulation with concentration 0.25% and 100% for formulation with concentration 0.5%. In case of third and fourth instars larvae the level of mortality was from 10.2% to 30.6% depends on the concentration of suspension. The adult insects in case all concentrations of Bitoxibacillin formulation were alive. Mortality was same as in pure control when potato leaves were treated only with sterile water. Our laboratory experiments allow to do conclusion, that potato beetle control can be realized only for the larvae first and second instars.

Tuesday, 14:00 - 16:00
POSTER SESSION II

FP 19 Compatibility between entomopathogenic *Beauveria bassiana* and pesticides used in coffee crop protection

C. N. de Oliveira[1], P. M. O. J. Neves[1], L. S. Kawazoe[1], and R. C. de Oliveira[1]

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The *in vitro* fungitoxic effect of ten fungicides, fourteen herbicides and nine insecticides, at three concentrations (AR= average field recommendation; 0.5 AR and 2 AR) to *Beauveria bassiana* (CG 425 strain) entomopathogenic fungi was evaluated. The effect of the products on conidia germination, vegetative growth and conidiagenesis was compared. As few as six herbicides and five insecticides at the smallest concentration promoted a higher than 70% germination. No fungicides were compatible. Conidia germination should be considered the most important factor to be evaluated since it is the first step within the infection process. The Zapp herbicide (Sulfosate) had no effect on conidia germination at the three study concentrations. The others herbicide treatments: Herburon (Diuron), Reglone (Diquat), Fusilade 125 (Fluazifop-P-Butil), Gramoxone (Paraquat) and Sencor 480 (Metribuzin), had no negative effect on germination but at the smallest concentration (0.5 RM). Vegetative growth was inhibited in all treatments, except for Reglone (Diquat) that showed no statistical variation in relation to control at the two smallest concentrations. As for conidia production all herbicides showed statistically significant inhibition as compared with the control in all study concentrations. The insecticides showed the best results for the three parameters evaluated. Fastac 100 (alpha-cypermethrin), Actara (Thiamethoxan) and Baytroid (Cyfluthrin) formulations caused the smallest inhibition level on conidia germination at the two smallest concentrations (0.5 RM and 2 RM), with no statistical difference in relation to the control. Upon vegetative growth analysis, the Actara (Thiamethoxan) formulation, at the two smallest concentrations, was found both not to cause inhibition on the radial growth and to be significantly higher as compared to the other insecticide formulations. Actara (Thiamethoxan) caused the smallest inhibition level in regard to conidia production. Upon such results we may recommend the use of Zapp (Sulfosate), Fastac 100 (Alfacipermetrina) and Actara (Thiamethoxan) in IPM coffee crop programs since these products were compatible with the entomopathogenic fungi *B. bassiana* (CG 425 strain), an important natural control agent of the coffee berry borer, *Hypothenemus hampei*.

FP 20 Influence of temperature in germination, vegetative growth and conidia prodution of *Beauveria bassiana* (Bals.) Vuill

P.M.O.J Neves[1], E.T. Ito[1], E.I. Yuki[2], P.H. Santoro[1], V.R. Chocorosqui[1], L. Koguishi[1], J.G.Z. Vieira[1]

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The development of six *Beauveria bassiana* strains previous selected for Coffee-berry-borer *Hypothenemus hampei* was compared in different temperatures (20, 25 and 30°). It was evaluated the % of germination, the vegetative growth and conidia production using PDA medium. The results showed that for the majority of the strains the best development temperature for the three parameters was 25°C. Germination is the parameter that was most affect by temperature with the best results for 25°C. However some strains did not show any statistical difference for some parameters. For instance, the strain CG 432 did not show statistical difference in conidia production at the three temperatures. Only one strain differs statistically from the others with lowest germination levels at 25°C. RAPD of these strains showed that CG 432 is the strain that less share genetic material with the other strains. This information is very important for formulation of a commercial product especially for strains blended that could be used for best efficiency in some climatic grow conditions. Also, for use of the pathogen in some conditions where temperatures are largest like in the yard.

FP 21 Inibition of coffe anthracnose *Colletotrichum* spp. by the entomopathogenic fungus *B. bassiana* (Bals.) Vuill. *in vitro*

D. C. Ribeiro[1], P. M. O. J. Neves[1], N. Massola[2] and P. H. Santoro[1]

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Colletotrichum spp. is an important disease of coffee plants. Two *Colletotrichum* sp. was isolated from coffee plants and its inhibition by *Beauveria bassiana* (strain CG 425) was studied under laboratory conditions. The two fungi was inoculated in PBA Petri dishes at the same time. The initial methodology was to inoculate the fungi in two points but *B. bassiana* was sprayed all over the dishes and the *Colletotrichum* was inoculated in only one point. Preliminary tests showed that the entomopathogenic fungus *B. bassiana* have a great capacity for *Colletotrichum* inhibition. This growth inhibition is probably biochemical caused by extracellular metabolites. The study showed a fungitoxic potential effect of *B. bassiana* toxins that should be explored. Future studies will define the inhibition mechanism, the toxin involved and its potential as a fungicide for plant diseases. **(STUDENT POSTER).**

FP 22 *Evlachovaea*: First reports of an unusual and little known entomopathogenic fungal genus from the New World

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Several collections of entomopathogenic fungi from Brazil have been found to be referable to a little known genus described from Russia. Borisov and Tarasov (1999. Mikologiya i Fitopatologia 33[4]: 248-256) described a new genus and species, *Evlachovaea kintrischica*, from alder beetle, *Agelastica alni* (Coleoptera.: Chrysomelidae) for a fungus with conidiophores bearing clusters of *Paecilomyces*-like, flask-shaped conidiogenous cells and dry chains of mussel- or wedge-shaped conidia. Unlike in *Paecilomyces*, the conidia are produced obliquely at the apex of the conidiogenous cell, and each successive conidia are usually produced in differing orientations thus resulting in conidial chains with a zipper- or ribbon-like appearance. The Brazilian collections of his fungus have ovoid to short-cylindrical conidia 4.2 ± 0.5 x 2.0 ± 0.3 µm (range: 2.9-6.0 x 1.4-3.1 µm), and an average length/width ratio of 2.1 ± 0.4 (range: 1.24-3.1). All of the cultures grow sparsely with little aerial mycelium on cornmeal agar, and more luxuriantly and with more aerial mycelium on quarter-strength Sabouraud dextrose agar + yeast extract; none of the cultures has been observed to produce distinctive pigments. To date, this fngus has been confirmed \from a probable species of *Chiromyza* (Diptera: Stratiomyidae) from Lavras (Goias), from *Brassolis saphorae* (Lepidoptera: Brassolidae) from both Muju (Para) and Aracaju (Sergipe), and from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from Londrina (Parana).

FP 23 Virulence of *Verticillium lecanii* varies with different developmental stages of cotton aphid (*Aphis gossypii*)

J. J. Kim[1,4], D. R. Choi[1], K. C. Kim[2], C. S. Yoon[3], and D. W. Roberts[4]

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Aphids are some of the most serious pests in the world. *Verticillium lecanii* has high virulence to aphids and whiteflies and is under consideration as a microbial control agent. *V. lecanii* isolate CS-625 isolated from Korea has high virulence to cotton aphid (*Aphis gossypii*). We studied virulence of this isolate to different developmental stages of cotton aphid. Spore suspensions (10^8 conidia/ml) were sprayed on 1st, 2nd instar nymphs and adults of cotton aphid. First-instar nymphs are 0~6 hr progeny, second-instar nymphs are about 60hr after birth and adults are about 7 days after birth. The mortality of cotton aphid was 100% 4, 5 and 7 days after spore treatment of adults, 2nd and 1st instar nymphs, respectively. Why does the *V. lecanii* isolate CS-625 have a different mortality rate depending on the stage of cotton aphid? After spore treatment, the number of attached spores on the 1st instar aphid surface are approximately one half of that on than 2nd instar nymphs and adults. Scanning electron microscope observations one day after treatment indicate spore germination on 1st instar nymphs also was lower than on the surface of other stages of the aphid. Penetration into the insect body was found on the first day after exposure with adults, the third day with 2nd instar nymphs, and second day with 1st instar nymphs: but 1st instar-infection levels were lower than for 2nd instar nymphs and adults. Although the 1st instar is the weakest stage, it has slow mortality because of small spore numbers on the cuticle plus lower germination and infection rates.

FP 24 Entomopathogens Associated with Soybean/Wheat Production Systems in Brazil and Argentina

D. R. Sosa-Gomez[1], R. A. Humber[2] & F. Moscardi[1]

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We summarize a ten year survey of eukaryotic pathogens recorded from insects and mites affecting soybean and winter wheat in Brazil and Argentina. The foremost disease in the soybean system was *Nomuraea rileyi* on *Anticarsia gemmatalis* (VBC), *Pseudoplusia includens* and *Rachiplusia nu* (Noctuidae) and, less commonly, in Argentina on *Spilosoma virginica* (Arctiidae). *Pandora gammae* (Entomophthorales) attacked larval Plusiinae in late December-January. *Paecilomyces tenuipes* occurred in seasons with prolonged wet periods; this species infects VBC and Plusiinae larvae, killing usually at the pupal stage. *Pseudoplusia includens* and VBC were sometimes attacked in January-February by a *Zoophthora* species. Among the seed pest insects, *Piezodorus guildinii* (Pentatomidae) nymphs host a *Furia* sp. (Entomophthorales). *Paecilomyces fumosoroseus*, a generalist fungal pathogen, attacked both root-feeding stinkbug *Scaptocoris castanea* (Cydnidae) and dense populations of *Bemisia argentifolii* in January. *Paecilomyces amoenoroseus* was highly prevalent on *Lagria villosa* (Lagriidae) (December–March). A species of *Neozygites* sp. affected the two-spotted spider mite, *Tetranychus urticae*. *Zoophthora radicans* infected *Nabis* sp. (Nabidae) and *Bemisia argentifolii*. An unidentified protozoan flagellate attacked the stinkbugs *Nezara viridula* (up to 30% infection) and *P. guildinii* (up to 7% infection). In wheat, a complex of fungal epizootics of *Entomophaga aulicae*, *Pandora* sp. and *Zoophthora* sp. were detected in *Pseudaletia sequax* (Noctuidae) in July-August; at the same time *Entomophaga grylli* (Entomophthorales) became epizootic on *Bacacris punctulatus* (Acrididae). Less specific fungi such as *Beauveria bassiana, Metarhizium anisopliae,* and *P. fumosoroseus* attacked diverse species in Chrysomelidae, Curculionidae, Cydnidae, Scarabaeidae, and Pentatomidae. *Batkoa apiculata* attacked *L. villosa* adults on soybean (January) and on wheat (August). We also found *Pandora neoaphidis* on the aphids *Metopolophium dirhodum* and *Rophalosipum padi* on wheat.

FP 25 Compatibility of entomopathogenic fungi with pesticides

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Compatibility of the following pesticides was evaluated: clorfenapyr, fenpyroximate, amitraz, fenbutation oxide, acrinathrin, pyridaben, hexythioazox, abamectin, in two dosages – average dosage: (AD) (*1x*) and half of AD (*0,5x*) in the germination, vegetative growth and conidia production of the entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces fumosoroseus*. For germination testes (step1), the products, in the pré-established concentrations, were mixed with sterile water, Tween 20 (0,02%) and 1 mL of conidia suspension ($1x10^6$ conidia/ml). After 60 minutes, 0,5 mL aliquots were spread into Petri dishes with water-agar medium. The dishes were incubate at 25±1°C and 12hrs (photophase).. Twenty hours after incubation the percentage of germination was quantify. For vegetative growth (V.G.) and conidia production (C.P.) (step 2), the P.D.A. medium was sterilized and at ±45°C the pesticides were added, in the pre-established dosages, with streptomycin (0,5 g/L) and turn into the dishes. After, the fungi were inoculated in three points per dish, and incubated at 25 ± 1°C and 12 h photophase. Eight days after inoculation the colony diameter were measured and colony disks from each treatment were collected for quantification of conidia production. For germination, fenbutation oxide affects drastically the germination of the three species. However, acrinathrin, abamectin e hexythioazox (*0,5x* e *1x*), did not differ statistically from the control, for the three fungi species. The formulations that cause the less inhibitory levels were selected for V.G. and C.P. studies. For these parameters acrinathrin, abamectin and hexythioazox (*0,5x* e *1x*) did not differ statistically from the control, for the three species. However, amitraz and pyridaben caused significative reductions for the three entomopathogens. So, acrinathrin, abamectin and hexythioazox formulations are the most compatible with *B. bassiana, M. anisopliae* and *Paecilomyces fumosoroseus* and may be utilized in IPM programs.

FP 26 Susceptibility of the Paraguay-tea borer *Hedyphates betulinus* (Coleoptera: Cerambycidae) to entomopathogenic fungi

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The virulence of six *Beauveria bassiana* strains (CG424, CG166, CG425, CG375, CG082, CB124) and one *Metarhizium anisopliae* (E9) was evaluated in the laboratory on Paraguay tea borer *Hedyphates betulinus* (Coleoptera: Cerambycidae) the key pest of Paraguay-tea (*Ilex paraguariensis*). The statistic design was the completely randomized with four replications/strain and the control. For the bioassay 2 liters "pet" bottles were cut in the middle and the two half parts were put together . For each bottle 5 adults borers were transferred and sprayed with 1 mL of a conidia suspension (10×10^7 conidia/mL) and after Paraguay-tea branches were add into the bottles. The bottles/cadges were then left at 25±1°C and 12hrs (photophase). During 15 days, daily, the branches were changed and died insects transferred to a humid chamber for mortality confirmation by the fungus. The general mortality data for strains of the two fungi species varied between 50% and 100% and 0% and 81,25 % for the total and confirmed mortality respectively. For the most virulent strains CG166 and 424 (*B. bassiana*) and E9 (*M. anisopliae*) that promoted confirmed mortality of 75,00; 81,25 and 81,25% respectively, one other bioassay was made, with the same statistical design. However, in this bioassay the branches without leaves were first put into the bottles and sprayed with 1 mL of a conidia suspension (10×10^7 conidia/mL) and after the adult insects. The bottles were maintained at 25±1°C and 12hrs (photophase). After the fourth day the branches were changed each two days when the dead borers were transferred to the humid chamber. The total correct mortality were 100%; 93,75% and 81,25% and the confirmed were 75,00%, 66,67% and 53,85% for the strains CG424, E9 and CG166 respectively. These data showed that these entomopathogenic fungi have a great potencial for the Paraguay tea borer control.

FP 27 Effect of the fungus *Metarhizium anisopliae* var. *acridum* on non-target arthropods in Brazil

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A biopesticide based on the fungus *Metarhizium anisopliae* var. *acridum*, isolate CG 423, has shown to be efficient against the grasshopper *Rhammatocerus schistocercoides* (Rehn), a serious agricultural pest in Mato Grosso, Brazil. In order to assess the effects of this biopesticide on non-target arthropods, field tests were carried out in Mato Grosso State, in natural vegetation, during the period of occurrence of young nymphs of *R. schistocercoides*, normal target of the mycopesticide. The experimental protocol consisted of two four-hectare blocks (200 x 200m). One block was sprayed with a dosage equivalent to $2.2x10^{12}$ viable conidia per hectare. This dosage causes approx. 46.7% mortality twelve days after application as shown in another experiment. The other block was used as control and no application was made. In each block the insect fauna was sampled 2-3 days before spraying and 9-10 to 17-18 days after spraying, using Malaise, "window" and "yellow bowl" traps. Data analysis was performed regarding insect orders and families. The results demonstrated that the biopesticide can strongly affect the grasshopper target populations, but has no or few impact on non target fauna.

FP 28 Hydrophobicity of conidia of *Metarhizium anisopliae* produced by submerged fermentation

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The compatibility of a mycopesticide product to an oil formulation is often an important consideration for effective application of the biocontrol agent in the field. In addition, as hydrophobic interaction is commonly the first, non-specific stage in the attachment process of a biopesticide to the target insect, the hydrophobic nature of the product is of particular interest. This study assesses

conidial hydrophobicity during liquid culture of *M.anisopliae* var. *acridum* and comparison is made with blastospore production. The possibility of enhancing hydrophobicity of submerged conidia by manipulation of the nutritional environment is also investigated.

FP 29 *Beauveria bassiana* yeast phase on agar medium and its pathogenicity against *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Tetranychus urticae* (Acari: Tetranychidae)

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Beauveria bassiana colonizes insect hosts initially through a yeast phase, which is common in some artificial liquid cultures, but not reported on artificial solid media. A yeast-like phase for *B. bassiana* isolate 447 (ATCC 20872) was produced on MacConkey agar, and the virulence of yeast-like cells was tested against *Diatraea saccharalis* and *Tetranychus urticae*. The yeast-like cells of *B. bassiana* developed by budding from germinating conidia after 24-h incubation. Cells were typically 5 to 10 µm and fungal colonies were initially circular, with a mucoid aspect, but later were covered with mycelia and conidia. Ability to produce yeast-like cells on MacConkey medium was relatively common among different *B. bassiana* isolates, but growth rate and timing of yeast-like cell production varied. *M. anisopliae* and *Paecilomyces* spp. isolates did not grow as yeast-like cells on MacConkey medium. Yeast-like cells were more virulent against *D. saccharalis* than conidia when 10^7 cells/ml were used. At 10^8 cells/ml, the estimated LT_{50} was 5 d for the yeast suspension and 9 d for an equivalent conidial suspension, perhaps due to faster germination for yeast-like cells. The LC_{50} was lower for yeast than conidial suspensions. Yeast-like cells and conidia had similar virulence against *T. urticae*: the average mortalities with yeast-like cells and conidia were, respectively, 42.8 and 45.0%, with 10^7 cells/ml, and 77.8 and 74.4%, with 10^8 cells/ml. The estimated LT_{50}'s were 3.4 and 4.0 for yeast and conidial suspensions, respectively. The bioassay results demonstrate that the yeast-like structures produced on MacConkey agar are effective as inoculum for *B. bassiana* applications against arthropod pests, and possibly mre virulent than conidia against some species. Obtaining well-defined yeast phase cultures of entomopathogenic hyphomycetes may be an important step in studies of the biology and nutrition, pathogenesis, and the genetic manipulation of these fungi.

FP 30 A new record of the *Entomophaga* (?) *grylli* (Fresenius) Batko species complex on Acrididae (Orthoptera) in southern Bahia, Brazil

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An entomophthoralean fungus (Zygomycotina: Zygomycetes), from the *Entomophaga grylli* species complex has been found and described from Brazil, on populations of *Rhammatocerus brasiliensis* Bruner, *Rhammatocerus brunneri* Giglio Tos, *Abracris dilecta* Walker, *Abracris flavolineata* De Geer, and on an unidentified species of grasshoper from the subfamily Ommatolampinae (Acrididae). All of these grasshopper species were attacked by an *Entomophaga* species presumably belonging to the *E. grylli* species complex; it is impossible to say at this time whether this Brazilian fungus represents collections of a known (described) taxon or a new and undescribed member of this species complex. This new finding of *E. grylli* is a scientific contribution to the knowledge of the Entomophthorales in Brazil and opens opportunites for further studies since this pathogen causes natural epizootics that, on many occasions, have had significant impacts by the reduction of acridid populations.

FP 31 Study of *Verticillium lecanii* culture at different temperatures and growth media

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The present experiment was conducted to study different in vitro culture conditions for CPAC H1 a virulent strain of *Verticillium lecanii*, tested for *Leptopharsa heveae* (Hemiptera: Tingidae). Three bioassays were conducted at temperatures of 21, 24 e 28 ±0,5°C. The photoperiod was 12 hours and it was compared two growth media: oat medium (OM) and potato, dextrose, agar and yeast medium (PDAY). The diameter colony growth were evaluated daily and the production and conidia viability were evaluated every 2 days up to 20 day after fungus inoculation. The results (except the last) obtained in the twenty day were: OM media had a higher growth than PDAY, at temperatures of 21 and 24°C. However at 28°C, PDAY media enable better growth than OM media. The largest fungus colony diameter was obtained on OM media at 24°C. The conidia production was higher on PDAY media than OM media at 21 and 28°C, but not at 24°C. It was observed a high production ($4,4 \times 10^8$ con./ml) on PDAY media at 28°C. Conidia viability from OM media showed higher values than PDAY media at 24°C. The highest viability was observed on PDAY media, at 28°C, in conidia collected from 4 days colonies the fungus inoculation.

FP 32 Determination of growth media for the viability test of entomopathogenic fungi

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The aim of this study was to evaluate the effect of different growth media on entomopathogenic fungi conidia germination utilized in viability tests. The

viability tests were carried out on microscopy slides with 4 ml of growth media. In each slides it was delimited 3 areas where were dropped 0,05 ml of conidia suspension, varying from 10^5 to 10^6. The fungi species used were: *Beauveria bassiana* (JAB 06), *Metarhizium anisopliae* (E 09), *Paecilomyces fumosoroseus* (JAB 12), *Verticillium lecanii* (CPAC H1). The growth media analyzed were: 1-Agar-Water, 2-Potato, Dextrose and Agar (PDA), 3-Potato, Dextrose, Agar and Yeast Extract (PDAY), 4-Sabouraud and 5-Complete Media with five replications. For *M. anisopliae* at 27±0,5 °C, the lowest germination rate (69,6%) was verified in PDA media and the highest (96,9%) in Sabouraud media. Analyzing *V. lecanii* at 26±0,5 °C, it was observed a germination rate of 84,8% and 92,4% for Sabouraud and PDAY media, respectively. For *P. fumosoroseus* raised at 26±0,5 °C the lowest germination rate (51,6%) was verified in Agar-Water, and the highest (86,0%) in the Complete Media. Utilizing *B. bassiana*, at 26±0,5 °C, the lowest germination rate (52,5%) was observed in Agar-Water and the highest (68,2%) in the Complete media. The results showed that growth media has influence on the conidia germination on viability tests. Thus, further experiments have to be conducted to establish adequate growth media to viability tests to each species of entomopathogenic fungi.

FP 33 Water Sorption Isotherms of *Beauveria bassiana* (Bals.) Vuill. Formulation

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The entomopathogenic fungi *Beauveria bassiana* (Balls. Vuill.) is largely studied and applied as a biological control agent (BCA). One of the main requirements for successful commercial production of BCA's is the maintenance of the conidia viability for long period in environmental storage conditions. Therefore, it is necessary to develop formulations and packages that will increase the conidia shelf life. The water activity (A_w) is an important parameter to study the stability during the storage of dehydrated products but direct A_w determination demands equipments not always available and the water sorption isotherms correlate the A_w with the moisture (X) of a product. Adsorption isotherm is important to define the packaging system and desorption isotherm can be a useful tool to study the drying process of BCA's. The objective of this work was to determine the sorption isotherms of *Beauveria bassiana* conidia and adjust them using a mathematical model. The adsorption and desorption were determinate by gravimetric technique at 25°C, and the data adjusted by the BET model. To construct the desorption isotherm, initially the conidia presented high moisture content (X = 98% in dry basis) and Aw (0,93). From X = 98% to 50% a low reduction in A_w was observed, because the water was in a free form. When X was around 50%, a small reduction on X resulted in a great depression in A_w, because the water was in a bound form. The adsorption curve showed that X varying from 9 to 50% a small increment in the conidia moisture resulted in a high increment in A_w. The BET model represented adequately the sorption isotherms of *Beauveria bassiana* conidia formulation and will be useful to specify the ideal moisture to storage the BCA and to define the product packaging system.

FP 34 Pathogenesis of Aphanocladium album on Leptopharsa heveae (Hemiptera: Tingidae)

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Aphanocladium album is a lace bug's pathogenic fungus that causes serious injuries to Brazil's rubber trees. A laboratory research was carried out to observe the fungus pathogenic action on Leptopharsa heveae. A conidia water suspension was prepared and left without handling for 2 hours under environmental temperature before the application over the insects, which were maintained in a incubator at 26 ± 0,5°C. Insects were fixed on glutaraldehyde, post-fixed on osmium tetroxide and prepared to be observed by a scanning electronic microscope at 3, 6, 9, 15, 24, 48, 60 and 72 hours after the fungus application. Adults insects were fixed at 3, 9 and 24hours. At the moment of fungi inoculation, it was observed that the conidia had absorbed water and presented a larger length and width, however, they had not emitted germ tubes. Three hours after application (AA), it was verified that the conidia adhered and germinated on nymph and adult's tegument. It was also observed micro extensions emitted by germ tubes, what increased fungus's adherence to the insect. Six hours AA, the majority part of germ tubes were three to four times the conidia's length, however, one of the insects presented already long hifas. Although apressorium's formation didn't occur, 9h AA symptoms of fungus penetration on the insect were evident. However in some bugs well-developed hifas took place in different insect body parts. At 15 h AA, it was observed the first hifas emerging from the insects. At 24 h AA, there were hifas on many insects being observed the beginning of sporulation. Fungus also developed and sporulated on bug's excrement. At sixty hours AA, there were nymphs completely covered by fungi and fixed to the leaf. By this time, there were some bugs that were not covered by the fungi yet, with the first hifas emerging by intersegmental regions. This research gives evidences to both the pathogenic and saprophytic action of this fungus.

Viruses

VP 22 How Cotton Foliage Impedes Lethal Infection of *Heliothis virescens* by *Ac*MNPV

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The success of a baculoviral infection is influenced by many factors, including the diet of the host. Previous work demonstrates that fourth instar *Heliothis virescens* (Noctuidae) fed cotton leaves are less susceptible to mortal infection by the *Autographa californica* M nucleopolyhedrovirus (*Ac*MNPV, Baculoviridae) than larvae fed iceberg lettuce or artificial diet. Ingested cotton foliage may prevent establishment of a successful infection by impeding direct and/or indirect infection processes. Cotton leaf chemicals may interact directly with occlusion bodies or occlusion-derived virus (ODV), debilitating the viral particles. Further, the integrity of

the peritrophic matrix may be altered, decreasing the number of viral particles that gain access to midgut epithelial cells. Finally, midgut cell surface receptors may be impaired such that they do not recognize ODV. In addition to interfering with direct infection of midgut epithelial cells, cotton leaf chemicals may lessen or prevent indirect infections by increasing midgut cell sloughing. Mediation of disease in larvae fed cotton foliage likely results from a combination of the above mechanisms. We hope to distinguish a primary cause of reduced viral efficacy by using a *lacZ*-expressing *Ac*MNPV construct to evaluate the ability of *Ac*MNPV to establish primary midgut infections in cotton-fed insects. We expect that cotton-fed *H. virescens* will display a reduced number of either midgut or tracheal infections, pointing to primarily direct or indirect host-plant effects on infection processes. **(STUDENT POSTER).**

VP 23 Biological relevance of intrastadial developmental resistance of *Lymantria dispar* to LdMNPV

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As diet-fed gypsy moths (*Lymantria dispar*) age within the fourth instar, they become increasingly resistant to mortal infection by *L. dispar* M nucleopolyhedrovirus (LdMNPV), a phenomenon we refer to as intrastadial developmental resistance. Interestingly, this form of resistance has a strong systemic component because it cannot be overcome by delivering the virus intrahemocoelically. By testing individual diet components, we found that the wheat germ component of the artificial diet was necessary to induce developmental resistance. In fourth instars, we found that intrastadial developmental resistance also occurs in insects fed on their natural host, oak foliage, whether they are confined to living trees or fed on detached foliage. Recently, we found that intrastadial developmental resistance occurs in third and fifth instars. Thus, intrastadial developmental resistance may play a role in the epizootic potential of LdMNPV.

VP 24 Isolation of new geographic variants of *Cydia pomonella* granulovirus (CpGV) from the Iran

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The *Cydia pomonella* granulovirus (CpGV) is a very effective biological control agent for the codling moth, *C. pomonella*. So far three different strains, originating from Mexico (CpGV-M), Russia (CpGV-R) and England (CpGV-E), have been described. In a survey of natural occurring CpGV in Iran, diapause larvae of the codling moth were trapped in cardboard bands on trees at different regions through the country and were checked for signs of infection. At least, 11 natural isolates from Northwest and Northeast of Iran were collected and surveyed. Light and electron microscopy studies confirmed the presence of granulovirus in codling moth populations of these regions. Viruses from single infected larvae or from pooled larvae were isolated and propagated in fourth instars of *C. pomonella*. After DNA isolation of the propagated viruses of 11 isolates restriction analyses were performed using EcoRI, XhoI, PstI. The restriction profiles were similar to the known CpGV profiles, but also showed significant variation: Two isolates shared similarity with CpGV-M restriction patterns, but one of them had at least a difference in the PstI profile. One isolate shared similarity with CpGV-R but showed at least six different fragments in PstI, EcoRI and XhoI profiles. Three other isolates showed unknown submolar bands in PstI and EcoRI profiles. It appeared that the analysed Iranian isolates show more restriction fragment polymorphism than the characterized M, R and E strains together. **(STUDENT POSTER).**

VP 25 Identification and analysis of two putative DNA-binding proteins from *Cf*MNPV

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The *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (*Cf*MNPV) is an ideal candidate as a bioinsecticide to control the eastern spruce budworm (*C. fumiferana*) due to its narrow host-range. We have completed sequencing of the *Cf*MNPV genome and through comparison with 11 sequenced NPVs and 4 sequenced Granuloviruses (GVs) we have identified 11 CfMNPV unique ORFs that have no baculovirus or other GenBank homologues. Since these 11 ORFs are found exclusively in *Cf*MNPV some may be involved in host specificity. Two of these unique ORFs encode polypeptides with putative DNA binding domains. The first, Cf-unique2, has an estimated molecular weight of 36.9K and contains a putative helix-loop-helix dimerization domain found in many transcription factors. The second, Cf-unique4, has an estimated molecular weight of 14.0K and contains a putative zinc-finger domain. Temporal transcriptional analysis has indicated that both Cf-unique2 and Cf-unique4 are transcribed within the first six hours of viral infection. We are currently investigating the transcriptional start sites of the corresponding mRNAs and the DNA-binding potential of the proteins. In addition we have begun to develop knockout viruses in an attempt to determine the role of the two proteins in the viral life cycle, particularly in viral gene transcription and DNA replication.

