Book of Abstracts

S I P 2 0 0 4

37th Annual Meeting of the Society for Invertebrate Pathology
7th International Conference on Bacillus thuringiensis

Helsinki, Finland
1-6 August 2004

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Society for Invertebrate Pathology

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The 7th International Conference on Bacillus thuringiensis
is gratefully acknowledged.

Jorgen Eilenberg, Susanne Vestergaard (DK), Ingeborg Klingen (NO)	**Fungi**
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Rudolf Wegensteiner (AT) , Regina Kleespies (DE)	**Microsporidia**
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Program Overview
SIP 2004

Saturday, July 31st, 2004
Time: 08:30 - 17:00, Hotel Grand Marina

SIP Council Meeting

Saturday, July 31st, 2004
Time: 13:00 - 18:00, Lecture Room 4

Registration

Sunday, August 1st, 2004
Time: 09:00 - 09:20, State Room

Opening plenary

Sunday, August 1st, 2004
Time: 09:20 - 10:15, State Room

Founder's Memorial Lecture

Honoree: Hans Boman
Lecturer: Kenneth Söderhäll

Sunday, August 1st, 2004
Time: 10:30 - 13:30, State Room

Plenary (Cross-Divisional)
SIP - the past, present and future

Sunday, August 1st, 2004
Time: 13:30 - 15:30, Corridor levels 2 and 3

Setting up posters

Sunday, August 1st, 2004
Time: 16:00 - 18:00, Lecture Room 1

Workshop (Cross-Divisional)
The graduate student's guide to the galaxy

Sunday, August 1st, 2004
Time: 19:00 - 22:00, Marina Congress Center

Welcoming reception

Monday, August 2^{nd}, 2004
Time: 08:00 - 09:30, Lecture Room 1

Plenary (Cross-Divisional)
Invertebrate pathogens as pests

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 1

Symposium (Division of Bacteria)
Second generation transgenic crops

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 12

Symposium (Division of Nematodes)
Significance of the entomopathogenic nematode infected-host in the soil ecosystem, and potential impact on microbial control

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 6

Symposium (Division of Viruses)
Virus ecology

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 10

Symposium (Cross-Divisional)
Honeybee pathology

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 6

Contributed Papers (Division of Viruses)
virus / contributed paper session 1

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 1

Symposium (Division of Nematodes)
Nematodes and cold adaptations

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 12

Contributed Papers (Division of Microbial Control)
microbial control / contributed paper session 1

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Corridor, II and III levels

Poster Session 1: Posters for fungi and bacteria

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 6

Contributed Papers (Division of Viruses)
virus / contributed papers session 2

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 1

Symposium (Division of Fungi)
Insect-fungal associations

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 12

Symposium (Division of Microbial Control)
Bringing pathogens from the laboratory to the field

Monday, August 2^{nd}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Symposium (Division of Bacteria)
Risk assessment and non-target effects of Cry toxins in sprays and transgenic plants

Monday, August 2^{nd}, 2004
Time: 18:30 - 20:30, Main Building, Lehtisali

Helsinki University reception

Monday, August 2^{nd}, 2004
Time: 20:00 - 22:00, Lecture Halls 1, 12, 6, 10

Division meetings: V, B, N, Ms

Tuesday, August 3rd, 2004
Time: 08:00 - 10:00, Lecture Room 12

Contributed Papers (Division of Fungi)
fungus / contributed paper session 1

Tuesday, August 3rd, 2004
Time: 08:00 - 09:30, Lecture Room 10

Symposium (Division of Microsporidia)
Can microsporidia be seriously considered as biological control agents?

Tuesday, August 3rd, 2004
Time: 08:00 - 10:00, Lecture Room 1

Symposium (Cross-Divisional)
Oryctes virus - from discovery to classical microbial control agent

Tuesday, August 3rd, 2004
Time: 08:00 - 10:00, Lecture Room 6

Contributed Papers (Division of Bacteria)
bacteria / contributed paper session 1

Tuesday, August 3rd, 2004
Time: 10:15 - 12:00, Lecture Room 1

Society General Meeting

Tuesday, August 3rd, 2004
Time: 12:00 - 14:30, Solvalla

5 k Fun Run

Note: Departure at 12:15 by bus from UH Main Building

Tuesday, August 3rd, 2004
Time: 13:00 - 18:00, Nuuksio

Excursion 1: Nuuksio National Park (off-path)

Host: Larry Huldén
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3rd, 2004
Time: 13:00 - 18:00, Nuuksio

Excursion 2: Nuuksio National Park (easy)

Host: Lena Huldén
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3rd, 2004
Time: 13:00 - 18:00,