VP 26 Construction of a baculovirus expression system based on gp50 of CfDEFNPV

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The spindlin protein (GP50, GP37) of the *Choristoneura fumiferana* defective nucleopolyhedrovirus (CfDEFNPV) is synthesized in large amounts and forms bipyramidal

crystals in the cytoplasm of permissive cells in the late phase of replication. This indicates that the gene is driven by a powerful promoter and has the potential to be used for heterologous protein expression. We initiated a study to construct an expression system based on gp50 of CfDEFNPV. We had earlier ascertained that the gene was not essential for virus replication. In order to construct a negative screening expression system, we first produced a CfDEFNPV expressing LacZ under the control of the gp50 promoter and also introduced a unique restriction site AurI to produce linearized viral DNA (DefLacZ). This in effect increased the efficiency of generating recombinants. A transfer vector (pUCAlacZ3.2) with pUC18 backbone containing the Laz ORF flanked by 1 kb CfDEFNPV gp50 ORF upstream sequence and a 3.2 kb XhoI/HindIII fragment containing gp50 downstream sequence was constructed. DefLacZ was produced by cotransfecting SF21 cells with the plasmid pUCAlacZ3.2 and DefGFPXhoI viral DNA followed by plaque purification. To make a transfer vector which can be used with DefLacZ linearized DNA, a 1 kb fragment containing CfDEFNPV gp50 promoter and an upstream sequence was produced by PCR with a pair of primers containing EcoRI and BamHI sites and was cloned to the EcoRI/BamHI sites in pUC18 (pUC18A). A 1 kb PCR fragment of LacZ gene 5' sequence with restriction sites of BamHI and HindIII was cloned into the BamHI/HindIII sites of pUC18A (pUC18AB). A polylinker from pBlueBac4.5 vector was retrieved by digestion with BamHI /SnabI and cloned into the BamHI/AurII (AurII was blunted with Klenow) to generate transfer vector pUC18ABMCS. Discussion on the gp50 promoter activity in comparison to the AcMNPV polyhedrin and p10 promoters will be discussed.

VP 27 Genetic stability and virulence of *Anticarsia gemmatalis* nucleopolyhedrovirus used as bioinsecticide in Brazil

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The *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) has been used as a viral pesticide, since early eighties, against the velvetbean caterpillar on soybean crops in Brazil. Genetic stability studies of this virus were carried out by comparison of the DNA profiles of the seasonal isolates using restriction endonuclease analysis (REN). These isolates were obtained from infected larvae collected in the region of Londrina-PR during successive crop seasons (years from 1984 to 1997). Comparison was also done in regard to the AgMNPV-79, a wild type virus that was used originally and subsequently in this program. For this purpose viral occlusion bodies were obtained from dead larvae and purified on sucrose gradients by ultracentrifugation. The viral DNA was extracted and then cleaved with restriction enzymes. The DNA profiles were analyzed by agarose gel electrophoresis after staining by ethidium bromide. They showed to be similar to the profiles described for AgMNPV-2D (Jonhson & Maruniak, J. Gen. Virol. 1989, 70:1877-1883) which is a viral clone plaque purified from the AgMNPV-79. Digestions with *Bst*EII and *Bgl*II generated the same number and size of DNA fragments among the seasonal isolates. There was also a great similarity on the DNA profiles of the isolates after cleavage by *Pst*I, *Hin*dIII, *Eco*RI and *Bam*HI. However, some small differences were detected. In special it was noticed that once a submolar band was present in a viral population in a certain year it became persistent during the following years. The variation observed among the seasonal isolates is to be expected since this program was started with a wild type isolate composed by a genotipicaly heterologous population (AgMNPV-79). Besides, the possibility that other viral variants could be brought to the collecting area by migrating insects should not be ruled out. In parallel, to investigate whether the analyzed viral isolates presented variations on their pathogenicity we determined the median lethal concentration (LC_{50}) for each isolate using six different viral concentrations. The results indicated that the efficacy of the bioinsecticide has been maintained through out the years, although slight differences on the isolates virulence have been observed.

VP 28 Molecular characterization and sequence analysis of the *Anticarsia gemmatalis* Multicapsid Nuclear Polyhedrosis Virus (AgMNPV) glycoprotein GP64

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Baculoviruses are enveloped viruses that contain large circular double-stranded DNA genomes ranging in size from 80 to 180 kbp. Transcription, DNA replication, and nucleocapsid assembly occur within the nuclei of infected host cells. Baculoviruses are characterized by an infection cycle that produces two virion phenotypes, budded virus (BV) and occlusion-derived virus (ODV), which are structurally and functionally distinct. BV is highly infectious in cell culture and mediates cell-to-cell transmission in the infected animal. The BV envelopes of Group I baculoviruses contain an abundant viral-encoded glycoprotein, GP64, which is not found in the ODV envelope. This protein is the responsible to mediate the viral entry into the cell via an endocytic pathway. In the same way, the baculoviruses are capable to efficiently entry into the mammalian cells and to direct its nucleocapsids to the nucleus, although there are not infectious for mammalian because there have not DNA replication. For these reasons, the baculoviruses constitute an interesting vehicle for efficient gene-transfer to mammalian cells. In this work, we show the identification, genomic location, cloning and sequencing of the AgMNPV *gp64* locus. The plasmid clones were functionally tested to assess the presence of a fusogenic activity similar to that expected for GP64 protein. In addition, the complete sequence of the *gp64* gene was obtained and comparative analyzed with the same *locus* of other baculoviruses using bioinformatics tools. These constitute the initial steps in the development of new vectors for mammalian cells transduction.

VP 29 A transfer vector for the generation of recombinant AgMNPVs Containing large insertions of foreign DNA

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We have recently developed a system for the genetic modification of *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) using a conventional transfer vector and taking advantage of homologous recombination within the polyhedrin locus. Here, we report the construction of a novel vector based on the positive selection supplied by the *ccd*B gene, toxic for the gyrase. The multiple cloning site is placed within the *ccd*B ORF and facilitates the cloning of large DNA inserts by supressing the background of bacteria bearing non recombinant plasmids. The recombinant plasmid contains the AgMNPV *polh* ORF under the control of its own promoter and a large insert of foreign DNA. The system was used to generate a library of *Eco*RI fragments encompassing the whole genome of *Epinotia aporema* granulovirus (EpapGV). It might prove useful for exploring host range determinants using permissive and non-permissive hosts for AgMNPV and EpapGV, respectively. After cotransfection with parental AgMNPV DNA, a series of rAgMNPVs will arise containing different segments of the EpapGV genome, some of which might eventually contain genes critical for the infection of *E. aporema*. Bearing in mind that the large block of inserted genes is under the control of their natural promoters, we set out to evaluate in parallel if EpapGV genes could be transactivated by AgMNPV transcription factors. To this end we used a limited set of immediate early, late and very late gene promoters followed by *E. coli lac Z* as reporter gene.

VP 30 Identification and sequence analysis of a putative EpapGV envolope fusion protein

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Epinotia aporema granulovirus (EpapGV) has a demonstated potential for the control of *E. aporema* Wals. (Lep. Tortricidae), a major pest of legume crops in South America. Previous studies revealed that EpapGV causes a polyorganotropic infection in its host. Analyses of temporal events of the disease in infected cells and tissues, showed that the nucleocapsids budded from membrane-bound structures might be involved in cell to cell spread of the infection. However, the mechanisms of budding and virus entry remain unexplored at the molecular level. As a first step in this direction, we cloned and sequenced a *ld130* homologue of EpapGV. The LD130 family comprises envelope proteins that proved to be critical for the spread of several Group II NPVs via low pH-induced fusion. However, the role of these proteins in GVs remains to be assessed. The EpapGV *ld130* homologue encodes a 523 amino acid long polypeptide, with a significant degree of homology relative to other LD130 family members (*i.e.* 64-54 % compared with GVs, and 45-43 %, with NPVs). In addition to the predicted signal peptide at the amino terminus and occurrence of a carboxy-terminal transmembrane domain, other conserved features were identified, including cysteine residue positions and a consensus cleavage site (K/RXXK/R) followed by coiled-coil-like domains. The transcriptional analysis of the gene was done by Northern blot and the mRNA initiation and termination sites were assessed by 5´and 3´ RACE (rapid amplification of cDNA ends). The sequence analysis of the major RT PCR band mapped the initiation to the first A in the TTAAG late promoter element. Analyses of secondary RT PCR bands are underway to explore alternative transcription start sites.

VP 31 A quick and simple method for the analysis of putative recombinants during plaque purification

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Amplification of specific segments of DNA by PCR can be extremely useful, both for identifying putative recombinants and for confirming that selected recombinants have the appropriate genome structure. The judicious choice of primers can enable the researcher to differentiate the desired recombinant virus from single-crossover recombinants and/or contaminating parental virus. However, in previous work, PCR amplification of viral DNA extracted directly from a plaque has not been considered efficient enough to permit the routine use of this approach. Instead, it has been stated that it is necessary to scale up the plaque pick by infection of cultured cells. In the present study, an easily reproducible method has been adapted for a rapid and highly sensitive analysis of DNA extracted directly from plaques, which greatly simplifies the inherently tedious task of screening for recombinants. The method consists in treating an aliquot of the resuspended plaque with a lysis buffer (10 mM Tris-Cl pH 7,6; 10 mM EDTA, 0.25% SDS), extracting the lysate with chloroform and precipitating the DNA with ethanol. The DNA pellet, redissolved in water, is suitable for PCR amplification. This method has been tested on wt and recombinant AgMNPV plaques formed on UFLAG-286 monolayers. The possibility of directly screening plaques by PCR represents a sensible reduction in time and cost involved in selecting recombinants, similar but more significant than that involved in the direct screening of recombinant *E. coli* colonies, since time lapses when working with insect cell cultures are inherently greater than those for bacteria.

VP 32 Identification and molecular characterization of *Epinotia aporema* granulovirus helicase genes

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Epinotia aporema (Lep. Tortricidae) is a major pest of legume crops in South America. A recently isolated granulovirus (EpapGV) was characterized in terms of its main biological and biochemical properties, indicating that it is a good candidate for the biological control of

this species. In order to contribute to the understanding of EpapGV virus-host interactions, diverse aspects of its molecular biology are being studied. In general, the genetic determinants and the molecular mechanisms related to baculovirus pathobiology and host range specificity, are still unknown. In this regard, baculovirus DNA helicases are thought to play an essential role for virus replication, and might be also involved in virus host range specificity. The present study includes the mapping of two genomic fragments (EcoRI-C and D) from the EpapGV DNA library, which were subcloned and partially sequenced. Two helicase genes (helicase I and II) and their flanking regions were identified within those fragments and characterized. Preliminary data indicate that EpapGV helicase I gene is 3.4 kb long and is more closely related to CpGV helicase I. EpapGV helicase II gene is 1.4 kb long and its product has 55% amino acid identity (71% homology) with the most closely related baculoviral helicase II, *i.e.* XcGV. In addition to this, we found one AAA motif in the EpapGV helicase II, which is also present in the CpGV homologue. The UvrD/Rep helicase motifs identified in XcGV and PxGV were not found. The AAA-ATPases are associated with a variety of cellular activities including helicases, whereas the UvrD/Rep helicases catalyze ATP-dependent unwinding of double stranded DNA. The upstream untranslated region of the helicase genes exhibits several consensus early promoter elements. The sequences were used in phylogenetic studies to assess relatedness of EpapGV with other members of the *Baculoviridae*.

VP 33 Localization and sequence analysis of an inhibitor of apoptosis gene in the EpapGV genome

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A partial gene organization of *Epinotia aporema* granulovirius (EpapGV) genome has been reported recently. Southern hybridization experiments were done in search for homologues of the *iap* genes in an EpapGV genomic library. A clone positive for a CpGV *iap-3* probe was selected and sequenced. An open reading frame corresponding to a 256 amino acid long protein. This polypeptide showed a high degree of similarity with the IAP-3 gene family, and contains one RING finger and two BIR motifs, characteristic for IAP proteins. The phylogenetic history of this IAP-3 and its relatives of the IAP family was analyzed. The EpapGV IAP-3 clusters with the rest of the IAP-3 polypeptides, clearly separate form the insect IAPs. In addition, we analyzed and compared the gene order in the EpapGV IAP-3 region with that of CpGV genome. The position of IAP-3 ORF could be derived from a common ancestor genome that suffered an invertion of a 22 kb cassette and a subsequent translocation.

VP 34 Comparison of DNA sequences of polyhedrin gene of the Latvian isolates of *Malacosoma neustria* nucleopolyhedrovirus

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Nucleopolyhedrovirus (NPV) isolated from different *Malacosoma neustria* populations were investigated in the Institute of Biology, University of Latvia. A study was conducted to assess phenotypic and genetic variation within and among populations of *M. neustria* NPV. Previous studies of intraspecific variation in Mn NPV populations have examined differences in size of polyhedra, size of nucleocapsids, target cells and tissues, virulence and host range. Our purpose was to develop molecular method for differentiation among NPV isolates and compare Mn NPV isolates obtained from the *M. neustria* larvae collected in different localities. We used sensitive technique of DNA amplification by the PCR using specific primers for Mn NPV DNA detection and following sequence analysis of specific DNA fragments. 987 bp DNA fragments, that include coding region of polyhedrin gene, were cloned into pUC57T vector. Sequence analysis of the polyhedrin gene fragment of Mn NPV (Saldus) isolate (AJ277555) shows that polyhedrin gene coding region is located between positions 127 to 867. Translated protein - Polyhedrine is 246 amino acids in length. Comparison of DNA sequences of Latvian isolates revealed a number of nucleotide exchanges. There were 5 exchanges in the coding region of polyhedrin gene, but only one of them leads to amino acid exchange, leucine was substituted with phenylalanine. Obtained results demonstrate that characterised Latvian isolates have a high DNA homology -99.3%.

VP 35 Further Results with Optical Brighteners as Enhancers to *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) Nucleopolyhedrovirus Activity

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Optical brighteners (OPs) are used in detergents, dyes, plastics, paper industries and as a fluorochrome for microorganisms. They have also been shown to enhance the biological activity of insect viruses. The objective of our study was to further evaluate the effect of *A. gemmatalis* nuclear polyhedrosis virus (AgMNPV) in combination with OPs against the insect larvae under laboratory and greenhouse conditions. Studies were also conducted to determine the effect of brighteners on AgMNPV activity against *Spodoptera frugiperda* and *Rachiplusia nu*, noctuids associated to soybean but that are not infected by AgMNPV concentrations aimed at *A. gemmatalis* control. In the laboratory, four OPs (Tinopal UNPA-GX, Tinopal DMS, BRY 10 D2 100 and Leukophor DUB) were employed and the AgMNPV was serially diluted in OP suspension (0.5 % wt:vol), or in distilled water. Virus concentrations were distributed on the surface of an artificial diet to feed 4th-instar larvae. Under greenhouse conditions, the AgMNPV doses (3.0×10^{10} OBs/ha and 1.5×10^{11} OBs/ha) were diluted in distilled water or in Tinopal DMS suspensions at 0.1 % and 1.0 % (wt:vol). The suspensions were applied to 50-day-old soybean plants and the leaves were offered to 2nd-instar larvae. The addition of Tinopal UNPA-GX to AgMNPV reduced the virus mean lethal concentration (LC_{50}) from 54,505.9 OBs/ml to 153.1 OBs/ml for 4th instar larvae, i.e. an activity ratio of 356.0 related to the virus alone. The activity ratios of other OPs + virus were 56.4, 22.3 and 5.1, for Tinopal DMS, BRY 10 D2 100 and Leukophor DUB, respectively. Mortality was negligible in the controls (untreated or treated only with

each OP). When Tinopal DMS 0.1 % + AgMNPV was applied to soybean plants at 3.0×10^{10} OBs/ha (ca. $^1/_5$ of the recommended dosage), *A. gemmatalis* larval mortality was enhanced from 16.5 % to 95.0 %. Four days after treatments, collected leaves from plants with AgMNPV alone caused 1.3 % of virus-mortality, nevertheless, when larvae were fed on leaves with AgMNPV+Tinopal 0.1 % or 1.0 %, larval mortalities were 31.7 % and 68.4 % respectively. The addition of 0.5 % OP also enhanced AgMNPV activity on alternative hosts. The mortality of *R. nu* by AgMNPV alone at 1.0×10^5 OBs/ml was 17.1 % and 86.4 % by virus + UNPA-GX. In the same concentration the AgMNPV did not cause *S. frugiperda* mortality even when associated with the OP. However, at 1.0×10^7 OBs/ml, mortality was 3.8 % by the virus alone, and 87.1 % by the virus + OP.

VP 36 Characterization of the ecdysteroid UDP-glucosyltransferase (*egt*) gene of *Spodoptera frugiperda* nucleopolyhedrovirus

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The baculovirus *egt* gene encodes UDP-glucosyltransferase, a protein that interferes with the hormonal process that control insect development. We have identified and characterized the *egt* gene from *Spodoptera frugiperda* nucleopolyhedrovirus. The ORF of the gene encodes a putative polypeptide of 525 aminoacids. A genomic region including the ORF and flanking regions were cloned into a plasmid. Transient expression assay using this plasmid showed that this novel *egt* gene has glucosyltransferase activity. Promoter sequences typical of baculovirus early genes were found in the 5'region of the SfMNPV *egt* gene. A polyadenylation signal was identified downstream the translation stop codon. The sequence of the *egt* gene from SfMNPV and the sequences available at Genbank from the *egt* genes of other baculoviruses were used in the construction of a phylogenetic tree. * This research was supported by a grant from FAPESP

VP 37 A recombinant *Cydia pomonella* granulovirus expressing green fluorescent protein

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The sequencing of several granuloviruses (GVs) has revealed that ORFs of GVs have homologues in the genomes of nucleopolyhedroviruses (NPVs) that have been published. In addition to these, GV specific ORFs have been identified, as well as species specific ORFs and the function of these is mostly unknown. A cell culture system is essential to study the transcription and function of these ORFs. Currently, the green fluorescent protein gene (*gfp*) from *Aequoria victoria* is being used to optimise the production of recombinant CpGVs, to study gene function and for infection studies. The *gfp* gene was placed behind the *Drosophila* heat shock promoter. Fluorescent and confocal microscopy was used to study transfection and infection in established GV permissive cell cultures. Transfection efficiencies as high as 20% were observed in CpGV infected *Cydia pomonella* cells. The course of infection of recombinant CpGV-hspGFP inoculated tortricid larvae was visualised using fluorescent microscopy. Recombinant viruses expressing GFP behind other promoters and to knock out genes will be invaluable in understanding the expression and function of GV proteins.

VP 38 Susceptibility of chilean target pests to entomopathogenic fungi

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Since 1996, INIA CRI Quilamapu started an Insect Pathology Program by collecting entomopathogenic organisms along Chile from soil samples and through the *Galleria* larvae bait technique. Among the entomopathogenic fungi the most frequent ones were *Metarhizium* spp. and *Beauveria* spp. Afterward 27 *Beauveria* and 26 *Metarhizium* isolates were screened in fifteen important insect pests of Central and South zones of Chile. Inoculated insects were evaluated daily and compared using a mortality index. Different pathogenicity levels among the isolates were detected, and the most effective ones were those obtained from naturally infected hosts. There were also strains that caused low or non-insect mortality or were not pathogenic at all, showing the specificity of the different isolates. Thus, this work warrants future surveys new and better isolates, as well as field evaluations, in order to provide appropriated isolates for different weather and soil conditions in Chile.

VP 39 Identification of a *Bombyx mori* multiple nucleopolyhedrovirus isolate (BmMNPV) in Parana State, Brazil

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Sericulture is an important agricultural activity in Brazil; 97% of the national production is allocated for exportation and Parana State contributes with 83.59% to the national production. Silk production in Brazil, however, is low when compared to that of other countries, a problem mainly due to the occurrence of silkworm diseases. Among these, the viral diseases have an important position. Healthy and diseased silkworm larvae were obtained from silk industries and silk farms in the Northern, Western and Southern regions of Parana State. These larvae, as well as larvae inoculated experimentally, were initially reared and maintained under laboratory conditions in order to observe the development of the symptoms and identify the causal agent of the disease. Analysis on optical and transmission electron microscopy (TEM) were also carried out to determine the taxonomy of the pathogen infecting *Bombyx mori* larvae. The symptoms observed are the

same those described in the literature as typical of the grasserie (jaundice) disease, caused by *Nucleopolyhedrovirus*. The ultrastructural analysis, which showed viral replication in the nuclei of target cells and the typical cytopathic effects, support the conclusion that the infection agent is a *Nucleopolyhedrovirus*, a genus of the Baculoviridae family. The occurrence of more than one rod-shaped nucleocapsid per envelope is diagnostic for the Multiple *Nucleopolyhedrovirus* (MNPV), a highly virulent phenotype. Nucleocapsids average 95 nm in diameter and 315 nm in length. These enveloped nucleocapsids or virions are embedded in a crystalline protein lattice called polyhedrin which forms the occlusion body or polyhedron. Polyhedra measure 2.6 to 4.10 µm in size, mature within nuclei of infected cells and contain many virions.

VP 40 Enhancement of *Agrotis ipsilon* nucleopolyhedrovirus activity by an optical brightener and potential for control of the black cutworm.

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The baculovirus, *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV) is highly active against third instar black cutworms *Agrotis ipsilon*. The optical brightener M2R significantly reduced LD50 estimates by at least 75-fold, but had no direct effect on ST50 estimates. AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon* in greenhouse and field studies, although the addition of M2R produced only marginal improvements in virus performance at the virus application rates used in this study. In an appropriately designed pest management program it seems likely that AgipMNPV could be used to suppress populations of early and mid instar *A. ipsilon*, although slow speed of kill and high production costs continue to make it difficult for baculoviruses such as AgipMNPV to compete with chemical insecticides. Nevertheless, the soaring cost of developing new chemical insecticides, coupled with increasing public concern about pesticide residues on food, may lead to greater interest in using baculoviruses for pest management.

VP 41 Improvement of the pathogenicity of the *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) by inactivating the *egt* gene

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All baculoviruses sequenced to date were shown to contain a gene called ecdysteroid glycosyl transferase (*egt*) that encodes for the EGT enzyme. These proteins belong to one group of enzymes relative to the mammalian UDP-glucuronosyl-transferases. The EGT enzyme of baculoviruses is responsible for the inactivation of the ecdysteroid hormones of the infected insect host. As a result, baculovirus-infected larvae do not molt but continue to feed, leading to enhanced weight gain after infection and increased viral progeny production . Some baculovirus have been genetically modified for the inactivation of their *egt* gene, and these viruses were shown to kill more rapidly the infected larvae, when compared to the wild-type virus. We have previously identified, cloned and sequenced the *egt* gene of *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) and now we present the data regarding the construction of an *egt*- AgMNPV virus. We have inserted the *hsp70-lacZ* (3,7 Kb) gene cassette from the plasmid pAcDZ1 into the *Bam* HI site present in the middle of the *egt* ORF in the plasmid ⌜pHHV. This plasmid was used, together with wild-type AgMNPV DNA, in a co-transfection of UFL-AG-286 cells. After 4 rounds of end-point dilution, we purified a recombinant AgMNPV containing the *egt* ORF interrupted with the *hsp70-lacZ* cassette. The purification was monitored by PCR-amplification of the *egt* gene region and the lack of the *LacZ* activity in the presence of X-gal. Polyhedral inclusion bodies (PIBs) of the *egt*- AgMNPV were used in bioassays for determination of the LC_{50} and mean time to death. The bioassays showed that PIB production were consistently lower for the *egt*-modified virus (mean 46.9 x 10^8 PIBs/g of larvae and 20.2 x 10^8 PIBs/g of larvae for the AgMNPV and *egt*- AgMNPV, respectively. The LC_{50} from 7th day after treatment for the wild type virus were 82,296 PIBs/mL of diet compared to 5,677 PIBs/mL for the modified virus. The mean time to death (MTD) of infected *A. gemmatalis* larvae was calculated and, on the average, MTD was 9.2 days and 8.0 days for the wild type and *egt*-modified virus, respectively.

VP 42 Identification, location and partial sequencing of an *Anticarsia gemmatalis* nucleopolyhedrovirus DNA polymerase gene

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The *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) has been widely employed as a bioinsecticide for the control of the velvetbean caterpillar *A. gemmatalis*, the major soybean pest in Brazil. However, very little is known about its genome structure and replication. The DNA polymerase protein plays an important role in determining both the level of genomic replication and host specificity of DNA viruses. In order to understand more about the AgMNPV genome replication, the DNA polymerase gene (*dnapol*) of AgMNPV was identified, located, and partially sequenced. The location of this gene was determined by PCR, using primers made from conserved regions of *dnapol* genes from different baculoviruses and Southern-blot hybridization using AgMNPV DNA restriction fragments and a probe prepared from the previously identified polymerase gene from the *Autographa californica* nucleopolyhedrovirus (AcMNPV). We have amplified, by PCR, a DNA fragment from the AgMNPV genome (*Hin*dIII-Q fragment), corresponding to the putative DNA polymerase gene. To more precisely locate the DNA polymerase gene, the ends of the *Hin*dIII-Q fragment from an AgMNPV genomic library were sequenced in both directions. An 1.4 kbp region was sequenced and computer analyzed, revealing a high homology to *Orgyia pseudotsugata* nucleopolyhedrovirus

(OpMNPV) and *Choristoneura fumiferana* nucleopolyhedrovirus (CfMNPV) DNA polymerase genes. Presently, the AgMNPV fragments *Hind*III-U and -V, and additional overlapping regions are being sequenced. Studies are in progress to determine the complete sequence and transcriptional analysis of AgMNPV *dnapol* gene.**(STUDENT POSTER).**

VP 43 Sequence and transcription analysis of the *Anticarsia gemmatalis* MNPV *p74 locus*

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Anticarsia gemmatalis multiple nuclear polhiedrovirus (AgMNPV) is an insect viral pathogen of the baculovirus family. *Anticarsia gemmatalis*, its hostage, is a big pest on the soybean crops in South America, so that AgMNPV has begun to use as biological control agent. AgMNPV and all of sequenced baculoviruses have the *p74* gene, coding an important protein of the occluded virus, needed to infection. In the regulatory pathway of gene expression, *p74* belong to the late gene group, and the coded product appear after the replication of viral genome. The P74 protein is only detected in the occluded virus and P74 minus mutants are not infective in a *per os* route. In order to increase the knowledge about P74, we have identified a specific genome segment of two different AgMNPV isolates, corresponding to Argentina and Brazil. In addition to the cloning, sequencing and molecular characterization (mRNAs ends determination, time course of transcription), we have comparative analyzed all known P74 sequences and predicted secondary structures using bioinformatic tools. The upstream sequence analyses show the existence of tandem repeats with units of 127 bp, and the typical late motif, TAAG. This sequence region is different in the two studied virus, finding deletions or insertions in the tandem repeat zone. With the goal to characterize the promoter in *p74* gene of AgMNPV (Brazil, Argentina). In order to test the promoter properties of the *p74* upstream region we generated different constructs carrying this sequence and reporter genes.

Bacteria

BP 18 Activity of chimeric Cry toxins against *Helicoverpa armigera*

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Helicoverpa armigera is a major agricultural pest in Australasia. It has proven adept at developing resistance to all the synthetic chemical insecticides used against it, severely limiting the range of options available for its control. Of the insecticidal δ-endotoxins (Cry proteins) of *Bacillus thuringiensis* (Bt), the most effective against *H. armigera* is Cry1Ac. However with the advent of transgenic cotton expressing Cry1Ac, there is a greatly increased risk of *H. armigera* developing resistance to Cry1Ac due to increased selection pressure. In our laboratory we have selected a line of Cry1Ac-resistant *H. armigera*, demonstrating that genes for resistance already exist in wild populations. There is therefore an urgent need for new insecticides to control *H. armigera*. We are taking several approaches to identifying new insecticidal proteins that can be used for this purpose, and one of these is to test a range of modified Cry toxins constructed by domain swapping. Cry toxins are produced in the form of a protoxin that is cleaved by proteases in the insect gut to produce the active form. The activated toxin has a three-domain structure in which domain I is recognised as the domain responsible for pore formation, while domains II and III are involved in receptor recognition and binding. By interchanging the individual domains from different members of the Cry protein family chimeric proteins with altered specificity or toxicity can be produced. We have tested a range of chimeric proteins by bioassay to measure their toxicity towards *H. armigera*.

BP 19 *Ex vivo* toxic potency of the *Bacillus thuringiensis* Cry4B protein on isolated midguts of *Aedes aegypti* larvae

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The pathological effect of the *Bacillus thuringiensis* Cry toxins on susceptible insects has been shown to be an extensive damage of the larval midgut epithelial cells. In our studies, an *ex vivo* assay has been devised for assessing insecticidal potency of the Cry4B mosquito-larvicidal protein. The assessment of toxicity was carried out *via* a cell viability assay on midguts dissected from 5-day old *Aedes aegypti* larvae. The assay is based on quantitative determination of the cellular conversion of tetrazolium compound that is directly proportional to the number of living cells. After incubation with the tested proteins, the number of viable epithelial cells was determined by monitoring the quantity of the soluble bioreduced formazan product at 490nm. The 3-hour kinetics reaction curve was analysed for the potency parameter. The results revealed that the 65-kDa trypsin-activated Cry4B toxin exhibited toxic potency 3.5 times higher than the 130-kDa Cry4B protoxin. However, the non-bioactive mutant (R158A) and the lepidopteran-active Cry1Aa toxin displayed relatively no *ex vivo* activity on the mosquito-larval midguts.

BP 20 Bioassay of *Bacillus thuringiensis* toxins against two major coffee pests, i.e. coffee berry borer (*Hypothenemus hampei*) and coffee white stem-borer (*Xylotrechus quadripes*)

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H. hampei and *X. quadripes* are both endocarpic coleopteran insects difficult to control by conventional means. The transgenic approach may represent an efficient complement to traditional pest control strategies. Trangenic coffee plants would be adapted to the control of borers since the insecticidal toxin produced by the plant would be present within the tissues on which the pest feeds. Bioassay procedures based on artificial diet were developped on *H. hampei* and *X. quadripes* for estimating the insecticidal activity of *B. thuringiensis* which is the most important source of genes for the development of pest-resistant transgenic plants. Artificial diets for *H. hampei* and *X. quadripes* have been standardized. Bioassays were conducted with 2nd instar larvae for *H. hampei* and with neonates for *X. quadripes*. Mortality was counted one week after the *B. thuringiensis* spores-crystals applications. Several *B. thuringiensis* isolates potentially active against coleopteran were tested for toxicity against both targeted pests.

BP 21 Comparison of the expression of Bacillus thuringiensis full-length and N-truncated vip3A gene in Escherichia coli

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The Vip3A protein toxic against Lepidopteran insect larvae is one of Bacillus thuringiensis vegetative insecticidal proteins. The full-length vip3A-S184 gene was cloned from a soil-isolated B. thuringiensis, and the vip3A□N was constructed by deleting 81 nucleotides at the 5'-terminus of vip3A-S184. Both were transformed and expressed in Escherichia coli. The Vip3A-S184 was not N-terminally processed when expressed in E. coli, which was the same as in B. thuringiensis. About 19.2% of Vip3A-S184 proteins were secreted soluble proteins and others formed the inclusion bodies in the periplasmic space. On the contrary, the Vip3A□N was insoluble and formed inclusion bodies in the cytoplasm, indicating that the deleted N-terminal sequences were essential for the secretion of Vip3A protein in E. coli. On the other hand, the Vip3A-S184 showed different toxicity against 4 tested pests, Spodoptera exigua, S. litura, Argyrogramma agnate and Heliocoverpa zea, but the Vip3A□N showed no toxicity to any of them due to the deletion of the first 27 amino acids at N-terminus. This suggested that the N-terminal 27 amino acids might also be required for toxicity.

BP 22 Characterization of strains of *Bacillus thuringiensis* effective against bolweevil *Anthonomus grandis* Boema, 1843

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Bacillus thuringiensis is a soil bacterium, gram-positive, anaerobic facultative and has the ability to produce protein crystalline inclusions, named δ-endotoxinas or Cry proteins. The crystal protein of *B. thuringiensis* doesn't have toxicity, itself. Considered therefore, a protoxin. It's dissolution under the alkaline environment of the larval midgut produces toxins of different sizes which are toxic to some insects, in that way the dissolution is crucial for the expression of toxicity, this could explain why only insects with alkaline midgut pH are affected. A strong candidate to be controlled by *B. thuringiensis* is Bolweevil (*Anthonomus grandis* Boema). This coleopteran is very difficult to be controlled due to the fact that it spends its whole larval life inside flower buds so they are protected from the action of insecticides. Embrapa Genetic Researches and Biotechnology keeps a collection of B. *thuringiensis*. The objective of this work was the identification and characterization of Bt strains, which show toxicity against Bolweevil. Dosage tests were realized with 12 strains, from witch the ones showing the lowest CL 50 were selected. In order to obtain the protein profiles SDS–Page was used and to identify cry genes, PCR was used. The crystals where photographed by electronic microscopy to visualize their crystallography. Besides protein dosage was done. The protein and molecular profiles showed difference when compared to Btt (*Bacillus thuringiensis tenebrionis*), suggesting that there are different proteins pathogenic to coleopteran.

BP 23 Evaluation and characterization of *Bacillus thuringiensis* for *Culex quinquefasciatus* and *Aedes aegypti* larvae control.

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Aedes aegypti and *Culex quinquefasciatus* are antropofilic mosquitoes and vectors of many diseases like: filariosis, dengue and yellow fever. In urban environment, they may find a lot of disposable places to bread, like natural or artificial wastes of water with organic material under decomposition, when *Culex quinquefasciatus* or clean water, when *Aedes aegypti*. For controlling of theses mosquitoes, the biological control is more indicated because it has low toxicity and high specificity. Embrapa Genetic researches and Biotechnology keeps a collection of Bacillus *thuringiensis* (Bt) from different regions of Brazil. The objective of this work was the identification of Brazilian Bts which produce protein crystalline inclusions that are different from the currently identified as pathogenic to mosquitoes. This work aims to offer new active ingredients based on Bt toxin with high efficiency and specificity. First selective tests were made with 210 Bts. From this total, 11 showed mortality bigger than 50% against *Aedes aegypti*, 7 were efficient against *Culex quinquefasciatus* e 3 were efficient against both. From these 21 Bts certainly pathogenic 11 were selected for their lower CL 50. These were submitted to dosage tests and characterized for their protein composition and the presence of genes *cry* 4, *cry* 11 e *cyt*, which codify the active toxins against mosquitoes. As a pattern control BTi H-14 (Bacillus *thuringiensis israelensis)* from Pasteur Institute was used. Many of the samples showed different proteins and molecular profiles suggesting that there are toxins not yet described but effective against mosquitoes.

BP 24 How vast is the *Bacillus thuringiensis* toxin arsenal to control the geranium bronze?

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Proteins in the parasporal crystal (Cry proteins) from *Bacillus thuringiensis* are the active ingredient of the environmentally clean insecticides based on this bacterium. Despite the fact that around 200 *cry* genes have already been cloned, only a handful of these Cry proteins are toxic against a given lepidopteran pest. A crucial step in the mode of action of Cry proteins is their binding to specific sites in the midgut of susceptible insects. Binding studies in insects that have developed cross-resistance indicate that, to preserve the long-term use of *B. thuringiensis*-based insecticides, combination of Cry proteins sharing the same binding site is discouraged. If resistance management strategies are to be implemented, the arsenal of Cry proteins suitable to control a given pest may be not so vast as it might seem at first. The present study evaluates the potential of *B. thuringiensis* for the control of a new pest, the geranium bronze (*Cacyreus marshalli*), a butterfly that is threatening the popularity of geraniums in Spain. Eleven of the most common Cry proteins from the three lepidopteran-active Cry families (Cry1, Cry2, and Cry9) have been tested with the geranium bronze for their toxicity and binding-site relationships. Using ^{125}I-labeled Cry1Ab and ^{125}I-labeled Cry1Ac with brush border membrane vesicles of the geranium bronze larvae we found that, of the 7 most active Cry proteins, 6 of them competed for binding to the same binding site. The results indicate that for the long-term control of the geranium bronze with *B. thuringiensis*-based insecticides it would be wise to combine any of the Cry proteins sharing the binding site (preferably Cry1Ab since it is the most toxic) with those not competing for the same site. Of these latter, Cry1Ba would be the best choice, since it is significantly more toxic than other Cry proteins not binding to the common site.

BP 25 Four aminopeptidase N loci are involved as Cry1A receptors in Lepidoptera

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Aminopeptidases catalyse the cleavage of amino terminus of many proteins with different porpoises. Although, their biological functions, these types of enzymes have recently brought the attention in Lepidoptera because of their involvement in the binding of *Bacillus thuringiensis* insecticidal crystal proteins. As much as forty-four aminopeptidases N (APN) protein sequences have been reported in lepidopteran species. The main part of the sequences have been obtained from cDNA clones and, in some of them for a single species, although not all of them have been obtained from a single population. Then, the variety of reported sequences can be attributed to a high amount of genomic loci or a reduced number of loci with populational and splicing variations. Four groups of APN have been deduced from the phylogenetic analysis based on similarity of the sequences. There are sequences identified as Cry1A receptors in all groups. The analysis of genomic DNA of insects from a single population of *Plutella xylostella*, using PCR partial amplification using primers based on described sequences, confirmed the presence the four sequences of APN. Results evidenced that each kind of sequence is codified by a specific genomic locus. The occurrence of four APN loci involved in Cry1A binding in Lepidoptera does not imply the involvement all of them in binding, or in toxicity, for all species. Present data suggest the need for more detailed APN analyses to determine their influence in resistant colonies.

BP 26 *Bacillus thuringiensis* Survey in Brazil: Geographical Distribution and Insecticidal Activity Against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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3408 strains of *Bacillus thuringiensis* were isolated from a total of 1448 soil samples from 10 Brazilian States, including 4 different geographical regions, covering about 80 visited mircroregions. From this total, 1758 strains showed 0% of mortality and only 62 strains showed mortality between 81 and 100% against *Spodoptera frugiperda*. Regarding to the number of efficient strains (larval mortality above 75%) per Region were: 2 in the Northeast (Alagoas State =1 and Ceara State=1), South Region (Parana State=4), Southeast Region (Minas Gerais State=12 and Sao Paulo=1) and Central East Region (Goias State=32 and Mato Grosso do Sul=2). The South Region showed the highest proportional amount of efficiente strains (16.6%), followed by West Central (3.1%), Southeast (1.1%) and Northeast (0.4%).

BP 27 Identification and analysis of *Bacillus thuringiensis* virulence genes

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The entomopathogenic bacterium *Bacillus thuringiensis* possesses virulence factors that aid its colonisation and destruction of susceptible insects. Among these, the delta endotoxins comprise a major class but others, including factors expressed in vegetative *Bt* cells, have been reported. In a bid to discover additional vegetative *Bt* virulence factors, Signature-Tagged Mutagenesis was employed. Using the tobacco hornworm (*Manduca sexta*) as a model of infection, 1152 *B. thuringiensis* Tn917 insertion mutants were screened for attenuation of virulence. From the first 2 rounds of screening 12 attenuated mutants have been identified. Following cloning and sequencing of the genome-transposon junctions, the disrupted open reading frames (orfs) and the adjacent DNA were sequenced. This analysis has implicated a transcriptional regulator, a histone-like DNA-binding protein, a bacteriophage-like gene, a transposon and several genes of no known function in *Bt* virulence. Two mutants, 1A9 and 1A12, were revealed to be mutated in neighbouring orfs on the genome. These two orfs, together with downstream sequences, may constitute a virulence-associated operon. The orf disrupted in 1A12 was closely related to the ArsR family of transcriptional regulators. Interestingly, the closest

homologue to this orf is a putative gene carried on the *Bacillus anthracis* virulence plasmid pXO1. **(STUDENT POSTER).**

BP 28 Environment stress and virulence in *B. thuringiensis* and *B. cereus*

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Bacillus cereus and *Bacillus thuringiensis* can be considered to be the same species at the genetic level yet display virulence towards very different host species. Identification of the factors which confer virulence upon these bacteria and the signals which trigger their expression would allow a greater understanding of the pathogenic mechanisms employed by these bacteria to invade their respective host organisms. We have utilised signature tagged mutagenesis (STM) and RNA arbitrarily primed PCR (RAP-PCR) to identify genes expressed *in vivo* and genes controlled by specific environmental stresses *in vitro*. We have demonstrated that changes in extracellular iron concentration bring about upregulation of a number of virulence factors including a non-haemolytic enterotoxin. We have also shown that an increase in growth temperature results in changes in expression level of a large number of genes including members of the ferric uptake repressor (Fur) family of transcriptional regulators. Analysis of the *B. cereus* genome has led to the identification of a number of genes whose transcriptional level is controlled by Fur levels. Several of these genes including adhesions, internalins and haemolytic toxins have been implicated in the virulence of other microbes. Fur can therefore be considered a regulator of virulence gene expression and probably works in tandem with other regulators such as PlcR. This work highlights the link between discreet environmental stresses such as iron availability and temperature change and the control of virulence factor expression by the bacteria. **(STUDENT POSTER).**

BP 29 Local alternatives to produces *Bacillus thuringiensis* Subsp. *israelensis*

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The entomopathogen *Bacillus thuringiensis* Susp. *israelensis* (Bti) is produced commercially using expensive, complex chemical fermentation media. We produced toxic Bti using coconuts (*Coco nucifera*) and yucca (*Manihot esculenta*). Raw coconut "water", and yucca infusion (15 g yucca, peeled, chopped, boiled 2' in 150 mL water, filtered), each 100 mL, received 0.18 g agar, were autoclaved and poured in Petri dishes.
Bti (strain IPS-82) was plated and incubated, 96 hours at 30°C. The bacteria were harvested, washed by centrifugation, and inactivated (75°C, 15 minutes). Coconut water agar yielded 80-85% sporulation, $3.49x10^6$ spores/mL, $LC_{50}=2.41x10^4$ spo/mL; yucca infusion agar, 87-90% sporulation, $1.18x10^6$ spo/mL, $LC_{50}=2.66x10^4$ spo/mL. Dr. de Barjac's Standard Medium yielded 85-90% sporulation, 10^6 spo/mL, $LC_{50}=10^4$ spo/mL. Bioassays used third-instar field-caught *Anopheles pseudopunctipennis* larvae. LC_{50}s showed no significant difference between the 3 media, p=0.6342. TPH, MYSM media take longer (120 h) to produce spores. Coconut and yucca agars are appropriate alternatives for local production of toxic Bti. * Funded by Rivers of the World (R.O.W.)

BP 30 Studies of *vip3a* gene of vegetative insecticidal protein form *Bacillus thuringensis*

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Three Bt genes L48811, L48812 and Y17158 in GenBank , showed high homology . With a pair of specific primers designed according to these sequenced , a part of *vip3A* gene of 41 Bt strains were identified by PCR . One product , 470bp in length, was purified and sequenced . It indicated that the sequence was 100% homologous with L48811, L48812 and Y17158 by Blast on-line software. 30 of 41 Bt strains were found harboring the *vip3A* gene . The VCL1 and VCL2 , primers designed for full-length *vip3A* gene , have sites for NotI and XhoI . The full-length vip3A gene , named vip7 , was obtained by PCR from strain WB7 which is high toxicity to lepidopterid . Cloned the vip7 gene into pMD18-T vector and sequenced .It indicated that the vip7 gene sequence was 99% homologous with L48811, L48812 and Y17158 by Blast on-line software .

BP 31 Bacillus thuringiensis serovar oswaldocruzi (H-38) and serovar brasiliensis (H-39): challenge against Culicidae family and crystal's protein profile

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In the early 1990's, three strains of Bacillus thuringiensis were isolated from commercial preparations of pepper powder in the city of Rio de Janeiro which, according to flagellar (H) antigens identification, didn't agglutinated by any antisera known at that time. Those strains were investigated at Pasteur Institute of Paris with regards to serotyping and two new antigens were described, H-38 (B. thuringiensis serovar oswaldocruzi or OSW) and H-39 (B. thuringiensis serovar brasiliensis or BRA). This serovarieties produces a paraesporal inclusion which, to OSW are an ovoid or rhomboidal-shape crystal with diameters range of 0.5 to 1 µm and to BRA are diamond or bipyramidal-shape with size range of 0.6 to 3 µm. After initial description, few data were obtained from those serovars and only recently they had being subjects of further investigations. On this research, OSW strains (LFB/FIOCRUZ 856, 1016, 1021, 1030, 1045 and 1063) from different geographical origins and BRA (LFB/FIOCRUZ 869) were challenged against larvae of Diptera vectors of tropical diseases from Culicidae family

and a comparison of strains crystal's protein profile were obtained by SDS-PAGE analysis. The bacteria were confronted against young L3 larvae of mosquitoes species: Aedes aegypti, Aedes fluviatilis Aedes albopictus, Anopheles aquasalis and Culex quinquefasciatus. Results of entomopathogenic activity's bioassays shown a low toxicity pattern (< 10% of mortality) for mosquitoes species evaluated, without significant difference between serovars and strains. Although, it was observed a significant difference into mosquitoes species response, this fact could not be due to serovarieties products, since there is no difference between serovars mortality and negative control mortality into experiments performed. SDS-PAGE analysis shown, to OSW a very homologous protein's pattern, that are complete distinguishable from the pattern obtained to BRA.

BP 32 Persistence of spores of *Bacillus thuringiensis* serovar *israelensis* in various biotopes of a saltmarsh ecosystem in Southern France

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Biological control with *Bacillus thuringiensis* serovar *israelensis* (*Bti*) has been used operationally to control nuisance mosquitoes *Ochlerotatus* (fomer *Aedes) caspius* and *Oc. detritus* in Southern France since 1998. But in order to evaluate eventual unintentional effects of *Bti* and the organophosphorous larvicide, temephos (Abate® 500E), in a sensitive area close to protected areaof Grande Camargue, an ecological survey has been undertaken during the breeding seasons 2000 and 2001.Within this context, the fate of *Bti* spores in saltmarsh ecosystems (soil, plants and water in larval breeding sites) and the impact toward several species of the natural aquatic fauna has been investigated. Preliminary studies in 2000 showed that *B. thuringiensis* strains were virtually absent from the area before treatment, which makes it ideal for this study. The present results concern the presence of *Bacillus* spores and particularly those of *Bti* in a typical breeding site of *Oc. caspius* which was treated twice (1.95 L/ha) with a liquid formulation (Vectobac® 12AS), during spring and summer 2001. Treated and control plots were composed of four different biotopes (four major plant species based on particular physical and chemical soil compositions). Sampling was done at various intervals before and after treatments. The presence of spores was estimated from both soil (0-5 cm surface), plants (leaves and branchs) and water (when present) of which the number of spores of all *Bacillus* was evaluated by plating following heat shock. All colonies with typical *B. cereus* morphology were observed in light microscope for presence of crystals and final identification was done by H14 flagellar serotyping and protein profile analysis by SDS-PAGE. Statistical analysis were based on the number of spores of *Bacillus* in general and those of *Bti* and were evaluated with respect to four aspects: sample type and preparation, time course, biotope and treatment. Preliminary and general results indicate that there is no accumulation and persistence of *Bti* in the four different biotopes (after the two treatments). Soil is more rich (10^6 spores/gram) than vegetation (10^4 spores /gram) and water is very poor for the presence of spores. There seems to be an effect on the number of spores liberated from dried soil samples compared to non dried. There is probably no difference on the number of spores of *Bacillus* from April to October, there might be a light effect of the kind of biotope and there seems to be less *Bacillus* in the *Bti* treated plot compared to both control and temephos plots.

BP 33 Genetic divergence between Bacillus thuringiensis and Bacillus cereus

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Little is known about genetic exchanges in natural populations of bacteria of the spore-forming Bacillus cereus group because no population genetics studies have been performed on local sympatric populations. We isolated strains of Bacillus thuringiensis and B. cereus from small samples of soil collected at the same time from two separate geographical sites, one within the forest, and the other at the edge of the forest. A total of 100 B. cereus and 98 B. thuringiensis strains were isolated and characterized by electrophoresis to determine allelic composition at nine enzymatic loci. We observed genetic differentiation between populations of B. cereus and B. thuringiensis. Populations of a given Bacillus species B. thuringiensis or B. cereus were genetically more similar to each other than to populations of the other Bacillus species. Hemolytic activity provided further evidence of this genetic divergence, which remained evident even if putative clones were removed from the data set. Our results suggest that the rate of gene flow was higher between strains of the same species but that exchanges between B. cereus and B. thuringiensis were nonetheless possible. Linkage disequilibrium analysis revealed sufficient recombination for B. cereus populations to be considered panmictic units. In B. thuringiensis, the balance between clonal proliferation and recombination seemed to depend on location. Overall, our data indicate that it is not important for risk assessment purposes to determine whether B. cereus and B. thuringiensis belong to a single or two species. Assessment of the biosafety of pest control based on B. thuringiensis requires evaluation of the extent of genetic exchange between strains in realistic natural conditions.
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BP 34 Association of PCR and feeding bioassays as first-tier screening method for *Bacillus thuringiensis* strains more efficient in the control of tropical fall armyworm larvae

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Fall armyworm, *Spodoptera frugiperda*, is the most important maize insect pest in Brazil. The objective of this work was to use the PCR technique with *cry*-specific

primers to identify *Bacillus thuringiensis* (B.t.) strains collected from brazilian soil samples that control *S. frugiperda* more efficiently in tropical maize crops, based upon results of artificial-diet feeding bioassays. A group of 37 B.t. entries from our tropical collection were employed in the analysis, from which 25 showed a larvae mortality above 75.0%. The *cry*-specific primers used amplified the *cry*1A(a), *cry*1A(b), *cry*1A(c), *cry*1B, *cry*1C, *cry*1D, *cry*1E, *cry*1F, and *cry*1G genes. Overall, more than one *cry* gene were detected in most strains. Results from simple regression analysis involving presence/absence of correct-size *cry*-specific amplicons and levels of larvae mortality indicated that the presence of *cry*1D was very significantly and positively associated with mortality, explaining alone 23% of the phenotypic variation for mortality found among strains. Despite showing lower probability *P* values, the presence of *cry*1B and *cry*1E also showed a significant association with mortality, with *cry*1B showing a clear tendency to detect strains with high levels of mortality. Contrariwise, *cry*1G was very significantly and negatively associated with mortality, explaining alone 59% of the phenotypic variation for low levels of mortality, although the number of strains observed for this gene was reduced. The results presented in this work essentially confirm previous analyses in which a different set of strains from the same collection showed a high association between *cry*1E and high mortality. Moreover, results from multiple regression analyses revealed the same pattern evidenced for single regression analysis. Therefore, the combined use of PCR and feeding bioassays experiments has demonstrated the possibility of using one or few *cry* genes as markers for high levels of B.t. mortality against tropical fall armyworm.

BP 35 Toxicity of *Bacillus thuringiensis kurstaki* HD68 ICPs to the *Spodoptera frugiperda* (Lepidoptera, Noctuidae) larvae from south Brazil

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The fall armyworm is one of the most important pests of maize in Brazil. The use of Insecticidal Crystal Proteins (ICPs) from *Bacillus thuringiensis* (*Bt*), have been an alternative to control this pest, due to its efficiency and safety for the environmental. The aim of this study was to determine the toxicity of *Bt kurstaki* HD68 ICPs to *S. frugiperda* larvae obtained from corn and rice fields, in south of Brazil. The crystals purification was performed using discontinuous sucrose gradients of 67 to 88% and centrifugation at 20,000g for 1.5h at 4°C. The crystal inclusions were solubilized in alkaline buffer, after the ICPs were visualized in SDS-PAGE and the protein concentrations were determined using the Bradford procedure with BSA as a protein standard. The toxicity was estimated through the determination of the LD_{50}. The larvae of 2nd instar were individualized in mini-plates (30 mm diameter), with disks of corn leaf, where the protein was applied in the concentrations of 0.4 to 10µg/cm^2. The control group was treated with sterile distilled water. Each test was repeated 3 times, totalizing 90 insects tested for treatment. The leaf area consumed by the larvae in each treatment was determined 24h after the treatments applications. Larval mortality was assessed after 7 days. After correction of the mortalities according to Abbott (1925), the LD_{50} value and confidence limits were obtained by probit analysis by Polo-PC (LeOra Software, 1987). These results reveal a LD_{50} of 0.95 µg/larvae (0.55 to 1.75) with 95% of probability. This results will be important in selecting *Bt* strains producing toxin highly powerful to be used for the biological control or to select genes will be most appropriate in transgenic plants generation.

Microbial Control

MC 14 Entomopathogens isolated from field collected termites including *Coptotermes formosanus*.

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As part of the USDA/ARS "Operation Fullstop" against the Formosan subterranean termite *Coptotermes formosanus* , live termites of several species including *C. formosanus* were collected from 155 urban and non-urban termitaries in China, Malaysia and Réunion island. Thousands of termites were cooled until dead, placed on chloramphenicol-treated liquid agar and observed for the emergence of pathogenic fungi, nematodes and bacteria. *Beauvaria, Metarhizium* and *Paecilomyces* species were recovered from apparently healthy individuals. These fungi were isolated from the cadavers and plated on to SDAY agar. The resulting conidia were then used to treat live *C. formosanus*, and high mortality was observed with some isolates. Nematodes and bacteria were isolated and are being prepared for bioassay tests. These results show that under field conditions and despite the known efficient grooming behavior of termites pathogens are present in termitaries and could play a significant role in termite ecology. Future work will be directed towards field application of the most efficacious pathogens against *Coptotermes* species in La Réunion and Indonesia.

MC 15 Susceptibility of 8 Types of Corn Grown in Northeast Mexico to Armyworm (*Spodoptera exigua* Hübner), and the Potency of *Bt*-based Products (Xen Tari® and Lepinox®)

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Four varieties and four hybrids of corn grown in Northeast Mexico, were selected to determine their susceptibility to the armyworm (*Spodoptera exigua*) attack, measured by oviposition and larval palatability. In addition, the LC_{50} of the Bt commercial products Xen Tari® and Lepinox WDG® against corn infestation by *S. exigua* was determined. In order to determine preference to oviposition, 10 adults of *S. exigua* (1:1) were released in an area of 4 rows, each one with the 8 corn types, placed randomly and covered with a white cheesecloth. After 48 and 72 h, the amount of egg masses/plant was determined. In order to evaluate the larval palatability, 3-

cm leaf samples were randomly placed in plastic petri dishes. Fifty *S. exigua* larvae were then placed at the center of the dish, and they were incubated for feeding under dark conditions; after this, they were frozen at -10°C for counting. All tests were performed in 4 replicates. The Bt (Xen Tari and Lepinox) potency, among corn varieties, was evaluated with LC_{50} using overlay and corn leaves bioassays. Results (ANOVA test) showed no significant differences among the corn types and ovoposition preference by *S. exigua* female moths, or larvae palatability. Both Bt bioinsecticides tested were shown to control armyworm among the four corn varieties, with no significant differences with either corn leaves or artificial diet bioassays (probit, POLO-PC test). Both products are recommended against *S. exigua*, but our results showed that Xen Tari (Bt *aizawai*) is significantly more potent than Lepinox (Bt *kurstaki*). Corn varieties showing the best results using Bt products against *S. exigua* were Nuevo León U127 and Blanco Purísima, whereas Blanco Hualahuises (main corn type grown) showed the lowest armyworm control after Bt application. These results demonstrated the potential of *S. exigua* control with Bt products and the differences on control that growers might observe with the corn varieties cultivated in Northeast Mexico.

MC 16 Comparative studies of *Bacillus thuringiensis* var. *israelensis* growth and spore production in tradicional and alternative media (Manipueira)

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The successful of bioinseticide production and comercialization from *Bacillus thuringiensis* var. *israelensis* is given by the culture media utilization, using natural materials, usually low cost industrial by-products. The aim of this study was the comparation of cultive medium, the tradicional one, from glucose and peptone, and the alternative one, from manipueira, a cassava industrial waste, in order to verified the celular growth and the spore production. Besides, the effects of a suplementar salt solution in both media was analysed. For this, the medium had the pH adjusted to 7 before autoclavege, and the celular growth and spore production was monitored by optical density, pH and pour-plate count during 120 hours. By the absorbance analysis, it was verified a few difference between growth and spore production, when it compared manipueira with and without salts. In the tradicional medium, it was found a great diference, favorable to salt medium. By the pour-plate analysis, the manipueira medium with salts had a little superior growth but inferior sporulation comparing with medium without salts. In the tradicional medium, the difference was accempted again. To the comparation by absorbance, the peptone with salts medium was a little superior to the manipueira medium. But, from pour-plate count, the best value of spore quántity was for manipueira without salts, with a little difference to the peptone-salts medium. It´s possible to conclude that the salt utilization on tradicional medium is necessary, but it isn´t to the manipueira medium. And, because the little difference between the best results, it´s factible to say that the use of manipueira, a without cost waste, is very promissor.