Excursion 3: Marimekko factory outlet

Host: Ingeborg Menzler-Hokkanen
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3rd, 2004
Time: 19:00 - 24:00, Tolkkinen

BBQ

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 10

Contributed Papers (Division of Microsporidia)
microsporidia / contibuted paper session 1

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 12

Workshops (Division of Viruses)
Genome analysis methodology -workshop

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 1

Symposium (Cross-Divisional)
Fungi and nematodes under unfavorable conditions

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Fungi)
fungus / contributed paper session 2

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 6

Contributed Papers (Division of Microbial Control)
microbial control / contributed paper session 2

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 1

Symposium (Division of Bacteria)
Genomics and pathogenesis of invertebrate pathogens

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Corridor leves II and III

Poster Session 2: ALL other than fungi and bacteria

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 10

Contributed Papers (Division of Bacteria)
bacteria / contributed paper session 2

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Contributed Papers (Division of Fungi)
fungi / contributed paper session 3

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Contributed Papers (Division of Nematodes)
nematodes / contributed paper session 1

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 6

Symposium (Division of Viruses)
Role of native immune systems/molecular host response

Wednesday, August 4^{th}, 2004
Time: 20:00 - 22:00, Lecture Rooms 1, 12

Division meetings: MC, F

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 12

Symposium (Division of Bacteria)
New advances in research and development of insecticidal proteins

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 1

Workshop (Cross-Divisional)
Risk assessment

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 6

Contributed Papers (Division of Viruses)
virus / contributed paper session 3

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Bacteria)
bacteria / contributed paper session 3

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 6

Contributed Papers (Division of Fungi)
fungi / contributed paper session 4

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 10

Contributed Papers (Division of Nematodes)
nematodes / contributed paper session 2

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 1

Symposium (Cross-Divisional)
Microbial control in greenhouses and nurseries

Thursday, August 5^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Workshops (Division of Microbial Control)
Status of microbial control products

Thursday, August 5^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Workshop (Cross-Divisional)
SIP education workshop

Thursday, August 5^{th}, 2004
Time: 19:00 - 24:00, Marina Congress Center

Banquet

Program
SIP 2004

STU indicates papers being judged for
graduate student presentation awards

Saturday, July 31^{st}, 2004
Time: 08:30 - 17:00, Hotel Grand Marina

SIP Council Meeting

Saturday, July 31^{st}, 2004
Time: 13:00 - 18:00, Lecture Room 4

Registration

Sunday, August 1^{st}, 2004
Time: 09:00 - 09:20, State Room

Opening plenary

Presenter: Harry Kaya

09:05 **EARLY NORDIC CONTRIBUTIONS TO INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL**

Jørgen Eilenberg, *Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*

Sunday, August 1^{st}, 2004
Time: 09:20 - 10:15, State Room

Founder's Memorial Lecture

Presenter: Dudley Pinnock, *Founder's Lecture Committee*
Honoree: Hans Boman
Lecturer: Kenneth Söderhäll

Sunday, August 1^{st}, 2004
Time: 10:30 - 13:30, State Room

Plenary (Cross-Divisional)
SIP - the past, present and future

Presenter: Just Vlak; Harry Kaya

10:40 **HISTORY OF THE SOCIETY FOR INVERTEBRATE PATHOLOGY**

Elizabeth W. Davidson, *School of Life Sciences, Arizona State University, U.S.A.*

11:00 **PAST, PRESENT AND FUTURE OF MICROSPORIDIA IN THE SIP**

Jaroslaw Weiser, *Praha 4, Heralecka 964, CZECH REPUBLIC*

11:20 **FROM METCHNIKOFF TO MONSANTO AND BEYOND: THE PATH OF MICROBIAL CONTROL**

Jeffrey Lord, *USDA-ARS, USA*

11:40 **INSECTICIDAL BACTERIA IN HISTORICAL PERSPECTIVE: AN OVERWHELMING SUCCESS FOR INVERTEBRATE PATHOLOGY**

Brian A. Federici, *Department of Entomology, University of California, UNITED STATES*

12:00 **FROM BERGOLD TO BURAND: A JOURNEY WITH INSECT VIRUSES**

Basil Arif, *Great Lakes Forestry Centre, CANADA*

12:20 **THE FUNGAL PAST, PRESENT AND FUTURE: GERMINATION, RAMIFICATION AND REPRODUCTION**

John D. Vandenberg, *USDA-ARS, U.S. Plant, Soil & Nutrition Laboratory, U.S.A.*

12:40 **INSECT PARASITIC NEMATODES: FROM LAB CURIOSITIES TO MODEL ORGANISMS**

S. Patricia Stock, *Department of Plant Sciences. University of Arizona. Tucson AZ 85721-0036, USA, USA*

Sunday, August 1^{st}, 2004
Time: 13:30 - 15:30, Corridor levels 2 and 3

Setting up posters

Sunday, August 1^{st}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Workshop (Cross-Divisional)