MC 17 Biochemical and molecular characterization of a native mosquitocidal strain of *Bacillus thuringiensis* from Argentina

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As a result of a screening carried out in Argentina, a new native strain of *Bacillus thuringiensis* (FBt41) was isolated from soil samples of Provincia de Buenos Aires. Phase contrast microscopy of the sporangia revealed the presence of rounded to irregular crystal protein inclusions. The analysis of the protein profile by polyacrylamide gel electrophoresis of the spore-crystal complex showed the presence of several proteins of similar relative abundance. Through laboratory bioassays this strain exhibited a high insecticidal activity against dipteran larvae, specifically *Aedes aegypti*. No insecticidal activity against coleopterans (*Diabrotica speciosa* and *Tenebrio mollitor*), or lepidopterans (*Spodoptera frugiperda* and *Anticarsia gemmatallis*) was observed. We used a PCR-based strategy for amplification of DNA fragments with degenerated oligonucleotides homologous to *cry* genes, followed by Tail-PCR methodology, in order to clone and sequence a toxin strain gene. BLASTp analysis (using the deduced amino-acid sequence of the Cry protein from the FBt41 strain) indicated that it is related to Cry 24Aa protein type. This result was confirmed by using a generated dendogram including the Clustal W method with the PAM 250 residue weight table and representative members of Cry proteins from public data bases. Our data indicate that *B. thuringiensis* strain FBt 41 is of interest for further studies on the control of *A. aegypti*, a human disease vector.

MC 18 PCR-based strategy for cloning a wide spectrum of new *Bacillus thuringiensis* toxin genes

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Since the first cloning of the *cry*1Aa gene from *Bacillus thuringiensis* (Bt) serovar. *kurstaki* HD-1, more than one hundred *Bt* toxin genes have been cloned. The vast majority of the toxins cloned are active against lepidopteran pest in agriculture. In contrast the number of toxins that are active against other pest in agriculture, and in human and animal health are limited. Therefore, the isolation and characterization of novel toxins are critical for increasing the diversity of *Bt* toxins for biological control of pests and for overcoming potential problems associated with resistance. In order to identify new *Bt* toxin genes, different strategies were developed taking advantage of PCR methodology. Most of those initiatives involved the design of large number of primer sets directed against specific regions of type-specific *cry* genes, and later size differentiation of the amplification products. The aim of this work was to design a small set of oligonucleotides that can amplify a wide spectrum of *cry* genes. After multiple sequence alignments of representative of the most distantly sequences of known

cry genes, we designed a set of primers suitable for PCR reactions. Different combinations of four of these primers in nested PCR reactions and with different PCR conditions, allowed us to amplify *cry* genes of all *Bt* isolations of our laboratory collection, including serovars. *israelensis*, *tenebrionis* and *kurstaki* strain HD1. Some of these PCR products were identified as *cry* genes after cloning and sequencing. Remarkably, this set of primers can be consider as universal *Bt* primers and constitutes an important tool to be used as a straight-forward alternative for the cloning of *Bt* genes.

MC 19 Soybean extracts and their interactions with the nucleopolyhedrovirus of *Anticarsia gemmatalis* in *Ag*MNPV susceptible and resistant populations of the insect

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The synchronism between host plant resistance and biological control agents on agricultural crops, including soybean, are very important to the success of integrated pest management. However, data about these interactions are relatively scarce. Experiments were carried out to evaluate the effect of the interaction among extracts of two insect resistant soybean genotypes (PI 274454 and PI 227687) and *Anticarsia gemmatalis* nucleopolyhedrovirus (*Ag*MNPV) on the mortality of *Ag*MNPV susceptible and resistant populations of *A. gemmatalis*. Since eclosion, the larvae were fed on a diet without (control) or with plant extracts. When larvae reached the end of the second instar, diets like the previous one were inoculated with different concentrations of *Ag*MNPV. The larval mortality rates were higher when young caterpillars were fed on diet with extracts of resistant genotypes compared to the control. There was positive correlation between larval mortality and *Ag*MNPV concentrations in all diets. In general, synergistic effect on larval mortality was observed when larvae were fed on PI 274454 extract plus *Ag*MNPV, in virus resistant and susceptible colonies. When larvae were fed on diet with PI 227687 extracts plus *Ag*MNPV, there was an antagonistic effect in most of the treatments, for both larval populations, except on the interaction between genotype and the concentration of 1,600 occlusion bodies/ml of diet, in the larval population susceptible to the virus. The interaction between plant extracts and *Ag*MNPV was dependent on the soybean genotype and its chemical profile.

MC 20 Pathogenicity of *Beauveria bassiana* (Moliniaceae), *Steinernama glaseri* and *S. carpocapsae* (Steinernematidae) Brazilian isolates against the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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The pathogenicity of *Beauveria bassiana* Brazilian isolates was evaluated in forced-contact bioassay in which larvae and adults of the lesser mealworm (*Alphitobius diaperinus*) were vortexed for 10 seconds in 1 mL of fungal suspension (1,0 X 10^9 conidia/mL; germination rates > 85%). Control was treated with sterile distilled water. Test insects were removed by hand and transferred to clean Petri dishes with filter paper on the bottom and held in a rearing chamber maintained at 26°C, 14:10 hours (L:D) photoperiod and 60±10% RH. Mortality was daily assessed for 10 days. The cadavers were removed and transferred to moist chambers to induce fungus sporulation. The nematodes were tested against larvae and adults by placing insects in a Petri dish lined with filter paper and adding 1 mL of nematodes suspension in water (60 and 120 IJ/insect). Mortality was daily assessed for 10 days. Cadavers were held for 4 days on moist filter paper in a Petri dish and inspected for nematode reproduction. The experiments have demonstrated that the insect was susceptible to the fungi isolates, which have shown different levels of virulence and many of them provided high level of larval mortality (90 to 100%). The adults were less susceptible to the fungi and few isolates caused maximal mortality of 60 to 70%. The insects (larvae and adults) were highly susceptible to the nematodes, especially to the *S. glaseri*, which provided mortality of 100% at the both concentration. *S. carpocapsae* was more infective to the adults than larvae (mortalities of 90 and 70%, respectively) and this was the first observation with *S. carpocapsae* and the lesser mealworm.

MC 21 Embrapa's culture collections of microorganisms potentially useful for biological control of agricultural pests

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The increasing demand for environmental protection has stimulated adoption of biological control methods into sustainable agricultural programs to improve food quality and reduce the use of toxic chemical pesticides. However, the development of biopesticides requires establishment of microorganism collections to support the research programmes. In this concern, Brazilian Agricultural Research Corporation – EMBRAPA, is promoting and empowering the establishment of culture collections of several biological control agents, including insect pathogens. At the beginning, a survey was carried out in order to identify the existence of culture collections and to evaluate their conditions, at the National Agricultural Research System - SNPA level. According to this survey, there are eleven collections of insect pathogens in different Research Centers of Embrapa across the country. Some of them are rated as service collections, involving a great volume of exchanged strains and collaborative research projects. Others, are individual collections that are actively engaged in research applying modern molecular systematic methods and other headlines. In the future, these collections will make up a network for study and exploitation of biological control agents, covering aspects such as isolation, maintenance, identification, taxonomy, and biological efficiency. Information about these collections will be available on-line.

MC 22 Microbial control of *Triatoma infestans* with *Beauveria bassiana* in field conditions

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Chagas disease constitutes a major human health problem in most Latin American countries. This endemic disease is transmitted by several species of triatomine bugs, being *Triatoma infestans* (Klug) the most important. In this work, two strains of *Beauveria bassiana* (Bb 10 and Bb 65) virulent to this insect species were cultured by a diphasic method and the biomass produced on solid substrate (conidia) was formulated in oil and water base. Both formulations were assayed in experimental chicken houses with nymphs of *T. infestans*, under natural climatic conditions. The mass production of the two fungal strains resulted in different yields of conidia per kilogram of initial dry matter (Bb 10= $8.8 \times 10^{11} \pm 2.20$ and Bb 65= $2,13 \times 10^{12} \pm 0.32$). Conidial suspensions were sprayed in experimental chicken houses at a dose of 5×10^8 conidia/m^2 (2 ml/m^2). The results allow us to select strain Bb 65 formulated in oil (1:1 sunflower oil:kerosene) to further develop a mycoinsecticide to control *T. infestans*.

MC 33 Study of the production of *Anticarsia gemmatalis* nucleopolyhedrovirus in airlift reactors of different geometrical design

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The suspension culture of insect cells and the propagation of baculoviruses in airlift reactors (ALRs) have been demonstrated, but systematic studies devoted to optimize the viral production in this type of reactor are very scarce. We have studied how modifications of the geometrical characteristics of ALRs (that determine changes in the fluid dynamics in the reactors) influence the baculovirus production process. The performance of three different concentric ALRs (ALR 1, 2 and 3), operated at the same superficial gas velocity was studied. The IPLB-Sf-21 cell line was infected with *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) in a serum-supplemented medium in each of the reactors. The cells cultivated in the reactors were infected in the early growth phase, at a multiplicity of infection of 5 $TCID_{50\%}$/cell. The kinetics of infection was clearly different in the three reactors. The fraction of bound virus ranged from 0.54 (ALR 1) to 0.72 (ALR 3) at the first hour post-infection (hpi), and from 0.67 (ALR 1) to 0.95 (ALR 3) at 4 hpi. The kinetics of production of both viral progenies was also affected by reactor geometry. The production of non-occluded virus (NOVs) was first detected at 24 hpi in ALR 3, and 12 hours later in ALR 1 and 2. The first polyhedra were seen at 36 hpi in ALR 3, and they were first observed at 48 hpi in ALR 1 and 2. The yields of NOVs and polyhedra were also strongly influenced by reactor geometry. The maximum NOVs titer obtained in ALR 3 (1.08×10^8 $TCID_{50\%}$/cell) was 12 and 8 times higher than that reached in ALR 1 and ALR 2, respectively. In addition, the maximum polyhedra yield reached in ALR 3 (1.9×10^7 polyhedra/ml) was 6.5 and 4 times higher than that obtained in ALR 1 and ALR 2, respectively. In conclusion, the behaviour of a baculovirus-infected insect cell system was deeply affected by changes of the geometry of ALRs, revealing the dependence of the cellular virus production capacity on the fluids dynamics. A proper reactor design, that carefully considers the characteristics of the fluid dynamics, could be essential to develop a feasible process for production of insecticide baculoviruses in ALRs. **(STUDENT PAPER).**

Tuesday, 17:30 - 18:30
Iguaçu I
CONTRIBUTED PAPERS - Fungi I
Chair: I. Delalibera Jr.

Dose acquisition by second-instar and adult female western flower thrips exposed to leaf disks and impatiens plants treated with *Beauveria bassiana*

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Bioassays evaluated the efficacy of three preparations of *B. bassiana* strain GHA against western flower thrips (WFT). Preparations included an unformulated technical powder (TP) suspended with 0.01% Silwet L-77, a clay-based wettable powder (BotaniGard 22WP), and an oil-based emulsifiable suspension (BotaniGard ES). Sixty second-instar nymphs and 30 adults were exposed to treated bean leaf disks. Four-dose assays were repeated four times using different generations of thrips. Mortality was assessed on day 5 post-treatment. The conidia acquisition profile (distribution of conidia on specific body regions) was determined across application rates and formulations for nymphs and across rates of the WP for adults. Thrips were exposed to treated foliage for 24 h, and conidia were stained and counted at 400x magnification. Formulation had no effect on the number of conidia on the whole insect or on specific body regions. Nymphs treated with TP, WP and ES had mean LD_{50}s (± SE) of 42 ± 11, 50 ± 6 and 50 ± 10 conidia/insect, and LC_{50}s of 74 ± 16, 178 ± 40 and 112 ± 44 conidia/mm^2, respectively. Conidia acquired after 24 h caused no significant increase in mycosis among nymphs. Adult females were more susceptible to the fungus (WP) than nymphs, with an LC_{50} of 19 ± 8 conidia/mm^2. The dose on adults after 24 h indicated an LD_{50} of only 5 ± 3 conidia/insect; which is an underestimate of the true LD_{50}, as dose acquisition was not interrupted by molting. Conidia acquisition efficiency (conidia per whole body divided by the conidia/mm^2 of leaf substrate) decreased with increasing application rate across formulations for both nymphs and adults. Legs of both nymphs and adults acquired the greatest numbers of conidia (32% and 46% of total conidia acquired), followed by the abdomen for nymphs (31% of total) and thorax (wings and legs excluded) for adults (26% of total). Potted *Impatiens walleriana*, (var. Impulse) infested with WFT were sprayed with the WP at a label rate of 0.45 kg/380 l water, and doses on the whole bodies of second-instar nymphs and adult females were quantified 24 h after treatment. Doses on nymphs collected from foliage and open flowers were 115 ± 321 and 452 ± 1,049conidia/insect, respectively, and the respective doses on adult females were 255 ± 428 and 204 ± 416 conidia/insect. Of the total adults and nymphs sampled, 20.8% and 16.6%, respectively, had fewer than 10 conidia/insect.

Studies on the use of entomopathogenic fungi for biological control of the sheep scab mite *Psoroptes ovis*

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The presence of the mite *Psoroptes ovis* in sheep causes an allergic dermatitis (sheep scab) with severe animal welfare implications. Non-chemical approaches to control are being sought as alternative strategies to the use of veterinary pesticides. It has been suggested that the neurotoxic insecticides used in sheep dips may have harmful side effects to human operators and the environment. Their continued use will eventually select for resistant strains. Biological control offers the potential of an effective and alternative means of control. This project aims to use fungal isolates that are known natural pathogens of arthropods. We have selected 3 isolates of *Metarhizium anisopliae* from 16 isolates of *M. anisopliae* and *Beauveria bassiana*, based on their growth rate at various temperatures, germination in various oils, and mite bioassays experiments against *Psoroptes ovis* derived from rabbits (syn. *P.cuniculi*). Mites become infected with fungi after walking over an infected surface, or when put in contact with other infected mites. Two of the isolates grow at temperatures close to normal sheep body temperature (32-34°C), and show high infectivity levels at higher temperatures. We have established a two-stage small-scale mass production protocol for all 3 isolates, developed silicone-based formulations and we are currently undertaking sheep trials to study the distribution and survival of the *M. anisopliae* spores applied to ectoparasite free full fleece sheep. These trials will enable us to monitor the spore persistence and ultimately their efficiency for biocontrol of mites when applied to infected sheep.

Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to entomopathogenic fungi from Ethiopia

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The efficacy of 13 isolates of entomopathogenic fungi belonging to *Beauveria, Metarhizium* or *Paecilomyces* spp. was assessed against *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Coleoptera: Bostrichidae) using a total immersion bioassay technique in the laboratory. Fungi were applied at concentrations of 1 x 10^7 and 1 x 10^8 conidia ml^{-1} for *P. truncatus* and *S. zeamais*, respectively. All isolates tested were virulent to *P. truncatus* (98 – 100 % mortality, and median survival time (MST) ranged from 2.85 – 4.05 days). *Metarhizium anisopliae* and *B. bassiana* were also virulent to *S. zeamais* (92-100 % mortality, MST ranged from 3.58 – 6.28 days). The isolate of *Paecilomyces* sp. was found to be the least virulent against *S. zeamais*, causing only 26.32 ± 4.29 % mortality with MST of 10.38 ± 0.29 days. *Prostephanus truncatus* proved more susceptible to the entomopathogenic fungi tested than *S. zeamais*. One *M. anisopliae* (PPRC-EE) and three *B. bassiana* isolates (PPRC-HH, PPRC-9609 and PPRC-9614) were selected for further study and dose-mortality relationships were assessed on *S. zeamais*. The tested concentrations ranged from 1 x 10^4 -1 x 10^7 conidia ml^{-1}. *Metarhizium anisopliae* (PPRC-EE) showed the lowest LC_{50} (3.39 x 10^5 conidia ml^{-1}) followed by *B. bassiana* PPRC-HH (2.04 x 10^6 conidia ml^{-1}). PPRC-9609 and PPRC-9614 showed slight differences in LC_{50} but not at LC_{95}. The results revealed the higher potency of *M. anisopliae* as compared with the *B. bassiana* isolates tested. Subsequently, *M. anisopliae* isolate (PPRC-EE) and *B. bassiana* isolate (PPRC-HH) were selected. Using mass produced aerial conidia or submerged spores, 9 different dustable powder (DP) formulations were developed. The DP formulations were applied at the rate of 1 x 10^8 spores g^{-1} of maize grain and mortality of the maize weevils were assessed for 20 days. Dustable powder formulation developed based on aerial conidia of *B. bassiana* and *M. anisopliae* showed the highest efficacy (> 80 % mortality and median survival time of lower than 6 days) compared to spray or freeze dried submerged spores of *B. bassiana* (produced in TKI medium), which showed lower than 60 % mortality within 20 days. Spray or freeze dried submerged spores of *B. bassiana*, produced in Catroux medium, showed 65-83 % mortality with longer (> 15 days median survival time). The study suggests that the use of entomopathogenic fungi hold promise as an alternative method to control pests of stored-product in Ethiopia.

Colonization of the Colorado potato beetle following infection by *Beauveria bassiana*

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The fungus *Beauveria bassiana* may play an important role in management of the Colorado potato beetle. A better understanding of the colonization of the host is needed to exploit the fungus to its fullest. We quantified the colonization of the haemolymph by the fungus. Beetles were allowed to feed on leaf discs that were inoculated with two concentrations of *B. bassiana* (10^4 and 10^5conidia/adult prepared in an oil suspension). Beetles were maintained for 15 days at 22°C and 8:16 LD in an incubator. To quantify fungal colonization of the haemolymph, sub-samples of beetles from the inoculated population were homogenized and plated onto an oatmeal-dodine selective agar medium, starting on day 2 and continuing up to day 15. Results show that when beetles are inoculated with 10^4 conidia/adult, an increased propagule load was observed by day 8 ($2.6x10^4$cfu) which peaked on day 12 ($2.4x10^5$cfu). However, beetles inoculated with 10^5 conidia/adult showed an increase in propagule numbers starting on day 5 with a rapid increase on day 8 ($1.2x10^5$cfu) which peaked by day 11 ($2.4x10^5$cfu). Mortality in the population was observed earlier at the 10^5 (day 6) as compared to day 8 for 10^4conidia/adult inoculation; the highest mortality was observed on days 10,11,12 (3 adults/day) and day 10 (13 adults) at 10^4 and 10^5 inoculation, respectively. Of the total number of beetles homogenized and plated, 14%

and 36% were found to be infected at 10^4 and 10^5 inoculation levels. The number found infected per day was always higher at 10^5 inoculation levels. We conclude that *B. bassiana* propagules colonize the haemolymph more rapidly when beetles are exposed to higher initial concentrations.

Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to 4th instar and adult *Blissus antillus* (Hemiptera: Lygaeidae)

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This study determined the pathogenicity and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* to 4th instar nymphs and adult female chinch bugs *Blissus antillus* (Hemiptera: Lygaeidae). Insects were inoculated under laboratory conditions by rapid immersion in concentrations of 5 x 10^8 conidia/ ml. Following inoculation, insects were maintained for 48h at >90% RH and 25°C. Following this initial period, insects were maintained at a RH of approximately 70% and 25°C. Evaluation of mortality was carried out on a daily basis for 10 days. *B. bassiana* isolate ARSEF 792 was found to be the most virulent to both nymphs and adults, causing 64 and 73.3 % infection respectively. The effect of humidity on mortality and infection was evaluated for adult females. Insects were infected as previously described and subsequently maintained at either 67-73% RH, 85-89% RH or 94-98% RH. The infection rates for *B. bassiana* CG24 and *M. anisopliae* ESALQ 818 were not effected by differences in humidity, however, the percentage infection of *B. bassiana* ARSEF 792 was adversely effected by lowering the humidity, even so, the mortality caused by ARSEF 792 was higher than that caused by the two other isolates at lowest humidity used in these experiments. The production of inoculum on the cadavers was evaluated following conidiogenesis. The highest value was observed following infection by *B. bassiana* isolate CG24 (14.9 x 10^7 conidia/ml). This isolate also produced the greatest number of conidia when cultured on rice (10.6 x 10^9 conidia/ml/g), an important factor in the mass production of biological control agents in Brazil.

Caged field trials with Hyphomycetes against the Asian longhorned beetle, *Anoplophora glabripennis*

T. Dubois[1], A. E. Hajek[1], H. Jiafu[2] and Z. Li[3]

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The Asian longhorned beetle, *Anoplophora glabripennis*, is an invasive pest, discovered in New York City in 1996, and in Chicago in 1998, posing a major threat to hardwood forests and the urban landscape. During caged field trials in July 2000 near Wuhe, China, two species of entomopathogenic fungi for control of *A. glabripennis* were applied in two different ways. The use of non-woven fiber bands impregnated with fungal cultures and attached around the tree trunks was compared with spraying comparable doses of fungal spores onto tree trunks, using a strain of *Beauveria bassiana* marketed by Mycotech (Butte, USA) and a strain of *B. brongniartii* marketed by Nitto Denko (Osaka, Japan). Thirty poplar trees were used for each treatment, with an additional 30 untreated trees as controls. For each tree, five field-collected adults were placed within a 70 cm-long cage made of window screening at 1.5 to 2 m up the tree trunk for 10 days, after which the beetles were checked further in the laboratory for infection. Oviposition rate per female and number of oviposition scars per tree were significantly lower for cages where *B. brongniartii* was applied compared with *B. bassiana*, but did not differ between application methods. Time to 50% mortality (LT_{50}) was significantly lower for cages where *B. brongniartii* was applied compared with *B. bassiana*, and there was a trend of lower LT_{50}s in cages with bands instead of sprays. In July 2001, new caged field trials were carried out near Bengbu, China, using four strains: a North American *Metarhizium anisopliae* strain and *B. bassiana* strain, an indigenous Chinese *B. brongniartii* strain and the commercialized *B. brongniartii* strain from Nitto Denko. The fungi were only applied as non-woven fabric bands. Forty willow trees were used for each treatment, and all other parameters were identical to the previous field season. The LT_{50}s were similar for *M. anisopliae*, *B. bassiana* and the Nitto Denko strain but were higher for the Chinese strain of *B. brongniartii* because this strain produced fewer spores on the bands. The Nitto Denko strain performed better in July 2000 when weather conditions were optimal. After 15 days, five new beetles were released in the same cages for only 6 days and with only 10 cages per treatment. The LT_{50}s for each of the strains were the same, showing that the bands retain their full virulence in the field for at least 15 days. These studies demonstrate the potential for an entomopathogenic fungus to control a devastating, cryptic pest in high-valued habitats where the use of conventional pesticides is difficult. In addition, applying the fungus by means of bands around trees appears to be an excellent means for inoculating beetles. **(STUDENT PAPER).**

Tuesday, 16:30 - 18:30
Iguaçu II
CONTRIBUTED PAPERS - Virus 1
Chair: J.E. Maruniak

Evolution of the *Baculoviridae*

E. A. Herniou [1,2], J. A. Olszewski [1], J. S. Cory [2], D. R. O'Reilly [1,3]

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Our understanding of the insect virus family *Baculoviridae* has greatly been improved by the completion of 13 genome sequences, including 1 virus from the mosquito *Culex nigripalpus* (CuniNPV), as well as 3 granuloviruses and 9 nucleopolyhedroviruses from lepidopteran hosts. Comparative genomics have shown that the lepidopteran viruses share 62 genes, of which only 30 are present in CuniNPV. This suggests that CuniNPV is distantly related to the lepidopteran baculoviruses, but interrelationships between these viruses have yet to be established. We have applied new

methods based on complete genomes to reconstruct baculovirus phylogenies. The history of gene acquisitions and losses was visualised by mapping the gene content onto the baculovirus phylogenetic tree. This highlighted key genetic innovations that have accompanied baculovirus diversification. Furthermore, the use of comparative genomics methods has shown functional linkage between a number of genes, revealing 2 putative DNA repair systems, Lymantrid host related factors and *Baculoviridae* core gene cluster. Unravelling the genomic evolutionary history of these viruses can help us understand their biology and how it is linked to their hosts. The broadening of the phylogeny to a wide range of viral field isolates allow for the study of macro-evolutionary patterns such as insect/baculovirus co-evolution. **(STUDENT PAPER).**

Characteristics and genome sequence of *Adoxophyes honmai* nucleopolyhedrovirus

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Newly discovered *Adoxophyes honmai* nucleopolyhedrovirus (AdhoNPV) was characterized and its complete genome was sequenced and analyzed. AdhoNPV is a single-nucleocapsid nucleopolyhedrovirus of the smaller tea tortrix, *Adoxophyes honmai*, which was isolated from larvae in a tea field at Tsukuba, Japan. AdhoNPV kills the host in the final instar (5 - 9 days after final molting), regardless of the timing of inoculation. However, when neonates are exposed to a high dose of AdhoNPV (greater than 100 times LD_{50}), their development is prevented and they die at an earlier stage. The AdhoNPV genome is 113 kbp with a G+C content of 36%. Many ORFs show homology to genes from other baculoviruses, while some ORFs are unique to AdhoNPV, with no homologs in GenBank database. Among the baculoviruses, the amino acid identity of the AdhoNPV ORFs shows the highest homology to genes of *Spodoptera exigua* MNPV, which is a Group II NPV. AdhoNPV genes homologous to those from other baculoviruses include genes involved in early and late gene expression, DNA replication, and structural functions. AdhoNPV does not contain typical homologous repeat sequences. Interestingly, AdhoNPV contains the *egt* gene, which has an early gene motif (CAGT), although molting of AdhoNPV-infected larvae occurs until final stage.

Sequence analysis of the potato tuber moth, *Phthorimaea operculella* granolovirus

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Laboratoire de Pathologie Comparée. UMR 5087 and IRD, UR 132. 30380 Saint Christol les Alès, France

The genome of the Potato Tuber Moth, *Phthorimaea operculella*, granulovirus (*Phop*GV) tunisian strain, has been completely sequenced. Its size is 119217 nucleotides. 130 ORFs have been selected following the criteria of maximum length, minimum overlap or maximum conservation with the other baculovirus ORFs. Global analysis confirms that *Phop*GV is phylogenetically closer to *Cp*GV than to *Xc*GV, as we previously suggested, but the GC ratio is 35.7%, the lowest yet found in baculoviruses. *Phop*GV shares 64 genes with all other lepidopteran baculoviruses sequenced. 24 genes appeared to be exclusively present in granuloviruses. PhopGV presents three repeated gene families: two copies of Fibroblast Growth Factor, three Inhibitor of Apoptosis, and three genes of the K11C6 family (homologous to the AcMNPV ORFs 145/150). A single *bro* gene is present, and no *vef*, *chi*, or *cath*. The knowledge of the sequence opens the way to the investigation at the gene level of the differences in host range or virulence between isolates of this virus. It should help to the standardization in the production of this bio-pesticide.

The sequence of the *Rachiplusia* ou multi-nucleocapsid nucleopolyhedrovirus genome

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Rachiplusia ou multi-nucleocapsid nucleopolyhedrovirus (RoMNPV) was first isolated in 1960 from the mint looper, *Rachiplusia ou*. A different isolate of this virus was identified in 1985 from the celery looper, *Anagrapha falcifera*, and described as the *Anagrapha falcifera* multi-nucleocapsid nucleopolyhedrovirus (AfMNPV). RoMNPV is closely related to *Autographa californica* multi-nucleocapsid nucleopolyhedrovirus (AcMNPV). RoMNPV and AcMNPV have broad, overlapping host ranges, but RoMNPV is more virulent than AcMNPV against a number of agriculturally significant species, including the corn earworm (*Helicoverpa zea*), the European corn borer (*Ostrinia nubilalis*), and the navel orangeworm (*Amylois transitella*). As a preliminary step towards identifying the molecular basis for the greater virulence of RoMNPV against these species, we sequenced the RoMNPV genome. A set of overlapping restriction fragments of the R1 isolate of RoMNPV was cloned into plasmid vectors and sequenced by primer walking. The RoMNPV genome is 131,526 bp with a 39.1% G + C content. The RoMNPV nucleotide sequence is almost totally co-linear with the sequence of AcMNPV, and contains homologues of 149 of the 154 ORFs described for AcMNPV-C6. The average sequence identity of RoMNPV ORFs with AcMNPV is 96.1%, with the lowest sequence identity observed with *ac70* (84.1%). Eleven RoMNPV ORFs are completely identical (100%) with their AcMNPV homologues. A 1275 bp region that in AcMNPV contains *ac2* (baculovirus repeated ORF, *bro*) and *ac3* (*ctl*) is missing from RoMNPV. No other *bro* homologues occur in the RoMNPV genome. Insertions, deletions, and substitutions have altered the size of 47 ORFs relative to their AcMNPV homologues. In three cases (*ac97*, *ac121*, and *ac140*), the RoMNPV homologues have been reduced below 50 codons. Four pairs of ORFs (*ac20/ac21*, *ac58/ac59*, *ac106/ac107*, and *ac112/ac113*) have been fused into single ORFs in RoMNPV. All homologous regions (*hrs*) present in AcMNPV-C6 are present in RoMNPV, but the RoMNPV hrs have one to four fewer palindromic repeats, and spacing between the repeats is not well conserved.

Construction of a random insertion mutant library of HaSNPV and target screening of specific mutants

S. Hou[1, 2], X. Chen[1] and Z. Hu[1]

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A random insertion mutant library of HaSNPV was constructed by introducing the transposon TnMax13 to HaSNPV Bacmid (HaBacHZ8). TnMax13 has a preference to randomly insert as a single copy into the plasmid in *Escherichia coli*. The transposon harboring a *Tet*^{R} gene was transformed into DH10B containing HaBacHZ8, resulting in a library of about 1300 clones. The clones were inoculated into fourteen 96-well plates and a three-rounds PCR method was used to target screen specific mutants. The *orf107*, a unique gene of HaSNPV, was chosen as a target site for investigating the feasibility of the library. After 3 rounds PCR amplification, several clones were identified that may contain insertions at different parts of the *orf107* region. One of the clones, HaBacHS2, was further characterized by sequencing and indicated that the TnMax13- *Tet*^{R} was inserted at 1017 nt downstream of the ATG of *orf107*. HaBacHS2 DNA had been used to transfect HzAM1 cell line and the BVs were harvested for infection. Electron microscopic analysis showed that mature ODVs were produced in the nucleus of the HaBacHS2 infected cells. In conclusion, an effective random insertion mutant library of HaSNPV has been generated. An insertion-mutant at the *orf107* region, HaBacHS2, was obtained by target screening the library. Transfection and infection analysis of HaBacHS2 indicated that the *orf107* is not a necessary gene for HaSNPV to replicate in HzAM1 cell. Other genes are being investigated by using this library in our laboratory.

Construction of a Transposon-mediated Baculovirus Vector Hanpvid and a New Cotton Worm Cell Line AM1-NB for Stably Expressing barnase Gene

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We developed a transposon-mediated shuttle vector disignated Hanpvid comprising of HaNPV genomic DNA and a transposon cassette from AcNPV Bac-to-Bac system, which could replicated in Escherichia coli cells as a large plasmid and also remain infectious if introduced into cotton worm cells. To verify the construction of Hanpvid and test its recombinant efficiency, recombinant virus rHa-Bar containing barnase gene was constructed by transposition of the shuttle vector Hanpvid together with a transposon plasmid pHa-Bar introduced. Because the expression vector carrying barnase gene in the absence of barstar gene, a specific inhibitor of barnase gene, can not replicate. Therefore, we construct a new cotton worm cell line AM1-NB in chromosome of which barstar and neomycin genes were integrated to persist expression of barnase gene . Normal cotton worm cells Hz-AM1 and transformed cells AM1-NB were infected separately with recombinant virus rHa-Bar. After 48hr and 72hr post-infection, viral progenies in AM1-NB cells were 23 and 160 times, respectively more than that in Hz-AM1 cells. With additional insertion of polyhedrin gene from AcNPV into Hanpvid genome, rHa-Bar regained phenotype of polyhedron and pest-killing toxicity was greatly improved. Toxic analysis show that lethal dose (LD_{50}) and lethal time (LT_{50}) of rHa-Bar were reduced by 20% and 30% respectively compared to wild-type HaNPV in the third instar larvae of cotton worm.

Differential expression of host cellular genes in Sf 9 cells infected with AcMNPV

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The effects of baculovirus infection on host cell gene expression was studied at the mRNA level, using a modified differential display approach (Display Systems Biotech). *Spodoptera frugiperda* (Sf9) cells were mock-infected (time point 0) or infected with *Autographa californica* Nucleopolyhedrovirus (AcMNPV), and harvested at 3, 6, 12, 18, 24, 36 and 48 hours post-infection (hpi). Total cellular RNA was harvested, and poly A+ mRNA was converted to double-stranded cDNAs by reverse transcription. These cDNAs were digested with *Taq*I, ligated with adaptors, and amplified with PCR in the presence of radioactive label. PCR products were visualised and isolated by long acrylamide gel electrophoresis. Differential expression was confirmed by northern blot analysis and quantitative RT-PCR. As described in the baculovirus literature, most host cell transcripts, present in mock-infected cells were found to be down-regulated beginning at 12 to 18 hpi. To identify any host cellular transcripts that are up-regulated at earlier times of infection, before this general shut off of host mRNA transcription occurs, we studied differential expression of transcripts from 0-6 hpi. We used AcMNPV genomic DNA as a probe in Southern hybridisation to differentiate up-regulated cellular sequences from viral transcripts. So far, no host transcripts have been found to be significantly up-regulated in all three assays (restriction fragment differential display, northern blot and quantitative RT-PCR). Further analysis is underway on remaining genes..

Different isolates of shrimp white spot syndrome virus are distinguishable by hypervariable genomic regions

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Since the 1990's shrimp culture has been hampered by mass mortalities in ponds throughout the world. Up to the present day, white spot syndrome virus (WSSV) continues to cause major losses and remains a problem in shrimp culture. WSSV was first reported in 1992 and has spread quickly through Southeast Asia and subsequently to shrimp farming areas in Texas, South Carolina and Central- and South-America. WSSV has a broad host range, as it not only infects shrimp, but also other crustaceans like crab and crayfish. The virus consists of an enveloped particle containing a large circular double stranded DNA genome and belongs to a new virus family (Nimaviridae). The WSSV genome contains nine homologous regions (*hrs*), dispersed along the genome,

each containing a variable number of 250-bp tandem repeats. Three complete genome sequences have now been reported varying in size from 293 kb to 307 kb (approx. 185 ORFs). Genetic analysis revealed that these viruses are isolates of the same virus. The major differences in the genome isolates are caused by a 12 kb region containing 14 ORFs, which is easily deleted from the genome, and the *hrs*, which have shown to be highly variable. The remaining areas in the virus genomes are highly homologous (over 99%) and the gene order is conserved. Analysis of the highly variable genomic regions can be used to shed light on the way this virus has spread worldwide. The presence of different genotypes should be taken into account for the WSSV diagnosis, which is often based on PCR amplification of single WSSV fragments only. The degree of genotypic variation and its biological effect with regard to virulence and host range was evaluated.

Wednesday, 8:00 - 10:00
Iguaçu I
CONTRIBUTED PAPERS - Fungi 2
Chair: R. Samuels

Polyclonal antibodies production to detection of Beauvericin from the entomopathogenic fungus: *Beauveria bassiana*

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The entomophatogenic fungus *Beauveria bassiana* (Vals.) is a good tool to biological control of insects that attacks economically important crops. This fungus produce a ciclohexadepsipeptide toxin that exhibit biological activity as insecticide. The aim of this research job was to produce polyclonal antibodies (Pabs), which could be used to develop a more sensitive immunological method in order to quantify the production of this toxin in seven strains of *B. bassiana* (Bb) collected in Cenicafé-Colombia. Liquid culture Sabouraud´s medium was inoculated with $3.0x10^5$ spores/ml of each isolation and these filtered extracts were evaluated at the 7, 9 and 11 days of growing. Pabs were obtained through two intramuscular injections at aprox. two-week interval. New Zealand white rabbits were given injections containing 2.5 mg of the emulsified immunogen, with Freund´s complete adjuvant for the first injection and with Freund´s incomplete adjuvant for the second one. The serological tests used in this experiment were ELISA and Dot-blot. The results show some differences in toxin production between seven isolation that were evaluated. All isolations showed a peak of production between 9 and 11 days of culturing. The levels of the Beauvericin detected in *B. bassiana* fluctuated between 1.16 (Bb 9610) and 1.57 µg/µl (Bb 9205). Polyclonal antibody-based immunoassays have been developed for detection of few quantities of Beauvericin from the entomopathogenus *B. bassiana*. This methodology will permit to select more efficient strains, which could be included in appropriate integrated pest management program.

Production and characterisation of Pr1 by fungus *Tolypocladium*

A. R. Bandani[1] and T. M. Butt[2]

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Entomopathogenic fungi secrete an array of cuticle-degrading enzymes including proteases, chitinases, and lipases that some of which have been shown to be important pathogenicity determinants. One key enzyme, the subtilisin Pr1, is secreted by a wide range of fungal invertebrate pathogens. In spite of its importance as a pathogenicity determinant nothing is known about the production of this enzyme by *Tolypocladium* species. The aim of this study was to examine production and characterisation of Pr1 by *Tolypocladium*. The fungus was grown in Sabouraud dextrose broth culture in which mycelium was harvested and transferred (total of 5 gram wet weight) to 100 ml basal medium containing 0.02 % KH_2PO_4, 0.01 % $MgSO_4$ supplemented with 1% insect cuticle and incubated for four days. . Protein was precipitated by adding solid ammonium sulphate to the culture filtrates and collected by centrifugation. Enzyme assay, inhibition studies and SDS-PAGE studies showed that there is not significant differences between Pr1 from *Metarhizium anisopliae* and *T. cylindrosporum* but IEF studies showed that *M. anisopliae* pI was around 9.00 but that of *T. cylindrosporum* was around 11.00.

Impact of genetic diversity of *Beauveria bassiana* on sustainable control of Masson's pine caterpillars in forest ecosystem

Z. Li, M. Fan, B. Han, D. Ding and B. Wang

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Seven investigations in an anniversary year on *Beauveria bassiana* infected cadavers of various invertebrates were conducted in a Masson's pine stand inoculatively treated with a strain of *B. bassiana* and 207 cadavers were obtained of 32 host insect species, including 7 lepidopterans, 7 hemipterans, 6 coleopterans, 3 hymnopterans, 3 orthopterans, 2 homopterans, 2 dipterans, 1 dremopteran and 1 odonatan. Altogether 102 isolates were derived from the cadavers, and 18 derived from air and soil. In an esterase isozyme test of the 120 isolates, 30 esterase types were revealed. Bioassay on the virulence of 16 isolates of in 12 esterase types on the caterpillars showed very significant difference, with their LD_{50} ranging from 4.69 to 30.51 days. Isolates from the gray pine weevil, *Brachyderes incanus* belonged to different esterase types and showed obviously different virulence. MGS980404 (Esterase type 05) and MGS980510 (Esterase type 06) were highly virulent, while MGS980507, the most common strain in the ecosystem, which and Bb13(the released and the most virulent strain) both belonged to Esterase type 02, was the least virulent. The abundant genetic diversity shown by the large number of *B. bassiana* isolates from various insect species indicated that *B. bassiana* persists for long period and causes enzootics along many paths instead of one. Due to wide host spectrum of *B. bassiana*, different foodchains consisting of different hosts can joint up at some link, making the foodweb in the pine ecosystem very complicated, which is very beneficial to the sustainable control of the Masson's pine caterpillar. In addition, *B. bassiana* isolated from soil, litter, foliage and air were attributed to various esterase types with different

virulence, suggesting that the complicated saprophytic foodchains also exist in the ecosystem and makes the foodweb more complex. Subsequently, appropriate management strategies must be considered to maintain the another kind of foodweb characterized by abundant genetic diversity of *B. bassiana* as well as other insect pathogens, while proper strategies are taken to maintain a comlex foodweb characterized by abundant species diversity, in order to keep a fair ecological equilibrium, and suppress the caterpillar populations over a long period at a low level by predation, parasitoidation as well as continual enzootics through contribution of predators, parasitoids for pests as well as hosts for pathogens. Based on the whole results of the studies on sustainable control of the Masson's pine caterpillar with *B. bassiana*, an inoculative release at appropriate frequency and dose is one of such proper strategies.

Molecular variation among isolates of the mite pathogenic fungi *Neozygites tanajoae* and *N. floridana*: development of RAPD, AFLP and SCAR markers

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Neozygites tanajoae is an important natural enemy of the cassava green mite in Brazil and it was experimentally released in cassava fields in Benin, Africa in 1988/1999. *N. floridana* is morphologically similar to *N. tanajoae* but is a widespread pathogen of tetranychid mites in temperate and tropical regions. In this study the genetic variability in six isolates of *N. tanajoae* from Brazil and one isolate from Benin and two isolates of *N. floridana*, from the US and Colombia was assessed by three molecular techniques. Sequencing information from the small subunit (SSU) ribosomal DNA demonstrated consistent level of variability between *N. tanajoae* and *N. floridana* and no intraspecific variation was observed in this conserved DNA region. We were not able to compare the internal transcribed spacer (ITS), a region of ribosomal DNA that presents intraspecific variation in several fungi, since none of the most common fungi-specific primers tested amplified *Neozygites* DNA. The polymerase chain reaction (PCR) based techniques, random amplified polymorphisms (RAPD) and amplified fragment length polymorphisms (AFLP) were then used to assess inter- and intraspecific variation in genomic DNA of *N. tanajoae* and *N. floridana* isolates. Similarity coefficients for pairwise combinations of RAPD amplification patterns ranged from 84 – 100% between *N. tanajoae* isolates and 90% between the two *N. floridana* isolates. However, similarity between these two fungal species ranged from 14 to 22%. No correlation could be established between the clustering of RAPD-PCR polymorphism of *N. tanajoae* isolates and geographical origin. The RAPD results were consistent with AFLP comparisons among one isolate of *N. tanajoae* from Brazil and other from Benin with *N. floridana* from the U.S. The distinction of these two species was initially proposed based on host specificity and physiological patterns and is supported by the genetic variation demonstrated in this study. Polymorphic RAPD markers are currently been sequenced characterized (SCAR) to be used as isolate-specific genomic DNA probes. These fragments may provide a tool for identifying Brazilian strains of *N. tanajoae* and monitoring their impact in CGM populations in the introduced areas in Africa.

Risk assessment of genetic recombination between introduced and indigenous strains of *Beauveria bassiana* in agricultural fields

L. A. Castrillo, J. D. Vandenberg, and M. H. Griggs

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As part of our research on the fate of introduced strains of the entomopathogenic fungus *Beauveria bassiana*, we are conducting a study on the risks of genetic recombination between mass-released and indigenous strains in agricultural fields. Genetic recombination in asexual fungi, including *B. bassiana*, can occur through the parasexual cycle, during which vegetatively compatible hyphae fuse to form heterokaryons and exchange genetic material. Genetic recombination, along with mutation, can alter virulence and host range, and should be considered when assessing the risks of wide-scale applications of a given mycoinsecticide. Using nitrate non-utilizing (*nit*) mutants, we assessed vegetative compatibility groups (VCG) among strains of *B. bassiana* representing naturally occurring strains collected throughout the US and strains, like GHA, that have been mass released as biological control agents against insect pests. Genetic similarity among these strains was analyzed using randomly amplified polymorphic DNA (RAPD) markers. Our data revealed a group of genetically similar strains isolated from Colorado potato beetles (CPB) from the northeastern part of the US and from Quebec and Ontario, Canada belonging to the same VCG. Following *in vitro* studies, we co-inoculated CPB larvae utilizing pairs of complementary *nit* mutants of genetically distinguishable strains from the same VCG and from different VCGs. It has been proposed that parasexual recombination in nature is likely to occur between co-infecting strains within an insect host where the fungus proliferates as hyphal bodies. During repeated field applications of a given mycoinsecticide it is likely that mixed infections of larvae by the introduced strain and native strain(s) occur. Co-inoculations of CPB larvae with *nit* mutants revealed heterokaryon formation only between strains of the same VCG, suggesting that this self/non-self recognition system is an effective barrier preventing genetic exchange between dissimilar strains in the field. Heterokaryons were screened initially by their prototrophic growth on minimal medium and confirmed by analyses using molecular markers. Further studies are being conducted to assess the stability of these heterokaryons and the frequency of their formation.

Using entomophthorales for control: maturation, dormancy and survival of laboratory-produced *Entomophaga maimaiga*

A. E. Hajek, J. R. McNeil and M. J. Filotas

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Species of Entomophthorales are able to cause dramatic epizootics but their manipulation for pest control can be difficult. We have successfully introduced resting spores of *Entomophaga maimaiga* against the important N. Am.

forest defoliator, gypsy moth (*Lymantria dispar*). However, our studies have been based on introductions of resting spores collected in cadavers or soil from the field. We can produce resting spores (azygospores) both in vitro or in vivo in the laboratory but making certain that these are germinable is as important as producing them. Resting spores mature to a stage with thick walls and few internal oil droplets and resting spores of most species require a dormant period before germination. Field collected gypsy moth cadavers containing resting spores will not cause infections immediately but only after storage at 4°C for 7-8 months. *E. maimaiga* resting spores germinate asynchronously, for a discrete time period when hosts are present and not all resting spores germinate each year. Surprisingly, when cadavers bearing resting spores were produced in vivo in the laboratory (using a Japanese isolate), stored on water agar, and larvae were exposed to them, infections began ca. 3 weeks after resting spores had matured; therefore, infections commenced without a dormant period. Fungus that had not undergone dormancy continued to cause infections for at least 206 days. Cadavers were stored at 4°C for varying lengths of time and after up to 8 months of cold storage, infection still occurred over 170 days, although infection levels decreased with increasing time in cold storage. Activity of 3 Japanese isolates was compared with 3 N. Am. isolates. All Japanese and two of three N. Am. isolates caused infections without cold treatment although variability occurred in infection levels among isolates. Resting spores of the N. Am. isolate not causing infections never appeared fully mature. Infections initiated by lab-produced *E. maimaiga* differed from infections resulting from *E. maimaiga* ovewintering in the field because resting spores were produced within cadavers of larvae killed by lab-produced fungus while fungus from the field yielded only conidia once infections began during spring. These results demonstrate that *E. maimaiga* produced in the laboratory does not need a prolonged chill before becoming infective.

Wednesday, 8:00 - 10:00
Iguaçu II
CONTRIBUTED PAPERS - Virus 2
Chair: B. M. Ribeiro

The Op-*iap* gene is required to prevent OpMNPV-induced apoptosis during infection of LD652Y cells

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The *Orgyia pseudotsugata* M nucleopolyhedrovirus (OpMNPV) gene *iap-3* (Op-*iap*) was one of the first *iap* genes discovered, and expression of Op-IAP has been shown to block apoptosis triggered by a variety of stimuli in both insect and mammalian cells. Although the anti-apoptotic function of Op-IAP has been studied extensively, the function of Op-IAP in its natural context, OpMNPV infection, has not been examined. An antibody was generated against Op-IAP and used to study the protein's expression during infection of LD652Y cells.

Op-IAP protein was detectable within 30 minutes post infection (pi). Op-IAP levels increased until 2 hours pi and then remained steady until 24 hours pi, after which levels declined. By 72 hours pi, Op-IAP protein was no longer detectable. To study the function of the Op-*iap* gene in OpMNPV infection, double stranded RNA-mediated interference (RNAi) was used to silence its expression. Cells that were infected with OpMNPV and received Op-*iap* dsRNA underwent apoptosis, while cells that received control dsRNA formed occlusion bodies, indicating that addition of dsRNA did not inhibit virus infection. In addition, cells that received Op-*iap* dsRNA in the absence of virus infection did not undergo apoptosis. These results indicate that Op-IAP is required to inhibit OpMNPV-induced apoptosis during infection of LD652Y cells.

Extensive tissue damage associated with apoptosis in *Spodoptera frugiperda* caterpillars infected with *p35* mutant AcMNPV

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Apoptosis is stimulated in cultured cells from the caterpillar *Spodoptera frugiperda* that are infected with mutants of *Autographa californica* M nucleopolyhedrovirus (AcMNPV) lacking the anti-apoptotic gene p35. Such mutants are also defective in their ability to infect *S. frugiperda* caterpillars. A study was carried out in which fourth instar *S. frugiperda* caterpillars were infected by intrahemocoelic injection with 2×10^5 PFU of a p35 mutant strain of AcMNPV expressing enhanced green fluorescent protein (eGFP) from the hsp70 promoter. Only 30% of the caterpillars showed any detectable eGFP expression, and of these only a third showed strong levels of eGFP. Infected caterpillars produced very little virus in the hemolymph, had very low levels of infected hemocytes, and underwent extensive tissue damage to the fat body and body wall epithelium. These areas of damage correlated very well with eGFP expression and extensive TUNEL staining, indicating that virus replication induced high levels of apoptosis in these tissues. In contrast, *S. frugiperda* larvae infected with wild type AcMNPV contained very few TUNEL-positive cells and exhibited normal fat body pathology. These results represent the first *in vivo* evidence of induction of apoptosis by a baculovirus, and strengthen the hypothesis that apoptosis can serve as an effective anti-viral defense in insects. However, the apoptotic response can be detrimental to the host if very high doses of virus are used.

Molecular characterization of an *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) mutant and the *iap-3* gene of AgMNPV

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We have previously isolated an AgMNPV mutant virus (vApAg) capable of inducing apoptosis in an *A. gemmatalis* cell line (UFL-AG-286) and replicating normally in another cell line (BTI-Tn-5B1-4), derived from *Trichoplusia ni*. Light and electron microscopy showed that the mutant virus induces membrane surface blebbing and apoptotic body formation in UFL-AG-286

cells. Despite massive apoptosis, the virus was still able to produce some progeny virus and therefore, we concluded that the apoptosis induced by the vApAg virus was partially blocked or delayed. In order to characterize the mutant virus, we digested the viral DNA with some restriction enzymes and found some differences in the restriction profile, when compared with the wild-type. Baculovirus are known to have 2 genes able to block apoptosis in insect cells (*p35* and *iap*). P35 is a broad-sprectrum caspase inhibitor and viral IAPs block the processing and activation of caspases. We have designed oligonucleotide primers, based on the sequence of different *iap* genes from baculoviruses and used in a PCR reaction with the AgMNPV DNA. A fragment of 600 bp was amplified and shown to have homology to the *iap-3* genes of baculoviruses. This fragment was used as a probe to locate this gene in the AgMNPV genome. The AgMNPV *Hind*III-B fragment hybridized with the probe and 2 *Pst* I subfragments, containing the *iap-3* gene, were cloned and the AgMNPV *iap-3* gene sequenced. Computer analysis have shown that the AgMNPV *iap-3* ORF has 861 nucleotides, coding for a protein of 287 amino acids. Two BIRs and 1 ring finger motifs were located in the sequence. We also designed oligonucleotide primers for the full amplification of the gene using the mutant vApAg and wild-type DNAs. The PCR from the wild-type DNA amplified a 0.9 kb fragment and the mutant DNA amplified a 3.5 kb fragment, indicating that the mutant virus had a insertion of 2.5 kb into the *iap* ORF. The 3.5 kb fragment was cloned and is now being sequenced in order to determined the origin of the insertion.

Baculovirus apoptotic suppressors trigger global translation arrest in AcMNPV-infected Ld652Y by stimulating viral DNA replication

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The gypsy moth, *Lymantria dispar*, and at least two cell lines derived from it, Ld652Y and LdFB, are non-permissive for AcMNPV infection. In both of these cell lines AcMNPV infection results in global protein synthesis arrest by 12-16 h pi. We previously isolated an LdMNPV gene, *hrf*-1, that when incorporated into the AcMNPV genome, precludes global protein synthesis arrest in Ld652Y cells and promotes AcMNPV replication in Ld652Y cells and *L. dispar* larvae. The hrf-1 conceptual protein has no motifs or known homologues that suggest how it prevents translation arrest and alters AcMNPV host range. Therefore we have sought to understand the mechanism(s) responsible for translation arrest in AcMNPV-infected Ld652Y cells as a means to gain insight into hrf-1 function. Infecting Ld652Y cells with wt AcMNPV results in a dramatic translation arrest, whereas infecting Ld652Y cells with v□p35, AcMNPV lacking the apoptotic suppressor p35, or with AcMNPV bearing a mutant p35 that cannot inhibit apoptosis does not. Recombinant AcMNPV bearing alternative baculovirus apoptotic suppressors, Cpiap, Opiap, or p49, in place of p35 also resulted in dramatic translation arrest in infected Ld652Y cells. In contrast, peptide caspase inhibitors blocked apoptosis but did not trigger global translation arrest in v□p35-infected Ld652Y cells. We previously found that treatment of AcMNPV-infected Ld652Y cells with the DNA synthesis inhibitor aphidicolin attenuated translation arrest when the virus carried any of the baculovirus apoptotic suppressors, translation arrest was not observed if the virus either lacked an apoptotic suppressor or carried the LdMNPV *hrf*-1 gene. Together these data suggested that blocking apoptosis per se was not sufficient to trigger translation arrest and that viral DNA replication or late gene expression might play a role. When Ld652Y cells were infected with temperature-sensitive-mutants defective in viral DNA synthesis and late gene expression, ts8 and ¯p35ts8, translation arrest occurred in ts8 and not in ts8¯p35-infected cells. Translation arrest correlated with the onset of viral DNA replication. Dot-blot analysis confirmed the correlation between translation arrest and viral DNA replication. These data suggest that viral DNA replication and/or late gene expression triggers global translation arrest.

Induction of apoptosis by wild-type AcMNPV in infected *Spodoptera litura* larvae blocks viral propagation

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Direct evidence of *in vivo* apoptosis of *Spodoptera litura* larva were demonstrated by hemocoel inoculation with wild-type (wt) *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) budded virus (BV). In sharp contrast to the natural infection, the cadavers did not melt, liquefy and melanize. Typical morphological changes of insect hemocytes apoptosis post infection (p.i.), including blebbing of cell surface, chromatin margination and condensation, vacuolization of the cytoplasm and formation of apoptotic bodies, were observed by light and electron microscopy. Total DNAs extracted from virus-infected hemocytes showed DNA ladders. Cleavage of chromatin DNA by endogenous endonucleases were detected in most tissues cells, such as epithelial cells and fat body cells, using terminal dUTP nick end labeling (TUNEL) assay. Virogenic stroma (VS) and viral nucleocapsids could be seen in the nuclei of a few hemocytes. Yields of BV and ODV (occlusion derived virus) produced from the infected larvae were very low. Our data suggests that host apoptotic response to viral infection blocks AcMNPV spread at the organismal level and that apoptosis could be a host range limiting factor for baculovirus infections. The study also provides direct evidence of the implication of *in vivo* apoptosis in viral replication and pathogenesis of baculoviruses.

Identification of six *Autographa californica* M NPV early genes that mediate nuclear localization of G-actin

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Nuclear filamentous actin (F-actin) is required for nucleopolyhedrovirus (NPV) progeny production in NPV-infected, cultured lepidopteran cells. We have

determined that monomeric G-actin is localized within the nuclei of host cells during the early stage of infection by *Autographa californica* M nucleopolyhedrovirus (AcMNPV). Using a library of cloned AcMNPV genomic fragments along with a plasmid engineered to express eGFP-*B. mori* actin in transient transfection experiments, we have identified six AcMNPV early genes that mediate nuclear localization of actin in TN-368 cells: *ie-1*, *pe38*, *he65*, *Ac004*, *Ac102*, and *Ac152*. Within this subset, *ie-1* and *pe38* encode immediate-early transcriptional transactivators, *he65* encodes a delayed-early product, and the products encoded by *Ac004*, *Ac102*, and *Ac152* are uncharacterized. We found that when driven by foreign promoters, *ie-1*, *pe38* and *Ac004* had to be expressed prior to *Ac102* or *he65* for nuclear actin to accumulate, and that expression of *Ac152* was no longer required. These results and others suggested that the product of *Ac152* was a transactivator (directly or indirectly) of both *Ac102* and *he65*, and that recruitment of actin to the nucleus was a temporally regulated process. Determining the functions of each of the six AcMNPV gene products with respect to our assay should provide valuable clues to basic cellular mechanisms of actin regulation, and how AcMNPV infection affects them.

Interaction of HaNPV capsid protein with host actin

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Toward constructing a *Heliothis armigera* cell complementary DNA (Ha-cDNA) library, using *Heliothis armigera* nuclear polyhedrosis virus (HaNPV) capsid protein VP39 as bait, the actin gene was isolated from the Ha-cDNA library with the yeast two-hybrid system. This demonstrated that HaNPV VP39 could interact with its host actin in yeast. In order to corroborate the interaction in the host cell, we then fused the HaNPV *vp*39 gene with green fluorescent protein (GFP) gene in plasmid pEGFP39 and expressed the fusion protein in the Hz-AM1 cell line under the control of the AcNPV immediate early gene (IE1) promoter. Observation under fluorescence microscopy showed that green fluorescence began to appear in the host cell 9hr after being transfected with pEGFP39 and that actin cytoskeleton, which was stained by tetramethy rhodamine isothicyanete (TRITC)-labeled phalloidin (red fluorescence), began to rearrange at the same time. Actin cables first formed in the cytoplasm, subsequently the cell rounded as the actin aggregated throughout the cell. Further observation indicated that green and red fluorescence always appeared in the same location of the cell demonstrating that HaNPV VP39 protein could combine with host actin and that this combination resulted in the actin cytoskeleton rearregement after the HaNPV infected *Heliothis armigera*.