The graduate student's guide to the galaxy

Chair: Todd Udine

Sunday, August 1^{st}, 2004
Time: 19:00 - 22:00, Marina Congress Center

Welcoming reception

Monday, August 2^{nd}, 2004
Time: 08:00 - 09:30, Lecture Room 1

Plenary (Cross-Divisional)

Invertebrate pathogens as pests

Presenter: Heikki Hokkanen

08:00 **EPIDEMIOLOGY IN HONEY BEES**

Ingemar Fries, *Department of Entomology, Swedish University of Agricultural Sciences, SWEDEN*

08:30 **CRAYFISH PLAQUE (APHANOMYCES ASTACI) IN FINLAND: PAST, PRESENT AND FUTURE**

Satu Viljamaa-Dirks, *National Veterinary and Food Research Institute, Kuopio Department, FINLAND*

09:00 **IMPORTANCE OF BLOOD CELLS AND HEMATOPOIESIS IN HOST DEFENCE IN CRUSTACEANS**

Irene Söderhäll, *Department of Comparative Physiology, Evolutionary Biology Centre, Uppsala University, SWEDEN*

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 1

Symposium (Division of Bacteria)

Second generation transgenic crops

Chair: Sarjeet Gill

10:00 **QUANTIFICATION OF LEPIDOPTERAN ACTIVITY IN COTTON EXPRESSING TWO BT CRY PROTEINS.**

Ty Vaughn, James Baum, Sakuntala Sivasupramaniam, John Greenplate, *Monsanto, USA*

10:25 **USING BT'S TO ACHIEVE ECONOMIC LEVELS OF HOST-PLANT NON-PREFERENCE: HERCULEX * I VS. BLACK CUTWORM**

Steve Lefko, Laura Higgins, Bill McCutchen, *DuPont Agriculture & Nutrition, USA*

10:50 **BACILLUS THURINGIENSIS BINARY INSECTICIDAL PROTEINS FOR CORN ROOTWORM CONTROL: MODE OF ACTION STUDIES**

Meibao Zhuang, Tarlochan S. Dhadialla, *Dow AgroSciences LLC, UNITED STATES*

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 12

Symposium (Division of Nematodes)

Significance of the entomopathogenic nematode infected-host in the soil ecosystem, and potential impact on microbial control

Chair: David Shapiro-Ilan

10:00 **INFECTED HOST'S ROLE IN INFECTION DYNAMICS OF ENTOMOPATHOGENIC NEMATODES**

Parwinder S. Grewal, *Department of Entomology, Ohio State University, U.S.A.*

10:20 **INFECTED HOST INTERACTION WITH ANTAGONISTS**

Harry Kaya, *University of California-Davis, USA*; Heidi Goodrich-Blair, *University of Wisconsin -Madison, USA*

10:40 **EMERGENCE DYNAMICS FROM THE INFECTED HOST AND QUALITY OF EMERGED NEMATODES**

Christine T. Griffin, Martin J. Downes, Alec N. Rolston, *Department of Biology, National University of Ireland, Maynooth, Co. Kildare, IRELAND*; Jon J. Ryder, *School of Biological Sciences, Queen Mary, University of London, London E1 4NS, ENGLAND*

11:00 **RESPONSE OF SOIL FAUNA TO INUNDATIVELY AND CADAVER-APPLIED ENTOMOPATHOGENIC NEMATODES**

Mary Barbercheck, *The Pennsylvania State University, USA*; C. Marie Greenwood, *North Carolina State University, USA*

11:20 **POTENTIAL FOR APPLICATION OF INFECTED HOSTS IN MICROBIAL CONTROL**

David I. Shapiro-Ilan, *USDA-ARS, SAA, U.S.A.*; Edwin E. Lewis, *Virginia Tech, U.S.A.*

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 6

Symposium (Division of Viruses)

Virus ecology

Chair: Linda King

10:00 **ECOLOGY AND EPIDEMIOLOGY OF WHITE SPOT SYNDROME VIRUS OF SHRIMP**

Just M. Vlak, *Wageningen University, NETHERLANDS*; Bui Thi Minh Dieu, *Can Tho University, VIETNAM*; Hendrik Marks, Angela Vermeesch, *Wageningen University, NETHERLANDS*; Tran Phuoc Duong, *Can Tho University, VIETNAM*; D. Zuidema, *Wageningen University, NETHERLANDS*