Study of the effect of deletions of ORF145 and ORF150 11K gene homologues in *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV)

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Homologues of the "11K" gene have been shown to be encoded by all lepidopteran baculoviruses sequenced to date. The AcMNPV genome contains two such homologues, orf145 and orf150. These genes encode for polypeptide products of 90 and 100 amino acid residues, respectively, each of which migrates at the expected M_rs of about 11000, by western blot analysis. The gene products contain an hydrophobic N-terminal domain similar to that of signal peptides and a "C6" motif that contains six cysteine residues in a conserved spacing. This motif is found in proteins that interact with chitin in various ways, but neither of the ORF145 or ORF150 gene products were found to bind to chitin *in vitro*, despite testing different forms of chitin and multiple binding conditions. The intracellular localization of orf145 and orf150 proteins during AcNPV infection of Sf21 cells was determined by immuno- electron microscopy. Additionally, single deletion mutants of either gene, and a double deletion mutant have been successfully isolated. These deletion mutants were assessed *in vivo* for infectivity to *Trichoplusia ni* and *Heliothis virescens* larvae.

Thursday, 8:00 - 10:00
Iguaçu I
CONTRIBUTED PAPERS - Fungi 3
Chair: J. Pell

Quantitative measurements for the fecundity of *Myzus persicae* apterae infected by the entomophthoraceous fungus *Zoophthora anhuiensis*

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To quantify the effect of the entomophthoraceous fungus *Zoophthora anhuiensis* (Li) Humber on the fecundity of aphids, newly emerged apterae of the green peach aphid *Myzus persicae* (Sulzer) were exposed to a shower of about 60 spores/mm^2 from sporulating mycelial mats of the fungal species. Based on daily records on aphid deaths after exposure, a latent period for *Z. anhuiensis* to kill the apterous adults was on average 7.2, 5.3, 4.9, and 3.9 days at 10, 15, 20, and 25°C, respectively. These estimates were significantly correlated to temperature (r^2=0.94). During the latent periods at the four temperature regimes, each infected apterae on average produced 7.97, 11.20, 11.86, and 11.20 nymphs. In contrast to those unexposed to the spore shower, the fecundity of the infected apterae decreased by 56.45, 41.58□39.98□and 49.02%, respectively. Based on life-fecundity table established with daily observations during the latent periods, the net reproductive rate (R_0) of the infected apterae dropped by 58.32□45.54, 43.11□and 50.84% at the temperature regimes considered. These contributed to reduction of their intrinsic increase rate (r_m) by 24.28, 16.98, 14.12, and 20.13%, respectively. The results indicate that infection of entomophthoraceous fungi such as *Z. anhuiensis* is of great significance in suppression of aphid population increase.

Time-specific infection rate of *Beauveria bassiana* conidia on *Myzus persicae* under controlled conditions

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Conidial suspension (5×10^6 conidia/mL) of the entomopathogenic fungus, *Beauveria bassiana* BB8702 was used to inoculate newly emerged apterous adults of the green peach aphid, *Myzus persicae* (Sulzer), on detached cabbage leaves in order to measure time-specific infection rate of the fungal agent. The aphids with attached conidia were disinfected using 0.2% chlorothalonil solution at intervals of 8-56 h after inoculation and then maintained at 10 and 20°C for daily observation. There was a significant difference ($P<0.05$) in mortality attributed to the fungal infection among the time intervals within 56 h at 10°C or within 40 h at 20°C after inoculation. In comparison with the control (not treated with 0.2% chlorothalonil after inoculation), time-specific infection rate at 10°C was estimated as 22.9, 48.8, 64.9, 80.4, 72.7, and 98.3% within 8, 16, 24, 32, 40, and 56 h after inoculation, respectively. The same estimates at 20°C were 31.6, 48.8, 58.6, 86.9, 97.2, and 98.7%, respectively. The results indicate that the first 24-h period after inoculation was crucial to the infection of *B. bassiana* to *M. persicae*. Effective infection during this specific period accumulated to about 60% at 10-20°C. This finding is useful for understanding the significance of *B. bassiana* as microbial control agent against aphids.

Phenoloxidase activity of diamondback moth *Plutella xylostella* and its changes during invasion of *Zoophthora radicans*

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Prophenoloxidase-activating system in insects plays an important role in their recognizing invasion of various pathogens. In the present study, the phenoloxidase activity detected was much higher in the precipitated cell debris of hemocytes of the diamondback moth *Plutella xylostella* than in the supernatant of hemocyte lysate (SHL), but was hardly detected in the plasma of the insect species. Moreover, the prophenoloxidase in hemolymph was activated by laminarin (a Γ-1,3-glucan), leading to increase of phenoloxidae activity. Adding the plasma to SHL containing laminarin, the activity of the phenoloxidase was greatly enhanced by four or five folds. This suggests that the prophenoloxidase be located primarily on the membrane of the hemocytes and less inside. Apparently, the plasma had a recognition protein that in the presence of laminarin could rapidly transfer the prophenoloxidase to the phenoloxidase in the hemocytes despite a hardly detected activity of the phenoloxidase. While *P. xylostella* larvae were infected by *Zoophthora radicans* after exposed to spore shower, a largely enhanced level of the phenoloxidase activity was detected in their hemolymph. During the first three-day period after exposure, the phenoloxidase activity in larval hemolymph steadily increased, peaked on day 3, and then decreased. The highly enhanced phenoloxidase activity in larval hemolymph in the process of *Z. radicans* invasion into *P. xylostella* larvae implies that the phenoloxidase is involved in *P. xylostella* defense. Better understanding this defensive mechanism would facilitate consideration of *Z. radicans* against *P. xylostella* and warrants further study.

Temperature gradients and viability of *Beauveria bassiana* conidia on leaf surfaces

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Persistence of fungal propagules in the canopy zone has significant importance for tactics and strategies of microbial pest control. Sometimes, the active ingredients of microbial formulations are quickly inactivated by different environmental influences including extreme temperatures, UV-radiation, phytotoxins, activity of epiphytic microorganisms etc. Ambient temperature is the most important factor influencing the viability of fungal propagules and productive interaction of the fungus with noxious macroorganisms. Based on these circumstances we estimated the impact of different temperatures on the viability of *Beauveria bassiana* conidia on the leaf surfaces of chrysanthemum. After spraying with conidia suspension, the plants were held at 15°C, 20°C, 25°C and 30°C. Viability of conidia was estimated immediately after spraying and also at 5, 10, 15 and 20 days. Leaf prints from the top and bottom sides of the leaves were prepared separately using the scotch tape method. These prints were placed on a nutrient medium for 24 hours at 20°C and viability was calculated directly on the scotch tape. This method is simple and rapid to apply. It was established that after 5 days more than 50% of conidia were inactivated on the upper leaf side and after 10 days, from only 1.5 to 7.0% conidia survived. A high level of viability for conidia located on the under surfaces of the leaves was observed during all 20 days of experiment. The reduction of viability after 20 days was following: 69.02±3.54 (15°C), 53.45±3.27 (20°C), 41.27±7.9 (25°C) and 39.65±8.15 (30°C). Statistical analysis indicated no significant differences of data within a temperature.

Molecular studies on intra-specific variation in the aphid pathogenic fungus *Erynia (= Pandora) neoaphidis*

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Ongoing research at Rothamsted is aimed at developing managed field margins as natural refugia for the aphid pathogenic fungus *Erynia neoaphidis*. For conservation biocontrol of pest aphid species it is important to be able to track the movement of particular isolates between the margin and adjacent crops using molecular techniques.
Random amplified microsatellite (RAMS) alternatively known as ISSR (inter simple sequence repeat) markers, have been used to detect genetic variation both in plants and fungi and the technique is highly reproducible. The aim of this study was to determine whether these methods, together with the use of random primers, were useful in detecting genetic variation among *E. neoaphidis* isolates.Using three RAMS primers, two primers based on the enterobacterial repetitive intergenic consensus (ERIC) sequence, two random primers and four RAPD primers, intra-specific variation was detected amongst 30

E. neoaphidis isolates. Various levels of polymorphism were detected depending on the primer used. Cluster analysis based on the amplified products was carried out to establish relatedness of the isolates. These techniques will now be used to develop diagnostic markers for tracking isolates.

A tiered approach for evaluating *Erynia neoaphidis* isolates against seven aphid species

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A tiered, or hierachical, testing system was devised for evaluating *E.neoaphidis* isolates from different aphid hosts, based on previous work by Milner (1992) with *Metarhizium* spp. The aims were to formalise a selection process for *E. neoaphidis* isolates and compare susceptibility of seven aphid species to infection. The aphids used were the legume aphids *Aphis fabae* and *Acyrthosiphon pisum*; the brassica aphids *Brevicoryne brassicae* and *Myzus persicae*, and the cereal aphids *Metopolophium dirhodum*, *Sitobion avenae*, and *Rhopalosiphum padi*. Isolates of *E. neoaphidis* were obtained from the Rothamsted and USDA-ARS culture collections. In Tier 1, 20 *E. neoaphidis* isolates were tested in unreplicated three-dose screens against the seven aphid species. Scoring maximum infections provided three categories or groups of isolates. Isolates with high scores were considered to have a high infectivity and/or infect a large number of the seven pest aphid species tested, whereas isolates with low scores were considered to have low infectivity and/or infect a small number of the seven aphid species. *Acyrthosiphon pisum* was the most susceptible species while *R. padi* was least susceptible to infection. In Tier 2, isolates NW314, NW316 and NW327 were used in dose-response experiments with *A. pisum*, *M. dirhodum* and *M. persicae*. Probit analyses with the three isolates resulted in median lethal doses (LD_{50}) of 8-105 conidia mm^{-2} for *A. pisum*, 38-92 conidia mm^{-2} for *M. dirhodum* and 105-626 mm^{-2} for *M. persicae*. For Tier 3 bioassays, the LD_{50} values obtained with the three isolates were tested against *A. fabae*, *B. brassicae*, *R. padi*, *S. avenae*, with *M. persicae* as the reference aphid species. Logistic regression analysis indicated *M. persicae* was more susceptible to *E. neoaphidis* infection compared with the four other aphid species, particularly *B. brassicae* and *R. padi*. The tiered testing procedure outlined above was suitable for testing and comparing the virulence of isolates against several host species. Obviously, other criteria besides virulence are necessary to develop a holistic selection process, such as the ability to withstand cryopreservation, growth characteristics on artificial media, temperature optima, pesticide tolerance, effects on non-targets, spore production and transmission efficiency.

Effect of dissolved oxygen concentration in submerged culture of entomopathogenic fungus *Paecilomyces fumosoroseus*

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Entomopathogenic fungi *Paecilomyces fumosoroseus* is used to control insect-pest populations around the world. The effect of dissolved oxygen on germination, growth and sporulation in submerged cultures with a medium that promotes conidiation was studied. Dissolved oxygen concentration (dO_2) was monitored with an electrode and controlled to 0%, 5% and 100% saturation. Although the electrode reading was constant, dO_2 in the bulk gradually changed from 0.3 $mg \cdot L^{-1}$ to 3.3 $mgO_2 \cdot L^{-1}$ (set point: 5%dO_2) and from 5.9 $mg \cdot L^{-1}$ to 45.9 $mg \cdot L^{-1}$ (set point: 100%dO_2) as free mycelia concentration increased. It was found that aerial conidia germination was delayed in cultures at 0.3 $mgO_2 \cdot L^{-1}$ compared with those at 5.9 $mgO_2 \cdot L^{-1}$. Through all the fermentation there were more germinated propagules at 100%dO_2 than at 5%dO_2, but germination was less than 10% of total propagules in both conditions, and the difference could not significantly affect propagule productivity or accumulation. It was also found a one to two-fold increase in conidia/blastospore ratio at 100%dO_2. Vacuolated, empty and deformed mycelium was observed at 100%dO_2, as a consequence of high dissolved oxygen concentrations. Experimental biomass yields at 100%dO_2 were of 64% compared to those obtained at 5%dO_2. There was no difference in maximal volumetric productivity of propagules, but maximal propagules concentration and maximal conidia concentration were $8x10^7$ $pro \cdot mL^{-1}$ and $6x10^7$ $con \cdot mL^{-1}$, two and three-times higher than at 100%dO_2. The experimental system showed the great deviations that can occur in fungal fermentations between the dissolved oxygen concentration detected by the electrode and the bulk dissolved oxygen concentration, which can be higher than 900%. **(STUDENT PAPER).**

Thursday, 8:00 - 10:00
Iguaçu II
CONTRIBUTED PAPERS - Virus 3
Chair: J.L.C. Wolff

Involvement of IE0 and IE1 in the replication of the *Autographa Californica* nucleopolyhedrovirus in *Spodoptera littoralis*

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Infection of *Spodoptera littoralis* SL2 cells with the *Autographa californica* nucleopolyhedrovirus (AcMNPV) results in abortion of the viral infection in contrast, to *S. littoralis* nucleopolyhedrovirus (SlNPV) infection. By transfecting SL2 cells with wild type AcMNPV DNA and cosmids from a cosmid library representing the entire SlNPV genome, we isolated vAcSL2, a recombinant AcMNPV able to replicate and form polyhedra in SL2 cells. The vAcSL2 genome contained a "foreign" DNA insert located between the AcMNPV genes *me53* and *ie0*Æ Concomitantly we observed a dramatic reduction in expression of the *ie0* product IE0 that correlated with high steady state levels of IE1 (product of the immediate early gene1©Æ Targeted mutagenesis of the *ie0* promoter replicated the phenotype of the vAcSL2 mutation. The above data suggests that IE0 modulates the "functional" IE1 steady state levels and that efficient replication of AcMNPV in SL2 cells require overexpression of the major viral transactivator IE1Æ

Interchange of OpMNPV and AcMNPV replication factors restores the replication function of chimeric IE1 proteins

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The regulatory protein IE1 of the baculovirus *Orgyia pseudotsugata* multiple nucleopolyhedrovirus (OpMNPV) contains a 135 amino acid acidic activation domain (AAD) that is essential for both transcriptional transactivation and DNA replication. The AAD contains separable domains required for DNA replication and transcription. Replacement of the OpMNPV IE1 AAD with the evolutionarily related domain from *Autographa californica* MNPV (AcMNPV) IE1 produces a chimeric protein IE1-AcAD that is a potent transactivator that surprisingly is unable to support OpMNPV transient DNA replication. This suggests that the AAD replication domain contains a host or virus specific determinant. One possibilty is that for replication the AAD interacts specifically with one or more of the essential viral factors required DNA replication, that is, LEF-1, LEF-2, LEF-3, DNA POL, or HEL. In this study we determined if we could restore the ability of the chimeric OpMNPV IE1-AcAD to support DNA replication by switching OpMNPV replication factors with the AcMNPV factors. Exchanging specific OpMNPV replication factors with the corresponding AcMNPV replication factors permitted the chimeric protein to regain functionality and support DNA replication. These results suggest that the IE1 acidic domain has specific interactions with viral replication factors, and may function as a origin binding protein in the viral replisome.

Deletion of the baculovirus non-*hr* origin of DNA replication prevents the accumulation of defective interfering particles

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The generation and accumulation of defective interfering particles (DIs) was studied by serial passage of *Spodoptera exigua* MNPV in Se301 insect cells. Sequences containing the non-*hr* origin of DNA replication (*ori*) became hypermolar in intracellular viral (ICV) DNA within 10 passages, concurrent with a dramatic drop in budded virus (BV) and polyhedra production. These predominant, non-*hr ori*-containing DI sequences replicated in larger concatenated forms. They were generated *de novo* in Se301 cells upon infection with a bacterial clone of SeMNPV (bacmid). Sequence overlaps were identified at the junctions of the non-*hr ori* units within the concatemers, which may be involved in recombination events leading to the rapid generation of DIs. We hypothesized that deletion of the non-*hr ori* from the SeMNPV genome would enhance virus stability in cell culture. The non-*hr ori* was deleted by an "ET-recombination" protocol. Homologous recombination occurs in the bacmid host-strain (*E. coli* DH10B) between the bacmid and a PCR product containing a bacterial resistance gene and viral sequences flanking the site to be deleted. Deletion of the non-*hr ori*, which proved not be essential for baculovirus DNA replication, strongly enhanced stability of budded virus and polyhedra production upon serial passage in Se301 insect cells. This suggests that the accumulation of DIs containing reiterated non-*hr oris* is due to the replication advantage of these sequences. Since baculovirus non-*hr oris* share structural properties with consensus eukaryotic *oris*, these sequences might play a role in baculovirus persistence *in vivo*. The SeMNPV bacmid allows detailed studies on gene function by targeted mutagenesis in *E. coli*. **(STUDENT PAPER).**

Analysis of the impact of deletion of the LdMNPV p24, 25K FP, and PE genes on polyhedron synthesis and morphology

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During studies on the formation of *Lymantria dispar* M nucleopolyhedrovirus (LdMNPV) few polyhedra mutants, a virus that produced abnormal polyhedra was identified and designated as polyhedron formation mutant-2 (PFM-2). LdMNPV isolate PFM-2 generates polyhedra with an average diameter of approximately 4 microns (vs. 2 microns for wild-type polyhedra), that either lack or possess a fragmented polyhedron envelope, and that contain virions. The mutation responsible for the abnormal polyhedron phenotype was localized to the *p24 capsid* gene. In the present study we have extended our investigations on this ge
ne through analysis of a recombinant virus lacking the gene. In addition, interaction of the p24 gene product with other viral gene products that affect polyhedron synthesis or morphology was investigated. The morphology and number of polyhedra produced by the recombinant virus p24del, which lacks the *p24* gene, was the same as isolate PFM-2. This result suggests that the single amino acid change in the *p24 capsid* gene of isolate PFM-2 was sufficient to eliminate all function that impacts polyhedron morphology and synthesis. The recombinant virus p24del25KFP, which lacks the *p24 capsid* gene and contains a mutation in the *25K few polyhedra* gene, produced fewer polyhedra than either p24del or the 25K few polyhedra mutant isolate. The polyhedra lacked occlusions, and were larger than 25K few polyhedra mutant polyhedra and smaller than p24del polyhedra. The recombinant virus p24minusPEdel, which contains a mutation in the p24 gene and lacks the *polyhedral envelope* gene, produces polyhedra that exhibit the same morphology and the same size as p24del polyhedra.

Cloning *Panolis flammea* nucleopolyhedrovirus genotypes from mixed populations

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The baculovirus, *Panolis flammea* multi-nucleopolyhedrovirus (PaflNPV) is an insect-specific

pathogen, containing a 145 kb double-stranded DNA genome. It was originally isolated from pine beauty moth (*P. flammea*) larvae, during a natural epizootic in Scotland, and has since been utilized as a biological insecticide for controlling *P. flammea* infestations of lodgepole pine (*Pinus contorta*). Previously, *in vivo* cloning of wild-type PaflNPV using an alternative host, *Mamestra brassicae* has shown the virus to consist of twenty-five different genotypes. Genotypic variation is observed when the virus genomes are digested with restriction enzymes and fractionated using agarose gel electrophoresis. Most genotypic variation appears to be located in specific areas on the PaflNPV genome. Hybridisation analysis suggested that PaflNPV genotypes could be categorised into two classes, PaflNPV (A) and PaflNPV (B), depending on the size of the *Hin*dIII F fragment encoding the PaflNPV polyhedrin gene. When PaflNPV (A) and PaflNPV (B) strains are propagated together within *M. brassicae*, one genotype gradually becomes more dominant. In this study a wild-type PaflNPV population was passaged three times through different insect host species and the proportions of genotypes after each passage were quantified using hybridisation analysis. Again, certain genotypes gradually dominated the virus population depending on the insect host species infected. The role of these genotypes and why so many are maintained in the insect population is unknown, but it may be speculated that certain genotypes have a replication advantage when infecting certain hosts. This may have implications when using PaflNPV as a bio-control agent as the infectivity of a mixed PaflNPV population may be reduced when compared to a single genotype. However, isolating PaflNPV genotypes from mixed populations is time consuming and difficult. Therefore, a novel system for cloning PaflNPV genotypes from mixed populations is presented using a modified bacterial artificial chromosome.

Functional analysis of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus envelope fusion protein

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Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HaSNPV) is a group II NPV and has a novel budded virus (BV) envelope fusion protein (F protein). This protein is a functional analogue of GP64-like fusion proteins in group I NPVs, such as AcMNPV. The F proteins are involved in BV infection by interaction with cellular receptors, in cell fusion with host cell membranes, and in virus budding. GP64 is present in the BVs as disulfide-linked trimers. Group II F proteins are present in BVs as two subunits, F_1 (≈ 60 kDa) and F_2 (≈ 20 kDa), which are disulfide-linked. A cellular furin is responsible for the cleavage of a F protein precursor F_0 into F_1 and F_2. The cleavage is essential for BV activity (Westenberg *et al.*, J.Virol. 2002, 76: 178-184). The group II F proteins can replace GP64 in AcMNPV pseudotype viruses (Long *et al.*, J.Virol. 2002, 76: in press). So, only group II MNPVs have been investigated (SeMNPV ORF8; LdMNPV ORF130). Here we report the functional analysis of a F protein of a single nucleocapsid NPV, *Helicoverpa armigera* SNPV. The F analogue in this virus, HaSNPV ORF133 (Ha133), encodes a 80 kDa precursor protein; the two subunits, F_1(62kDa) and F_2 (18kDa), were present in the HaSNPV BVs by using two specific antibodies against F_1 and against F_2, respectively, raised in chicken. No oligomerized fusion could be found in HaSNPV BVs, a situation similar to Se8 but different from AcGP64. Carbohydrate staining indicated that F_1 and F_2 are glycosylated. This was confirmed by the use of tunicamycin (inhibitor of glycosylation) in HaSNPV-infected HzAM1 cells. Insertion of Ha133 into an AcMNPV GP64-null mutant and reintroducing the resulting bacmid into Sf9 cells showed, that Ha133 can rescue the infectivity of AcMNPV. This suggests that the SNPV F proteins are functional analogues of the group I GP64 proteins.

Genetic requirements for homologous recombination in *Autographa californica* nucleopolyhedrovirus

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It is known that baculovirus infection promotes high-frequency recombination between its genomes and plasmid DNA during the construction of recombinant viruses for foreign gene expression. However, little is known about the viral genes necessary to promote homologous recombination (HR). We developed an assay to identify viral genes that are necessary to stimulate HR. In this assay, we used two plasmids containing extensive sequence homology that yielded a visible and quantifiable phenotype if HR occurred. The plasmids contained the green fluorescent protein (*gfp*) gene that was mutated at either the N- or C-terminus and a viral origin of DNA replication. When the plasmids containing these mutant *gfp* genes were transfected into insect cells alone or together, few GFP-positive cells were observed, confirming that the host cell machinery alone was not able to promote high levels of HR. However, if viral DNA or viral genes involved in DNA replication were cotransfected into cells along with the mutant *gfp*-containing plasmids, a dramatic increase in GFP-positive cells was observed. The viral genes *ie-1*, *ie-2*, *lef-7*, and *p35* were found to be important for efficient HR in the presence of all other DNA replication genes. However, *ie-1* and *ie-2* were sufficient to promote HR in the absence of other viral genes. Recombination substrates lacking a viral origin of replication had similar genetic requirements for recombination but were less dependent on *ie-1*. Interestingly, even though HR was stimulated by the presence of a viral origin of DNA replication, virally stimulated HR could proceed in the presence of the DNA synthesis inhibitor aphidicolin.

Characterization of *Mamestra configurata* nucleopolyhedrovirus enhancin and its functional analysis via expression in an AcMNPV recombinant

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Viral enhancing factor (VEF) or *enhancin* has been identified in a number of *Granulovirus* (GV) species and is characterized by its ability to enhance the oral infectivity of *Nucleopolyhedrovirus* (NPV) in lepidopteran insects. Until now, LdMNPV was the only NPV shown to encode *enhancin* and it contains two copies of the gene. Here, we describe the putative *enhancin* gene from *Mamestra configurata* nucleopolyhedrovirus (MacoNPV), only the second NPV in which an *enhancin*-like ORF has been identified. The putative *enhancin* gene from MacoNPV has a typical baculovirus late promoter (ATAAG) 15 bp upstream from the ATG codon. The *enhancin* open reading frame encodes an 847 amino acid protein with a predicted molecular weight of 98kDa and contains a conserved zinc-binding domain (HEIAH) common to metalloproteases. The MacoNPV enhancin shows approximately 20% amino acid identity with other baculovirus enhancins. An AcMNPV recombinant, AcMNPV-enMP2, expressing the MacoNPV *enhancin* gene under control of its native promoter was developed and characterized. Northern blot analysis showed expression of *enhancin* from 24 through 72 hours post infection. In 2nd- instar *Trichoplusia ni* larvae, the LD_{50} of AcMNPV-enMP2 recombinant was 2.8 polyhedral inclusion bodies (PIB)/larva, 4.4 times lower than that of AcMNPV E2 wild type virus (12.4 PIB/larva). At biologically equivalent doses, i.e. LD_{90}, the survival time 50% (ST_{50}) of AcMNPV-enMP2 recombinant and AcMNPV E2 wild type viruses were not significantly different.

Thursday, 16:30 - 18:30
Iguaçu II
CONTRIBUTED PAPERS – Virus 4
Chair: A.S. de Cap

Origin and host range of the *Choristoneura fumiferana* defective nucleopolyhedorvirus, CfDEFNPV

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The *Choristoneura fumiferana* defective nucleopolyhedorvirus, CfDEFNPV, is intriguing for a number of reasons. (i) It was originally found to be unable to infect the spruce budworm natural host by the normal *per os* route. (ii) It is infective to a number of hosts only when virions are injected directly into the haemolymph. (iii) Total genomic sequencing did not reveal an obvious deletion to explain its aberrant properties. (iv) The genome appears to have all the properties typically of other baculovirus genomes. (v) Even though it was isolated from *C. fumiferana*, amino acid identity analyses have shown that CfDEFNPV was more closely related to NPVs from *Orgyia pseudotsugata* (OpMNPV) and from *Anticarsia gemmatalis* (AgMNPV) than to CfMNPV. AgMNPV infects an insect of tropical and subtropical regions. CfDEFNPV and AgMNPV were found to be so closely related in terms of amino acid identity and viral biology that they must be considered variants of the same virus. At some point in their evolution, the two viruses became associated with two unrelated hosts and environments. From data analyzed so far, it appears that their genomes have not grossly diverged as they evolved in two different natural hosts. It is also possible that the two variants have arisen from one parental virus that infected two different hosts occupying a common but narrow geographical area in North America.

Construction of HaSNPV polyhedra with polyhedrin-fusolin fusion protein

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Fusolin is a protein that forms the matrix of the spindle body in certain emtomopoxviruses. Reports showed that the formation of spindle bodies by fusolin protein is consistent with a role in enhancing baculoviral infection of insects. In this paper, a *polyhedrin-fusolin* fusion gene was introduced into the *egt* locus of the *Helicoverpa armigera* SNPV (*Ha*SNPV) genome to produce a novel recombinant baculovirus. A fusion gene was constructed by combining the HaSNPV *polyhedrin* and the CfEPV *fusolin*, where the stop codon TAA of *polyhedrin* was deleted and the start codon ATG and the 20 amino acid-long signal peptide sequence of the *fusolin* were truncated. A transfer vector pPXY4 was constructed which contained the fusion gene and a *lacZ* cassette in between of the upstream and downstream sequences of the HaSNPV *egt* gene. After co-transfection of pPXY4 with the wild-type HaSNPV DNA, recombinant viruses were selected, plaque purified and further characterized by PCR amplification and REN profile analysis. One of the recombinants, HaPXY4 was investigated for its infectivity to HzAM1 cells and *H. armigera* larvae. Results showed that HaPXY4 was able to form polyhedra within the infected cells, but most polyhedra contained less ODVs and there were unidentified black dots in the polyhedral matrix. Sometimes, the envelope of the polyhedra was not completed and some of the polyhedra had irregular shapes. The average size of the polyhedra produced by HaPXY4 was found to be smaller than that of the wild type virus. Western blot analysis indicated that the fusion gene was expressed in the infected cells and that the expression products were occluded into the polyhedra. Bioassays of HaPXY4 are now being conducted by using *H. armigera* larvae to determine the biological activity of the recombinant.

Further characteristics of developmental resistance of *Lymantria dispar* (Lepidoptera, Lymantriidae) to its baculovirus, *Ld*MNPV

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We previously described intrastadial developmental resistance of *Lymantria dispar* to *Ld*MNPV. Fourth instar larvae become progressively resistant to viral challenge from 12 hours post-molt until 72 hours post molt (4^{12} - 4^{72}), after which resistance decreases slightly until the 4^{120} stage. Developmental resistance to budded virus indicates a strong systemic resistance mechanism, which has not been reported for other Lepidoptera unless they are in the penultimate instar. We are currently exploring several facets of developmental resistance in *L. dispar*. We have conducted two types of experiments to corroborate the importance of systemic resistance in *L. dispar*. A fluorescent brightener, M2R, which damages the peritrophic membrane and appears to block sloughing of infected midgut cells, eliminated developmental resistance in orally challenged 4^{th} instar larvae at 250 OBs/larva (an LD80 in newly molted (4^0) larvae without M2R). In contrast, developmental resistance did occur against a viral dose about 90% lower (20 OBs/larva) in the presence of 1% M2R. M2R had no effect on sensitivity of larvae inoculated intrahemocoelically as 4^0s or at 24 h post-molt (4^{24}). We also partially transferred resistance against budded virus from 4^{48} to 4^0 larvae by hemolymph transfusion. These observations agree with our hypothesis that the mechanism of intrastadial developmental resistance in *L. dispar* is largely systemic. We are investigating the hypothesis that developmental resistance is partially mediated by juvenile hormone (JH). The JH agonist fenoxycarb synergized the activity of *Ld*MNPV against 4^{48} larvae. These results are consistent with a hypothetical role for JH in modulating intrastadial developmental resistance. Other experiments designed to investigate the roles of JH and ecdysone in this form of resistance are underway. Finally, we are conducting experiments to investigate whether the mechanism of developmental resistance in *L. dispar* is specific to *Ld*MNPV. We recently challenged a series of developmentally staged 4^{th} instar larvae with the entomopox virus *Amsacta moorei* (*Am*EPV) in an effort to determine if developmental resistance generalizes to non-baculoviral pathogens, and whether the pattern of resistance parallels that of *Ld*MNPV. Preliminary results indicate that there is a similar pattern of developmental resistance in *L. dispar* larvae intrahemocoelically injected with *Am*EPV extracellular virus (ECV) to that seen in *L. dispar* larvae injected with BV of *Ld*MNPV.

Vertical transmission of HaNPV Marked with Green Fluorescent Protein Gene in the Larvae of Cotton Bollworm and its Infectious Course in the host

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A recombinan *Helioverpa armigera* nuclear polyhedrosis virus (HaNPV) rHa-FGP carried green fluorescent protein (*gfp*) gene was constucted by transposon/shuttle system. Third instar larvae of cotton bollworm were fed with the virus rHa-FGP and reared in the laboratory for three successive generations without repeated infection. The percentages of larvae emitted green fluorescence in the three generations were about 34%, 20%, 8%, respectively. Recombinant baculovirus DNA was extracted from the larvae of each generation, which emitted green fluorescence, and analyzed by PCR and dot blot. The results indicated that the green fluorescent protein gene was expressed in every generation and baculovirus was transmitted vertically from parent to progeny. The infectious course of HaNPV in larvae of cotton bollworm was observed also using the virus rHa-FGP. The time at which the third instar larvae were placed on diet with the rHa-FGP was counted as 0h p.i. (hour post infection), samples were obtained at different time after the infection, then various tissues such as the hemocytes, epidermis, midgud and fat body were dissected and examined GFP expression under a fluorescent microscope. The results showed that the infectious course of HaNPV was: midgud hemocytes tracheae system fat body epidermis. Our results also supported the view that the budded virion could cause the secondary infection through the tracheae system. The expression of the very late gene (*gfp*) was observed in the midgut 12h p.i. other than 20h p.i., which indicated that there may be other approach for the virion to transfer from the midgut to the hemocytes more quickly.

Vertical transmission of nucleopolyhedrovirus in *Spodoptera exempta*

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The mechanisms by which pathogens persist in field populations is still poorly understood. Insect baculoviruses are primarily transmitted by horizontal transmission, however this does not explain how the virus persists between epizootics when host densities are low or when the insect is highly mobile. The African armyworm, *Spodoptera exempta*, is a migratory pest of graminaceous crops in Eastern Africa. A nucleopolyhedrovirus (SpexNPV) frequently causes epizootics in this species and is being studied as a potential control agent. As part of our studies on the ecology of this virus, we are examining whether SpexNPV is vertically transmitted in the field and the conditions under which this occurs. We have developed a polymerase chain reaction (PCR)-based method for the detection of viral DNA in infected insects targeting the polyhedrin gene and *lef8*, which is an essential gene required in late gene transcription. Larval and adult stages of laboratory cultures of *S. exempta* insects have been found to harbour a persistent baculovirus infection. Data will also be presented on the prevalence of sublethal SpexNPV infections in insects collected in outbreaks in Tanzania. *S. exempta* can exist in two morphs, solitary and gregarious (which causes the outbreaks). Previous work has shown that the morphs vary in their susceptibility to NPV, with the gregarious morph being 10 times more resistant to SpexNPV than the solitary morph. Preliminary studies using PCR indicate that in our laboratory colony, the insects reared gregariously have higher levels of sublethal NPV infection that solitary insects. Initial studies on the transmission of overt NPV disease in late instar larvae indicate that the virus is not vertically transmitted if insects are exposed to low levels of NPV.

Developments in the use of endemic baculoviruses of *Plutella xylostella* (diamondback moth) for control of DBM in East Africa

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Increasing resistance to chemical pesticides by insect pests is creating new opportunities for viral control world-wide. In Kenya the horticultural export market is seeking to replace its existing control systems based on chemicals with new biological approaches that minimise pesticide residues on produce. A major target is diamondback moth (DBM) as its control using chemicals alone is becoming increasingly expensive, uncertain and environmentally damaging. A project to develop non-chemical methods of DBM control on brassica crops in Kenya has been exploring the use of endemic pathogens as potential control agents. Initial surveys for endemic pathogens identified *Plutella xylostella* granulovirus (*Plx*yGV) on farms in Kenya. Subsequently 14 genetically distinguishable isolates were identified from field collected material by REN analysis. The distinct isolates were purified and dose range bioassays showed these isolates were pathogenic to Kenyan strains of DBM with $LC_{50's}$ varying from $2.36x10^6$ to $3.95x10^7$ occlusion bodies (OB) per ml for second instar DBM. One isolate (Nya-01) was selected and subsequently used for field trials in Kenya. The trials showed that un-formulated *Plx*yGV applied at weekly intervals at a rate of $3.0 x10^{13}$ OB/ha could control DBM on kale more effectively than a chemical insecticide (Karate, lamda-cyhalothrin). The field trials also showed that at an application rate of $3.0x10^{14}$ OB/ml, *Plx*yGV applied once a week gave rise to a significantly higher kale-yield over a growing season than Karate or any other treatment did. Subsequent laboratory bioassays have shown that simple formulation ingredients added to *Plx*yGV suspension can reduce the LC_{50} value by 10 to 100 times. As a follow on to this work, a project to establish a commercial viral production facility in Kenya to produce *Plx*yGV has also been approved and us due to start in 2002. This will be the first commercial production of a baculovirus-based product in Sub-Saharan Africa to date. Evaluation trials of *Plx*yGV are now also underway in West Africa with a view to similar commercialisation. If successful this initiative should advance prospects for commercialisation of other viral pesticides in Africa and world-wide.

Research on the environmental release and ecological effects of gene manipulation bacoluvirus pesticide application in vegetable pests control

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The multiple-character recombinant bacoluvirus vAcphBtTPE with polyhedrin-positive and polyhedra envolope-negative and expression of truncated CryIA(b) gene from *Bacillius thuringiensis* was constructed successfully. With the action of the truncated endotoxin crystal protein and improvement of the speed of viorons release in midgut, vAcphBtTPE showed more efficiency on pest control than wild type AcNPV. Its LC50 value was 50% that of wtAcNPV, and the LT_{50} was 3 days less than that of wtAcNPV. At the same time, a series of toxicity tests of the recombinant bacoluvirus pesticide had done on the rabbits rats pigeons and fishes. The results indicated the pesticide had no harm to the animals, and it was safe in application in the vegetable pests biological control. In order to learn its ecology in the field, we conducted a 70 ha. pesticide release trial from 1998 to 2001,which indicated that recombinant AcNPV could greatly reduce population of target insect, while did not damage the structure of insect community in the field. Upon application in cauliflowers, cabbages and cowpeas, the insect community diversity and evenness went up gradually, showing that its application stabilized insect community. Insect community similarity of spraying recombinant AcNPV to no-spray was higher than that of spraying recombinant AcNPV to chemical insecticide, demonstrating that recombinant AcNPV do little harm to pest enemy and eco-system, and that it can balance insect community, not resulting in pest recurrence. 1,5,9 days after release, target gene of recombinant AcNPV was detected in leaf sample by PCR, whereas from 5 to 17 days after release, target gene was detected in larvae sample by the same methods, with no target gene being detected in soil and water sample. Upon application and 3,6,9,12 days after application, contaminated leaf sample were taken for bioassay by 3rd instar Spodoptera exigua, with the pest mortality being 89,74,58,26,7% correspondingly, which justified that environmental factors such as ultraviolet rays, rains, dews and wind have significant effect on its virulence. The yield of cauliflower and cabbage sprayed with recombinant AcNPV increased 14.1% and 26.4% respectively than that of farmer practice. The nutrient ingredients and quality of vegetables was not damaged after application. An examination report by food quality administration of Agricultural Department of China indicated that the nutrient ingredients such as carbohydrates, amino acids, proteins, vitamin C and calcium of cabbages sprayed with recombinant AcNPV was the same as that sprayed with water. There is still no story about unhealthy effect after eating those vegetables by volunteers and consumers.

Incidence and impact of *Entomophaga aulicae* and a Nucleopolyhedrovirus in an outbreak of the whitemarked Tussock Moth (Lepidoptera: Lymantriidae)

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In Nova Scotia, the whitemarked tussock moth, *Orgyia leucostigma* Fitch, periodically erupts in outbreaks that typically last 3-5 years. Population changes during a recent outbreak were monitored by means of aerial defoliation surveys and fall egg mass surveys conducted between 1997 and 2000. The area of moderate-severe defoliation increased rapidly from ~250 ha in 1996 to hundreds of thousands ha in 1998, and then dropped to ~41 000 ha in 2000. The total infested area (> 0.01 egg

masses per three *Abies balsamea* L. branches) decreased from ~1.4 million ha in 1997 to ~547 200 ha in 1998 and ~12 400 ha in 1999. The collapse of larval populations during 1998 was associated with widespread prevalence of a singly embedded nucleopolyhedrovirus (NPV) and *Entomophaga aulicae* (Reichardt in Bail) Humber, which were found to infect >75% of larvae in many sites throughout central and northern Nova Scotia when sampled in late-July and August 1998. Pathogen impact on larval survival was studied in 1999 in a persisting pocket of moderate infestation (2 sites in Hants County). Larvae were collected every 3 days from balsam fir branch samples between 17 June and 21 July and reared to determine cause of death. The two pathogens together accounted for at least 50% of cohort mortality, calculated as marginal mortality rates according to Royama (2001). Although cohort mortality due to disease on balsam fir was significantly correlated with the between-generation reduction in mean egg mass density, overall pathogen-induced mortality was not high enough to drive the populations into an endemic state, and a moderate infestation persisted into 2000.

Thursday, 16:30 - 18:30
Cataratas I
CONTRIBUTED PAPERS - Bacteria 4
Chair: J.E. Ibarra

Phylogenetic relationship between the *Bacillus thuringiensis* type strains, based on the sequence of the flagellin gene

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During the last 40 years, strains of *Bacillus thuringiensis* have been serologically characterized and identified according to the cross-reaction of specific anti-sera developed from flagellin antigens. This technique has been widely used to differentiate between strains and was once the bases for sub-specific classification of the species. Unfortunately, there are several constrains that limit the technique, like: a)the cross-reactivity with some *B. cereus* strains; b) the so-called "auto-agglutination" response of some strains; c) the impossible serotyping of non-flagellar (or poorly flagellar) strains; and, more significantly, d) the impossibility of establishing phylogenetic relationships between serovars. These reasons have promoted the development of alternative techniques that may overcome the shortcomings of serotyping, as well as make the typing of strains available to many laboratories. These alternative techniques are based on several molecular methods, like various RFLPs, PCR-based techniques, and sequence analyses. This contribution deals with the development of a typing technique that tries to combine the advantages of serotyping and sequence analysis. For this purpose, flagellin genes from all the serovar type strains were partially sequenced from amplicons generated by primers designed from the highly conserved termini of the genes. Sequencing was followed by sequence analysis and the development of phylogenetic trees. Results indicated that, although some sequences showed a very high degree of identity, none showed identical sequences. Also, as many other flagellin genes, regions at the 3' and 5' ends of *B. thuringiensis* flagellins are very conserved, while the internal region is highly variable. It was clear that flagellins from *B. cereus* and *B. thuringiensis* are closely related, as amplicons were obtained from two out of three *B. cereus* strains, using the same primers designed for *B. thuringiensis*. Also, their sequences intermingle with those from *B. thuringiensis* strains, in the phylogram. On the contrary, flagellins from *B. licheniformis*, *B. megaterium*, *B. mycoides*, and *B. subtilis* may not be that closely related to those from *B. thuringiensis*, as no amplification was detected from these species. Sequence diversity between the serovars of *B. thuringiensis* was high. Even the amplicon obtained from PCRs varied from 800 to 1200 bp. The best phylogram obtained from the best alignment shows an arrangement of discrete groups of serovars. Serovars within the same serological group (i.e. H3, H5, H11, H20, etc.) also grouped together in the phylogram.

Molecular analysis of a brazilian *Bacillus thuringiensis* strain collection*

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Brazil is located in a transition zone between tropical and subtropical regions and contains a rich and unique biodiversity. The genetic diversity of a brazilian *Bacillus thuringiensis* strain collection was assessed by PCR analysis of the *cry* genes, ribotyping, RAPD, Enterobacterial Repetitive Intergenic Consensus (ERIC) and Repetitive Extragenic Palindromes (REP), by isoenzymes and plasmid patterns. Strains containing *cry*1 genes were the most abundant in our collection (48%). *B. thuringiensis* strains harboring *cry*11 and *cyt* genes represented 7.0% of the strains, and 2% of the strains containing *cry*8 genes. Ribotyping and RAPD fingerprints discriminated the strains, showing high genetic variability. Much of the results involving the zymograms and the rep-PCR results provided material for the description of some putative special strains. The comparative analysis of the plasmid content of the 95 field isolates was done, suggesting heterogeneity in the plasmid DNA content from the different *B. thuringiensis* isolates. The analysis of these results showed no correlation between the origin of the *B. thuringiensis* strains and distribution of genetic parameters that were considered.

Could the low concentration of Cry protein be the consequence of accumulation of citrate or lactate in fed batch cultures ?

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The effect of accumulation of citrate and lactate on expression of Γ-galactosidase and final spore count were investigated in batch (BC) and fed-batch cultures (FBC), using a *Bacillus thuringiensis* HD73 Cry (-)BPHT1KA strain with the fused to *lacZ* plasmid pHT1KAc containing the promoter of *Cry*1Ac plus 1000 pb upstream. Time courses of cell growth, spores, lactate,

citrate concentrations and -galactosidase activity were investigated. When feeding log phase culture (3h batch) with concentrated medium during 12 hours at 83.3 mL per minute, the culture accumulated citrate and lactate up to 18 and 10 g/L respectively. The cell concentration increased up to 7 times compared to BC and ¯-galactosidase activity increased up to 6 times, but the final spore count were only one half the batch concentration. To study the effect of citrate and lactate accumulation over the final spore count, -galactosidase and cell number, a pulse of 30 g or 20 g of lactate was added to a 3 h batch culture. A BC without pulse was used as control. When lactate was added neither the final spore count or cell growth were affected, but the ⌜-galactosidase activity was increased up to 30%. However, the addition of citrate had a negative effect on the sporulation only 12% of bacilli sporulated and one half of them released a spore. Also ⌜-galactosidase activity, it diminished 12%. No matter these decreases, the maxim cell count rose up to 30%. The obtained results suggested no relationship between the end of the sporulation process and the expression of *Cry*1Ac gene, because citrate affected dramatically the final spore count, both in the BC and FBC, but it did not affect l*acZ* gene expression. **(STUDENT PAPER).**

Does exist a relationship between enthalpy consumed, PHB accumulated and Cry production in *Bacillus thuringiensis*?

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Many different parameters have been reported to affect the toxicity and crystal protein production by *Bacillus thuringiensis* in sumerged culture: the strain, the culture parameters and conditions and the numbers of *cry* gene copies. Aim of this work was to verify the effect of intracellular energy accumulated during vegetative growth on Cry formation. From energy balance, a relationship between the quantity of cristal protein synthetisized and accumulated energy (ΔH_D) by *B. thuringiensis* during its growth, was established, considering as initial stage the beginning of fermentation and as final stage, the moment when first spores were detected. Poly-β-Hydroxybutirate (PHB) has been reported to be a carbon and endogenous energy resource for many microorganisms and presumably the genus baillus use this polymer during esporulation. Therefore, PHB quantification during fermentation was made and a relationship was established between this polymer and the quantity of Cry protein synthesized. Our results shows that maximun PHB accumulated is proportional to ΔH_D and to the Cry protein production. We conclude that PHB is one of the principal energy resource during sporulation and Cry production in cultures with a initial C:N ratio of 7:1, varying glucose from 13.4 to 68.5 g/l and the initial total nutrients concentration from 40 to 150 g/l. **(STUDENT PAPER).**

Shaking flask fermentation of *Bacillus thuringiensis*

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The orthogonal design had been developed for the superior experiment of *Bacillus thuringiensis* TS16 strain with shaking flasks. TS16 was grown in fermentation media using different agricultural by-products as the main carbon and nitrogen sources. These agricultural by-products included defatted soy flour, corn meal, defatted peanut flour, fishmeal, starch, peptone and yeast. Of these tests, fishmeal was the most efficient substrate for Bt while starch didn't has evident influence on Bt. With orthogonal experiments, a superior combinatorial medium (defatted soy flour 32g/L, corn meal 14g/L, defatted peanut flour 32g/L, fishmeal 14g/L, peptone 4g/L, yeasts 6g/L, $FeSO_4 \cdot 7H_2O$ 0.04g/L and $ZnSO_4 \cdot 7H_2O$ 0.08g/L) was obtained. Fermentation condition tests showed that the pH value at 8.5 was better than 8.0 and 9.0 before the media were disinfected. As far as the temperature was concerned, it was best at 25~30° for Bt toxicity. All media required enough air(O_2) for Bt fermentation. No difference existed for the efficacy of Bt fermentation liquid with inoculation quantity between 1 and 5%.

Comparative studies between *Bacillus thuringiensis thuringiensis* and *Bacillus thuringiensis israelensis* production by solid state fermentation to use as a bioinsecticide

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Bacillus thuringiensis biopesticides production are being studied in this brazilian group since 1970. Initial studies were done in submerged fermentation but with the international renewed interest in solid state fermentation, since 1980 it was decided to employ this methodology. Between the advantages are the use of particulate material, mainly solid residues and the integration of fermentation and residue disposal is amenable in terms of several commercial products. The process is carried out in a reduced water level and this also favours a cleaner industrial operation, with low levels of waste water. This work had as objective the biopesticide production to be used in public health. To do the solid state fermentation it was used an alternative reactor, a plastic bag (polipropilene), one liter volume, and as substrate sugar cane bagasse (25 or 15%) and corn grits (75 or 85%). This culture medium was pasteurized in a domestic microwave owen (high potency 2 minutes x 2 minutes wait). Two *Bacillus thuringiensis* varieties were studied,*Bacillus thuringiensis thuringiensis (Btt)* and *Bacillus thuringiensis israelensis (Bti).* The growth temperature of the microorganisms in study were tried at 28 ° C and 32 ° C and the initial inoculum volume was 10 and 15 mL. Water activity was 0.955. The behavior of the microbial growth was followed.These conditions favoured the *Bacillus thuringiensis israelensis (Bti)* whose production was 4,10% superior to that of *Bacillus thuringiensis thuringiensis (Btt).*

Acknowledgement: FAPESP, CNPq

Production and commercialization of biological insecticides in Colombia

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Production of biological insecticides in Colombia has been mainly focused on entomopathogenic fungi. Two approaches have been used. Farmer production leaded by the Colombian Coffee Federation, and directed towards the control of the coffee berry borer, and the industrial approach developed mainly by private companies as Live Systems Technology and a few other companies. The industrial production of products based on entomopathogenic fungi and antagonists of phytopathogenic fungi has increased significantly in the last 10 years. Stable and homogeneous products that reach an efficacy in the field comparable to chemical products are starting to appear in the national market. The commercialization goes along with technical support for users, that does not only demonstrate the technical performance of the product but the crop profitability by the implementation of integrated pest management or under organic production. In the case of *Bacillus thuringiensis* (Bt), there is no local production. At the present time, the active ingredient is imported and formulated or the final formulations are imported. The size of the market is small compared to that of the chemical insecticides. These two factors may account for the high prices that range from 12 to 14 USD per liter of a 3.5% formulation. Between 1995 and 2002, the government has financed several R&D projects with the aim to develop production and formulation technology to be transferred to the industry. It is expected that Bt based products developed with local strains and technology, will be released to the market at the end of 2002, at a competitive price, that may help to increase the demand for these type of products.

The expression of *gfp* gene in *Bacillus thuringiensis*

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This paper described a method to express *gfp* gene in *Bacillus thuringiensis*. The gene *gfpmut3a* with specific promoter from *B.cereus* were cloned into a shuttle plasmid pHT304. It was then introduced into acrystalliferous strains BMB171, CryB, IPS78-11 and 4Q7 by eletroporation. The results showed that green fluorecence could been detected in those recipient strains. Two kinds of *B.thuringiensis* promoters, *cry3A* promoter and *BtI-BtII* promoter, were chosen to construct fusion genes to drive the expression of *gfp* gene in *B. thuringiensis* strain. Each recombinant plasmids, pGFPExpA containing *cry3Apro-gfp* fusion gene and pGFPExpB containing *BtI-BtIIpro-gfp* fusion gene, were transferred into *E.coli* and plasmidless *B.thuringiensis* strains 4Q7, respectively. The *BtI-BtII* promoter was found to drive *gfp* gene to express strong green fluorescence in not only *B.thuringiensis* but also *E.coli* strain. However, *cry3A* promoter can not drive *gfp* gene expression in *E.coli*, and the expression in *B.thuringiensis* strain is much weaker than that drived by *BtI-BtII* promoter. RT-PCR experiment demostrated that *gfp* gene drived by *cry3A* promoter had strong transcription at early stage of sporulation and that drived by *BtI-BtII* promoter showed strong transcription at both of early stage and late stage of sporulation. But the fluorescent microscope observation showed that the cells carrying *cry3Apro-gfp* fusion emitted green fluorescence before sporulation while the cells containing *BtI-BtIIpro-gfp* fusion gene did before early stage of sporulation.

Friday, 9:00 - 11:00
Iguaçu I
CONTRIBUTED PAPERS - Fungi 4
Chair: A. Hajek

Importance of initial inocula and host density for development of *Zoophthora anhuiensis* induced epizootic in *Myzus persicae* colonies

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In an attempt to evaluate the impact of initial inocula and host density on the development of *Zoophthora anhuiensis* (Li) Humber (Entomophthoraceae) epizootic in *Myzus persicae* (Sulzer) population, colonies consisting of six newly emerged apterae were initiated at different ratios of artificially infected and uninfected ones from 0:6 to 6:0. The colonies then were allowed freely for reproduction, mycosis development and dissemination on cabbage leaves at an optimal regime of 15°C and 100% RH. A high-level epizootic occurred in the colonies initiated with ≥50% infected apterae (including the ratios of 3:3, 4:2, 5:1, and 6:0). The density of living aphids (apterae and their offspring) in the colonies never exceeded 50 aphids per 90 cm^2 during a 22-day period of observation, and was controlled by 93-100% compared to 656 aphids per 90 cm^2 in uninfected (control) colonies (0:6). In the colonies including fewer infected apterae (1:5 and 2:4), the aphid density decreased by 46-68% (356 and 207 aphids per 90 cm^2) compared to that in the control though the epizootic was insufficient to suppress the increase of aphid colonies. The development of *Z. anhuiensis* epizootic in each of the colonies was well described using a modified Gompertz model ($r^2 \geq 0.97$), which is usually used for modeling plant epidemic. The initial ratio of infected and uninfected apterae in the colonies was highly correlated to R ($r^2=0.89$) and K ($r^2=0.90$), the two parameters for the apparent infection rate and the capacity of epizootic estimated from fitting the model. This indicates that fungal inocula and host density are highly important for the development of *Z. anhuiensis* induced epizootic in *M. persicae* population.

Interactive effect of water content and temperature on viability of *Beauveria bassiana* conidia and metabolism of internally reserved nutrients in storage

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Water content of powdery conidia of entomopathogenic hyphomycetes is considered an important factor to affect shelf life of conidial products in storage. In this study, fresh powder of *Beauveria bassiana* SG8702 conidia with water content of 58.9± 1.6% was dried using a vacuum-freeze drying procedure, resulting in a water content of 7.4±0.9%. The fresh powder and the vacuum-freeze-dried (VFD) powder were then stored at 4 and 20°C, and examined for their viability and contents of internally reserved saccharides and proteins at 5-day intervals. It was found during one-month storage that the water content and temperature interactively affected the viability of the conidia and the contents of their reserved saccharides and proteins, which in turn correlated considerably well to the viability. Stored at 4 and 20°C, the VFD conidia lost content of saccharides by 13.4% and 14.1%, content of proteins by 39.2% and 38.2%, and viability by 32.0% and 55.8% when germinating in water only and 6.7% and 10.4% when germinating in 2% glucose solution plus 0.5% peptone, respectively. In contrast, the four estimates for the fresh conidia stored at 4 and 20°C decreased by 42.4% and 43.2%, 66.3% and 65.4%, 96.4.8% and 99.4%, and 9.9% and 98.4%, respectively. Thus, water content of *B. bassiana* conidia affected the ranges of variation in the viability and the content of internally reserved nutrients whereas storage temperature influenced the rates of their variation. However, depletion of the internally reserved nutrients did not necessarily inactivate the conidia. Instead, such conidia may germinate at relatively high level when being supplied with sufficient nutrients. In another experiment, the VFD powder of *B. bassiana* conidia with water content of 4.0±0.9% was half-monthly examined for viability during a 12-month period of storage. The viability slightly decreased to 90.2% from 99.0% at 4°C at the end of storage. Stored at 20°C, the viability also declined slowly during the first 165-day period but then dropped dramatically with little being detected on day 240. A modeling analysis showed that the time for the VFD conidia to lose 50% viability was 1006 day at 4°C and 197 day at 20°C, respectively. Conclusively, the powder of *B. bassiana* conidia, even though its water content was controlled below 5%, can be safely stored for one year or longer at low temperature only but no longer than 6 months at ambient temperature.

Impact of temperature and humidity regimes on the development of *Zoophthora anhuiensis* induced epizootic in *Myzus persicae* population

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This experiment was conducted to examine the effect of different temperature and humidity regimes on the development of *Zoophthora anhuiensis* induced epizootic in the population of the green peach aphid *Myzus persicae* (Sulzer). Aphid colonies consisting of 3 infected and 3 uninfected apterae were maintained on cabbage leaves for free reproduction, and mycosis development and dissemination at each of 16 regimes of temperature (10, 15, 20, and 25°C) and relative humidity (90, 95, 98, and 100% RH). The temperatures of 10 and 15°C were found most favorable to the development of epizootic in the aphid colonies regardless of the humidity regimes concerned. At 90-100% RH, the percentage of mycosis-killed aphids (apterae and their offspring) ranged from 72.88 to 98.16% at 10°C on day 26, and from 78.66% to 94.35% at 15°C on day 24, representing very high levels of epizootic. At 20°C, a high level of epizootic occurred only at 100% RH with a maximal mortality of 71.64% on day 24 whereas much smaller mortality (5.12-12.83%) was observed at the other three humidity regimes. However, the maximal mortality at 25°C on day 20 was only 27.21% even at 100% RH. The results indicate that *Z. anhuiensis* caused aphid epizootic more easily at the lower temperatures, at which it was to less degree affected by humidity. The disease prevalence (represented by the cumulative mortality) was correlated quite well to temperature, relative humidity, the number of days from initiation, and interaction among the variables (r^2=0.82, P<0.01).

Isolation of two β-1,3-glucan binding proteins from hemolymph of *Plutella xylostella* larvae

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The compound, β-1,3-glucan, is an essential component of *Zoophthora radicans* (Entomophthorales) cell wall and has been reported in association with insect immune system. A few recognition proteins that recognize β-1,3-glucan have been previously isolated from hemolymph of large-size insects such as lucusts *Locusta migratoria*, the silkworm *Bombyx mori* and the cockroach *Blaberus craniifer* or crustaceans such as the freshwater crayfish *Pacifastacus leniusculus*. In the present study, two β-1,3-glucan binding proteins were successfully isolated from the larval plasma of the diamondback moth *Plutella xylostella* but not from hemocytes. The hemolymph was collected from about 10,000 larvae. Precipitated with laminarin (β-1,3-glucan), the two proteins isolated were identified as glycoproteins with molecular weight of approximately 75.9 kD and 83.2 kD determined via SDS-electrophoresis, respectively. The two proteins from the plasma of *P. xylostella* larvae were in molecular weight similar to that from *B. craniifer* adults (90 kD) and one protein from *L. migratoria* adults (85 kD). However, both proteins were larger than that from *B. mori* larvae (62 kD), and smaller than another protein from *L. migratoria* adults (260 kD) and that from *P. leniusculus* (100 kD). Further SDS-PAGE analysis of the protein complex under reducing (containing 0.5% β-2-mercaptoethanol) and non-reducing conditions showed that the two proteins isolated could be two single-chain proteins. In addition, the two proteins were confirmed to be associated with host proPO-system, which can induce enhancement of a phenoloxidase activity in a laminarin-activated hemocyte lysate. Thus, the β-1,3-glucan binding proteins in the host are speculated in relation to immune response of the host to *Z. radicans* infection.

Preliminary studies on bioactivities of metabolites from the entomogenous Fungus *Aschersonia aleyrodis*

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The entomogenous fungus *A.aleyrodis* is a pathogen parasiting on whiteflies and scales. Because its host range

includes a number of important pest species , *A. aleyrodis* has been under investigation as potential biological control agent. As a consequence, its secondary metabolites are of great interest. The metabolites of a strain of entomopathogenic fungus belonging to *Aschersonia* grown in 100ml potato sucrose (potato $200gl^{-1}$, sucrose $20gl^{-1}$, in distilled water) in Erlenmeyer flasks (250ml) were prepared, and their effect on the growth of wheat etiolated coleoptile was studied. At the dilution of 1:10, the crude preparations of metabolites of *A. aleyrodis* caused the elongation of wheat coleoptile by 35.7%, whereas those of grown in 100ml potato sucrose (potato $200gl^{-1}$, sucrose $20gl^{-1}$, in distilled water) in Erlenmeyer flasks (250ml) cause the elongation of wheat coleoptile by 35.7%, whereas those of *A. aleyrodis* at middle and low concentration (1:1000 and 1:100000) inhibited the elongation by 7.1% and 35.7%, respectively. The combination of Naphthyl acetic acid (NAA) 2 ppm and metabolites of *A.aleyrodis* diluted at 1:100000 could significantly increase the elongation of wheat etiolated coleoptile by 21.4%, while NAA (2ppm) is used alone, the growth rate is 7.1%. It indicated that the suppressive effect could be counteracted by each other in the results. The metabolites of *A.aleyrodis* cultured in Czapek liquid medium obviously suppressed the elongation at high concentration (1:10) and middle concentration (1:1000) by 14.3%, but could significantly increase the elongation of wheat etiolated coleoptile by 35.7% NAA (0.02ppm) in several bioassay on their physiological effects as well. The combination of NAA (2ppm) and metabolites of *A. aleyrodis* cultured in Czapek at low concentration (1:100000) showed no physiological activities. The results showed hyperplastic effects could be partially counteracted by each other and the metabolites had the same physiological function as NAA.

Diversity of Subterranean entomopathogenic fungi in Southern coastal belt of Sri Lanka

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Southern part of Sri Lanka divide into three ecological zones mainly according to the monsoon rainy seasons and the amount of rainfall receive during each season. Faunal and floral diversity vary accordingly from wet zone (WZ), intermediate zone (IZ) to dry zone (DZ). Survey on occurrence of soil entomopathogenic fungi (EPF) were conducted at four major sampling sites (WZ, wet-IZ, dry-IZ and DZ) selected along the southern coastal belt from period of 1999 to 2001. Entomopathogenic fungi was isolated by baiting the soil with wax-moth larvae, *Galleria mellonella* (L.) and the physicochemical parameters of the soil was measured using the standard soil analysis methods. Pathogenicity of extracted fungi was confirmed by the reinfection of wax-moth larvae using the extracted fungal spores. EPF belong to different groups were isolated and *Beauveria bassiana* (Bals.) was the most abundant species. *Entomopthora* sp. *Paecilomyces* sp and *Aspergillus* sp. were also isolated from infected larvae.Abundance of EPF was significantly higher in the wet zone compared to that of the dry zone. Samples collected from coastal sites had more EPF isolates than those samples collected from 1km away from the coastal sites. Simple correlation studies revealed a positive correlation between the abundance of EPF and the moisture content of the soil and the negative correlation with the soil temperature.

Impact of species diversity on sustainable control of Masson's pine caterpillars with *Beauveria bassiana* in forest ecosystem

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Different application strategies of *Beauveria bassiana* were used against the Masson's pine caterpillar, *Dendrolimus punctatus*, at 3 forest farms in South Anhui, Southeast China, of similar natural conditions, and occurrence area of 4 years, caterpillar population dynamics of the 3 anniversary years and species diversity of community of animal and entomogenous fungi of an anniversary year were monitored. The results showed that the caterpillar populations of the 3 farms were all suppressed at low levels during the 4 years, but population dynamics were completely different. In Magushan Farm, the pine stands were treated inundatively with *B. bassiana* primarily and chemical insecticides alternatively, and the average population density was 3.16±4.94 per tree with sharp fluctuation and sometimes with a population close to or above the control threshold. In Jinsishan Farm, small area epicenters of the caterpillars were accurately detected and treated inundatively with chemicals primarily and *B. bassiana* alternatively, with average density at 1.45±2.29 and highest density slightly above the threshold. In Daigongshan Farm, however, inoculative application of *B. bassiana* had been kept up for 20 years, and the population had been at a very low level with average density at 0.09±0.14, and never above 0.6. Investigation revealed that in order of Magushan, Jinsishan and Daigongshan, total individual number of animals and entomogenous fungi decreased, while total species number, ration of total species number to total individual number, species ratio of natural enemies to pests, individual ratio of natural enemies to pests, and community diversity index all increased, indicating a tendency of species diversity getting richer, the food chain getting more complicated, and community stability and ability of self-regulation of pests getting stronger. A primary component analysis showed that in the same order, community evolution progressed in clearer sequence, and tended to be stabler, with stronger self-regulation ability. Based on the above results, a conclusion is drawn that the long used application strategy of inundative release of fungal insecticides should be substituted by a strategy of inoculative release.

Impact of *Beauveria bassiana* on species diversity and sustainable control of Masson's pine caterpillars in forest ecosystem

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Twelve plots for *B. bassiana* application at frequencies of twice a year, once a Year and once every 2 years, and at doses of 300, 150, 75 and 37.5 g/ha, respectively, were designed in Magushan Forest Farm, South Anhui, Southeast China. Each plot with similar stand factors covered 13.3 ha. Caterpillar population dynamics and species diversity of community of animal and entomogenous fungi were monitored for an anniversary

year in March, April, May, July, September and November and dynamics of species diversity index, regularity index and dominant index were acquired for the 12 plots. Dynamics of some entomogenous fungi, including *B. bassiana*, *Paecilomyces farinosus*, *P. cateniannulatus*, *Metarhizium flavoviride* var. *minus* and *Verticillium* sp., were investigated in details. The results showed that microthermal fungi such as *Paecilomyces farinosus* and *P. cateniannulatus* were dominant species of entomogenous fungi in January, and suppressed insect populations in the forest together with *B. bassiana*. As temperature rose, *B. bassiana* became the dominant population gradually in April, May, July and September. Species diversity index was the highest in January with richest species component and even distribution of entomogenous fungi in the pine ecosystem; the second highest index appeared in April, and the lowest one in September. Vertical distribution pattern of entomogenous fungi in the stand showed that the number of infected cadavers was in the following order: litter layer> tree layer> shrub and herb layer> soil layer. The general trend of species diversity index of animal and communication was in the following order: shrub and herb layer>tree layer>litter layer>soil layer, with slight variation in different seasons. In terms of annual sum of species diversity indices in the 12 plots, the sum for the plot was the highest where *B. bassiana* was released once every 2 years and at a dose of 37.5 g/ha, followed by once every 2 years at 75 g dose. The sum for the plot was the lowest where *B. bassiana* was released once a year at 300 g/ha (nearly inundative release), followed by once a year at 150 g/ha. The results suggested that the application frequency and dose of *B. bassiana* impacted species diversity and sustainable control of the Masson's pine caterpillar in the forest ecosystem. The optimal inoculation frequency and dose were determined to be once a year and 37.5 g/ha, based on gray decision-making analysis on the index, caterpillar density and control cost.

ADDENDUM

(Added after the Program and Abstracts had already been printed)

Tuesday, 14:00- 16:00
POSTER SESSION II

VP 44 Transgenic risk assessment: Potential effects of transgenic chitinase and 1,3-glucanase expression on grape vine arthropods

Hugo M. Arends, Claudia Vogel and Johannes A. Jehle

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

Recently, a wide variety of transgenic plants with enhanced resistance against different pests have been developed. A strategy to obtain improved resistance against fungal diseases is the combinative transgenic expression of chitinase (CHI), 1,3-glucanase (GLU) and a ribosomal inhibiting protein (RIP). These fungal resistance genes have been introduced in different plant species including grape vine (*Vitis vinifera*). The aim of this project is to investigate the possible effects of transgenic expressed CHI, GLU and RIP in grape vine on non target organisms. This is done by using the plant feeding grape berry moth *Lobesia botrana* and the beneficial predatory mite *Typhlodromus pyri*. Beside the possible direct toxic effect, the investigations focus on the question whether the constitutive expression of CHI, GLU and RIP have an adverse or a synergistic effect on the activity of naturally occurring insect pathogens like viruses, bacteria and fungi. For analysis of direct and indirect effects of CHI, the chi gene was cloned and expressed in the baculovirus expression system. Isolated protein is used in bioassays to determine toxic levels of the enzyme. The results from this project will provide data to determine possible non-target effects of transgenic fungal resistant grape vine plants on arthropods.

VP 45 *In vitro* replication of the balsam fir sawfly, *Neodiprion abietis*, nucleopolyhedrovirus (NeabNPV) in a cell line (Cf70) derived from the spruce budworm

Beatrixe Whittome[1], Benoit Morin[2], Christopher Lucarotti[2], and David B. Levin[1]

[1]Department of Biology, University of Victoria, P.O. Box 3020 STN CSC, Victoria, British Columbia, V8W 3N5, Canada; [2]Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, P.O. Box 4000, Fredericton, New Brunswick, Canada, E3B 5P7

The balsam fir sawfly, *Neodiprion abietis* (Harr.), was first identified as a defoliator of balsam fir and white and black spruce in 1936. Sawfly populations have periodically reached outbreak levels and have resulted in the defoliation of over 250,000 hectares of forests within the last 10 years (1991-2001). Canadian legislation restricts the use of many pesticides, promoting the need to find natural biological control agents. The nucleopolyhedrovirus of *Neodiprion abietis* (NeabNPV) shows promising potential as a control agent based on field trials. To study the viral characteristics *in vitro*, a model system needed to be created. Cell lines (NL18, NL28, and Cf70) derived from the Red-headed pine sawfly, *Neodiprion lecontei* (Order:Hymenoptera), and from the Spruce budworm, *Choristoneura fumiferana* were tranfected with DNA extracted from polyhedral inclusion bodies of NeabNPV. NL18 and NL28 cells do not support productive infection by NeabNPV, and studies to identify the stage at which NeabNPV replication is blocked in these cells are underway. Suprisingly, Cf70 cells did support productive infection by NeabNPV. Fresh Cf 70 cells cultured in medium from NeabNPV-infected cells developed cytopathic effects indicative of NPV infection (rounded cell morphology with hypertrophied nuclei) within three days. NeabNPV-specifc polymerase chain reaction (PCR) products were observed after amplification using DNA extracted from either NeabNPV-infected Cf70 cells or pellets derived from media in which NeabNPV-infected cells were cultured. Analysis of the replication kinetics and of NeabNPV progeny derived from infected Cf70 will be presented.

BP 36 Inheritance of Bacillus thuringiensis spore-crystal complex (HD-1 kurstaki) in a mexican Plutella xylostella population

Marcelino Vázquez García[1] and Trinidad López Pérez[1]

[1] Universidad de Guadalajara. Centro Universitario de Ciencias Biológicas y Agropecuarias, Centro de Investigación en Parasitología Vegetal, Guadalajara, México.

The diamondback moth Plutella xylostella (L) is one of the most important pest of cruciferous crops in Guanajuato, México. This insect is controlled by using not only synthetic chemicals but also Bacillus thuringiensis based insecticides. There are many reports of resistance to Bt from several parts of the world and also from Guanajuato, Mexico. This piece of work was made to determine the type of inheritance of the resistance character, besides the determination of increment of resistance through accelerated selection pressure under laboratory conditions. Results showed that an average of 70% selection pressure during four consecutive generations, caused a rather low level of

resistance (2.4x). Crosses of selected adult females with non selected adult males showed that the character of resistance in F1 is partially recessive. The gene frequency of the backcrosses of F1 adult males with parental selected adult females showed that the resistance is probably due to the presence of more than one single gene.

BP 37 Detection of virulence factors in strains of *Bacillus thuringiensis* isolated in Brazil

Diana Aparecida Cabral [1], Sonia Ermelinda Alves da Silva [2], Anna Lúcia Rocha China Leal,[1] Leon Rabinovitch [2] and Viviane Zahner [1]

[1] Laboratório de Sistemática Bioquímica, Departamento de Bioquímica e Biologia Molecular, IOC, FIOCRUZ and [2] Laboratório de Fisiologia Bacteriana, IOC, FIOCRUZ, Rio de janeiro, Brasil

Bacillus thuringiensis is a well characterized bacteria with some serovars being used in biological control programs. This bacteria is closely related to *Bacillus cereus* which is a Gram-positive, spore-forming, motile, aerobic rod that also grows well anaerobically. *B. cereus* can cause two different types of food poisoning: diarrhoeal or emetic. In this context *B. cereus* has been implicated in the intoxication and death of three people in France. The only difference between *B. cereus* and *B. thuringiensis* is the production of entomotoxins in *B. thuringiensis*. Although the safety of *B. thuringiensis* has been extensively tested and demonstrated over several years some papers have appeared in the literature showing the presence of enterotoxin genes in this species. As we have a big collection of *B. thuringiensis* isolated in Brazil, we decided to study the presence and distribution of *hbl*A, *bce*T, *nhe*BC, and *cyt* K genes, by PCR, in a sample of strains, given that these genes are encoded for a number of the virulence factors of *B. cereus*. Determination of hemolytic activity is being determined at 30°C on sheep blood agar plates. In addition we are analyzing strains from other collections in order to extend the scope of this study. To date we have analysed 102 strains of *B. thuringiensis* representing different serotypes. The *hbl*A and *nhe*BC genes are present in the majority of the samples analysed while the *bce*t gene is present in only one. We could not detect the *cyt*k gene in any of the strains analysed. Strains of the same serotype present different patterns of hemolisys on agar blood plates and presence/absence of *hbl*A and *nhe*BC genes. The results presented here have importance for a number of aspects: in the taxonomy of *B. cereus* group, the study of the genetic flow in *B. thuringiensis*, the study of the *plc*R operon and for food safety regulations.

NP 9 Diversity and Distribution of Entomopathogenic Nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey

Selçuk Hazir[1], Nevin Keskin[1], S. Patricia Stock[2], Harry K. Kaya[3], and Servet Özcan[4]

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The diversity and distribution of entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae were assessed throughout an extensive soil survey in Turkey during 1999 and 2000. Entomopathogenic nematodes were recovered from 6 out of 7 regions sampled, with 22 positive sites (2%) out of 1080 soil samples of which 15 were steinernematid isolates and 7 were heterorhabditid isolates representing a total of four species. Based on morphometric and molecular data, the nematode species were identified as *Heterorhabditis bacteriophora*, *Steinernema feltiae*, *S. affine*, and *Steinernema* n. sp. The most common species was *S. feltiae* which was isolated from 10 sites in 6 regions, followed by *H. bacteriophora* from 7 sites in 5 regions, *S. affine* from 4 sites in 2 regions, and *Steinernema* n. sp. from 1 site. *H. bacteriophora* and *S. feltiae* have been found in many parts of the world, whereas *S. affine*, so far, has only been recovered in Europe until our survey. *S. affine* was isolated from the European (Marmara) as well as the Asiatic region (Middle Anatolia) of Turkey. A new undescribed *Steinernema* sp. was isolated from the most eastern region (East Anatolia) of Turkey. Soils of the positive sites were classified as sandy, sandy loam, or loam (68.2%) and sandy-clay-loam or clay loam (31.8%) and the pH ranged from 5.6 to 7.9. The habitats from which the entomopathogenic nematodes were isolated were broadly classified as disturbed (59.1%) which included agricultural fields and poplar planted for lumber and wind breaks, and undisturbed (40.9%) which included pine forest, grassland, marsh and reed sites. *S. feltiae*, *S. affine*, and *H. bacteriophora* were recovered from both disturbed and undisturbed habitats. The new *Steinernema* sp. was recovered from grassland. Our survey showed that these nematodes occur widely throughout Turkey, but at a frequency below that reported for other parts of the world.

NP 10 Displacement and parasitism of entomopathogenic nematodes to berries infested by coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae)

Juan P. Molina A[1].; Juan C. López N.[2].

[1] Master student of Universidade Federal de Lavras (MG) Brasil. [2] National Centre of Coffee Research, Cenicafé. Chinchiná, Caldas, Colombia. Apartado Aéreo: 2427 Manizales, Colombia (Sur América).

The use of entomopathogenic nematodes (EPN's), is a possible alternativefor the control of coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae) (CBB), in berries that have fallen to the ground. The objective of this study was to determine the displacement and parasitism of the EPN's *Heterorahbditis bacteriophora* (Rhabditida: Heterorhabditidae) (Hb) and *Steinernema feltiae* (Rhabditida: Steinernematidae) (Sf) in berries infested with stages of CBB. Different situations were simulated (found in field) in which infective juveniles (IJ) of EPN's will have access to bored berries. In the first experiment EPN's displacement index (DI) was evaluated in coffee berries of two degrees of berry maturity: ripe and dry. In the experiment using sterile sand and acrylic displacement chamber, the dry berry was more attractive for IJ's of both species 21.13 ± 3.3 % (mean ± S.E), while the DI for ripe berry was 5.63 ± 1.48 %. The effect of two texture clays, sandy loam (CSL) and Loam (L) was evaluated, on the displacement of EPN's in dry berries, demonstrated that texture doesn't have any effect. The second experiment evaluated different IJ concentrations (5, 25, 125 and 625 IJ / 35 µl of solution) applied on berry, and the mortality percent of CBB (MPCB) was determined. The penetration index in berry (PIB) did not exceed 23% for both EPN's in all concentrations. Nevertheless its average of MPCB was 46.9 % to Hb and 28.8 % to Sf, being highest evaluated concentrations presented a high for both species. This work demonstrates the possibility of using EPN's as another alternative in the Integrated coffee berry borer management (IBM) in Colombia.

NP 11 Entomopathogenic nematodes; promissory tool in integrated management of coffee berry borer, in soil.

Juan C. López N.;[1] Juan P. Molina A;[2] Juan C. Lara G.;[1] Alex E. Bustillo P.

[1] National Centre of Coffee Research, Cenicafé. Chinchiná, Caldas, Colombia. Apartado Aéreo: 2427 Manizales, Colombia (Sur América). [2] Master student of Universidade Federal de Lavras (MG) Brasil.

The Coffee Berry Borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) (CBB), is the most economically important pest worldwide in coffee crops. The fallen bored berries, are known to be a very important reservoir of re-infestation to the healthy ones and therefore to the next harvests, therefore the cycle continues. Its control either by chemicals, fungi or parasitoids is not possible because none of them penetrates into the bored berries. The manual collection of these berries from the ground is difficult. There is a need to find and evaluate a natural enemy that could actively search and enter into the berry and decimate the CBB population in it, such as entomopathogenic nematodes (EPNs). The Laboratory of Entomology at Cenicafé, have conducted studies to obtain data on the effect of EPN's, mainly *Steinernematidae* and *Heterorhabditidae* families, in CBB since 1997. The work involved studies related to susceptibility of different stages of CBB to EPN's, strategies of host finding, life cycle, search and selection of native isolations adapted to Colombian soil coffee regions, conventional application systems, controlled green house and small field trials. Efforts continue to evaluate its doses and application time, mass production among others experiments, in order to perform larger field trials. All works are framed within the concept of Integrated Pest Management (IPM).

Tuesday, 16:30 – 18:45
Iguassu II
CONTRIBUTED PAPERS - Virus 1
Chair: J.E. Maruniak

18:30 h

The genome of the *Cryptophlebia leucotreta* granulovirus (CrleGV)

Martin Lange and Johannes A. Jehle

State Education and Research Center for Agriculture, Viticulture and Horticulture (SLFA), Section Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt an der Weinstraße, Germany

The *Cryptophlebia leucotreta* granulovirus (CrleGV) is a tortricid specific Baculovirus, that is highly pathogenic to the false codling moth *Cryptophlebia leucotreta*, an important pest of citrus, cotton, maize and other economically important crops in tropic and subtropic Africa. We have sequenced the genome of the genotype CrleGV-CV3, which was originally in-vivo cloned from CrleGV isolate derived from the Cape Verde Islands. The double stranded circular genome comprises 110.9 kb and encodes 134 predicted open reading frames (ORFs). 124 ORFs are similar to other baculoviruses of which 27 ORFs were present only within the granuloviruses PxGV, CpGV and XcnGV. The A+T content of the genome is 67.6% and is the highest found so far in baculoviruses. A striking feature of the CrleGV genome is the presence of an extended hyper-variable non-*hr ori*-like region consisting of direct repeats, palindromes and an unusual AT-rich region. Genes involved in DNA replication and expression include

dna-pol, *dna-ligase*, *lef* (1-3, 4-6, 8-11), *helicase*, *helicase2*, *ie-1*, *p47*, *pe-38*, *vlf-1*, and *39K*. Structural protein genes were *gp41*, *granulin*, *odv-e18*, *odv-e25*, *odv-ec27*, *odv-e56*, *odv-e66*, *p6.9*, *p74*, *vp1054*, *vp39*, *and vp91*. Auxiliary genes identified were *egt*, *fgf*, *PTP*, *PK-1*, *sod*, *v-ubi*, and cathepsin, whereas only a truncated *chitinase* gene was found. Other genes identified were *alk-exo*, *desmoplakin*, *fp*, *me53*, *mp-nase*, *p13*, *p24*, *p49*, *tlp20*, and *38.7kd*. Several ORFs with similarity to each other were identified including three genes belonging to the *iap* family. CrleGV is next closely related to *Cydia pomonella* granulovirus (CpGV) as revealed by genome order comparisons and phylogenetic analyses. Major differences to the CpGV genome are the absence of ORFs *Cp13 (gp37)*, *Cp38*, *Cp63 (bro)*, *Cp65*, *Cp66 (ptp-2)*, *Cp127 (rr1)*, *Cp128 (rr2)* and 11 ORFs which are unique in the CpGV genome.

Wednesday, 08:00 – 10:15
Iguassu II
CONTRIBUTED PAPERS - Virus 2
Chair: B. M. Ribeiro

10:00 h

Molecular evidence for insect host transposon activation during CpGV infection (STUDENT PAPER)

Hugo M. Arends[1], Doreen Winstanley[2] and Johannes A. Jehle[1]

[1]State Education and Research Center for Agriculture, Viticulture and Horticulture (SLFA), Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Weinstr., Germany; [2]Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

The horizontal transfer of endogenous Lepidopteran transposons into baculovirus genomes occurs during infection of insect cell cultures and of insect larvae. In previous i*n vivo* infection experiments of the Mexican strain of *Cydia pomonella* granulovirus (CpGV-M) in larvae of *Cydia pomonella* and *Cryptophlebia leucotreta* two virus mutants, named MCp4 and MCp5, containing transposons, named TCp3.2 and TCl4.7, were isolated. In this study we addressed the question whether the transposon harboring viruses had a replication advantage over the wild-type and became dominant in the virus population or whether the activity of the host transposable elements is stimulated by virus infection. Biological characterization studies which included lethal dose (LD) and survival time bioassays (ST) were performed. The LD_{50} values for MCp4, MCp5 and CpGV-M were not different, the ST_{50} values however, demonstrated that the transposon containing viruses killed *C. pomonella* larvae slower than CpGV-M. In order to compare the replication rate of CpGV-M and the mutants, co-infection experiments of fifth instar *C. pomonella* were performed using a mixture of CpGV-M and mutant viruses as inoculum. By comparing the CpGV-M : mutant ratio in the virus inoculum with the ratio in the virus progeny, it was shown that MCp4 and MCp5 were out-competed quickly by CpGV-M. This demonstrated a significant selection disadvantage for the transposon carrying mutants compared to CpGV-M. In order to test whether the virus infection has an influence on transposition activity, the transcription levels of the putative transposase gene of TCp3.2 was investigated in virus infected and uninfected larvae. Transcription levels of host TCp3.2 transposase were determined by quantitative real time RT-PCR of cDNA generated by reverse transcription of isolated mRNA of *C. pomonella* larvae. The experiments demonstrated that a higher level of transposase transcription was detectable in CpGV-M infected than in mock infected control larvae. This observation gave strong evidence that CpGV-M infection might trigger the activity of transposon TCp3.2 within the genome of *Cydia pomonella*. Our results suggest that horizontal transfer of insect host transposable elements into baculovirus genomes might be activated by virus infection

Thursday, 8:00- 10:15
Iguassu II
CONTRIBUTED PAPERS - Virus 3
Chair: J. L. C. Wolff

10:00h

Nucleotide sequence and phylogentic analyses of the balsam fir sawfly, *Neodiprion abietis*, nucleopolyhedrovirus (NeabNPV)

Aaron Young[1], Simon Duffy[1], David Harrison[1], Benoit Morin[2], Christopher Lucarotti[2], and David B. Levin[1]

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Information concerning the structure, organization, and evolutionary relationships among viruses of the family Baculoviridae have derived primarily from studies of nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) of host insects in the Order: Lepidoptera. Little, however, is known about the evolutionary relationships of baculoviruses isolated from non-lepidopteran insect hosts. Recent phylogenetic analyses using nucleotide and amino acid sequences of the *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV) suggest that this virus forms a clade that is separate from the lepidopteran NPVs and GVs. We are in the process of conducting a complete genome sequence analysis of the *Neodiprion abietis* (Order: Hymenoptera) nucleopolyhedrovirus (NeabNPV). We have identified numerous putative baculovirus genes and open reading frames (ORFs), including polyhedrin, p74, odv-e56, iap-3, lef-5, lef-8, lef-9, and DNA polymerase. In order to determine the evolutionary relationship of the NeabNPV to known NPVs and GVs, a comprehensive phylogenetic analysis

was performed using both complete ORF sequence data and conserved amino acid sequence motifs from NeabNPV and other baculoviruses. Our analyses indicate that the Hymenopteran NPV assorts as an independent clade that is more closely linked with the lepidoteran granuloviruses than with the lepidopteran nucleopolyhedroviruses.

Thursday, 16:30 – 18:45
Iguassu II
CONTRIBUTED PAPERS - Virus 4
Chair: A. S. de Cap

18:30h

Infection of late instars, horizontal transmission and persistence of *Cydia pomonella* granulovirus in the field

Susanne B. Steineke and Johannes A. Jehle

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The *Cydia pomonella* granulovirus (CpGV) is used to control the codling moth in apple orchards. The larvae have limited contact with the virus because the virus is rapidly inactivated by UV radiation and codling moth larvae spend only a short time on the apple's surface as first instars. Although this leaves only a small window, relatively irregular applications can result in an effective control of the codling moth. We have conducted experiments to help explain this observation. Using larval cadavers as a source of infection in laboratory experiments, we found horizontal transmission of CpGV to be as high as 40%. We set up an experiment to test whether the same applied under field conditions. In order to work with a sufficient host density under defined conditions in the field, we applied all larvae to the apples individually and subjected them to different treatments. Their survival was determined after four weeks by harvesting the apples and extracting the surviving larvae. Results indicate that horizontal transmission may have taken place, but quantification was difficult due to a low host density. More importantly, we found that active virus can persist more than 5 times longer on the apple than previously reported by others, indicating that the quantification of UV inactivation may depend strongly on the method used. In a further experiment, we found substantial virus related mortalities in apples that had been treated with CpGV two weeks after placing the larvae on apples. This finding shows that later instars can also be controlled effectively by CpGV, although fruit damage cannot be prevented.

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