10:25 **THE ECOLOGY OF INVERTEBRATE IRIDESCENT VIRUSES (IRIDOVIRIDAE): RECENT ADVANCES**

Trevor Williams, *Univ. Publica Navarra, SPAIN*; Carlos F. Marina, *CIP, MEXICO*; Anaximandro Gomez, Alvaro Hernandez, *ECOSUR, MEXICO*; Peter Christian, *Nat. Inst Standards & Biol Contr., UK*

10:50 **FUNCTIONAL IMPORTANCE OF DELETION MUTANT GENOTYPES IN A NUCELOPOLYHEDROVIRUS POPULATION**

Oihane Simón, Trevor Williams, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Miguel López-Ferber, *Laboratoire de Patologie Comparée, UMR 5087, INRA-CNRS-Université de Montpellier II, FRANCE*; Primitivo Caballero, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

11:15 **PERSISTENT INFECTIONS OF BACULOVIRUSES AND CYPOVIRUSES**

Rosie Hails, John Burden, *NERC Centre for Ecology and Hydrology, UK*; Clare Nixon, *School of Biological and Molecular Sciences, Oxford Brookes University, UK*; Rob Graham, *NERC Centre for Ecology and Hydrology, UK*; Steve Sait, *Centre for Biodiversity and Conservation, University of Leeds, UK*; Mike Bonsall, *Imperial College, London, UK*; Linda King, *School of Biological and Molecular Sciences, Oxford Brookes University, UK*; Robert Possee, *NERC Centre for Ecology and Hydrology, UK*

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 10

Symposium (Cross-Divisional)

Honeybee pathology

Chair: Ingemar Fries

10:00 **MOLECULAR CHARACTERISATION OF THE EUROPEAN BUMBLE BEE MICROSPORIDIAN PARASITE NOSEMA BOMBI BASED ON RIBOSOMAL RNA AND BETA-TUBULIN GENES**

W. T. Tay, *School of Biology and Biochemistry, Queens University Belfast, UNITED KINGDOM*

10:20 **FUNGAL DISEASES IN BEES: A STORY OF ASCOSPHAERA**

Rosalind James, Craig Huntzinger, Ellen Klinger, *USDA, ARS, Bee Biology & Systematics Laboratory, USA*; Jeff Skinner, *Oregon State University, USA*

10:40 **MOLECULAR AND BIOCHEMICAL DIFFERENTIATION BETWEEN PAENIBACILLUS LARVAE SUBSP. LARVAE AND PAENIBACILLUS LARVAE SUBSP. PULVIFACIENS.**

Elke Genersch, Ainura Ashiralieva, *Institute for Bee Research, GERMANY*; Jochen Kilwinski, *SVUA Arnsberg, GERMANY*

11:00 **SAMPLING OF ADULT BEES FOR DETECTION OF AMERICAN FOULBROOD (PAENIBACILLUS LARVAE SUBSP. LARVAE) SPORES IN HONEY BEE (APIS MELLIFERA) COLONIES**

Anders Lindström, Ingemar Fries, *Swedish University of Agricultural Sciences, Department of Entomology., SWEDEN*

11:20 **INVESTIGATING INTERACTIONS BETWEEN VARROA DESTRUCTOR, VIRUSES AND HONEY BEES**

Brenda Ball, Judith Wilson, Norman Carreck, *Rothamsted Research, UK*

11:40 **VARROA MITES (VARROA DESTRUCTOR) AND HONEY BEES (APIS MELLIFERA) A DYNAMIC RELATIONSHIP**

Ingemar Fries, *Department of Entomology, Swedish University of Agricultural Sciences, SWEDEN*

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 6

Contributed Papers (Division of Viruses)
virus / contributed paper session 1

Chair: H. J. R. Popham; K. Hoover

13:30 **THE PERITROPHIC MATRIX AS A BARRIER TO FATAL BACULOVIRUS INFECTION IN COTTON-FED HELIOTHIS VIRESCENS**

Ruth Plymale, Diana Cox-Foster, Dan Jones, Kelli Hoover, *Penn State University, USA*

13:45 **SELENIUM IMPACTS THE INFECTIVITY OF ACMNPV IN TRICHOPLUSIA NI**

Holly Popham, *USDA ARS Biological Control of Insects Research Laboratory, UNITED STATES*; Kent Shelby, *USDA ARS Biologcial Control of Insects Research Laboratory, UNITED STATES*

14:00 **INACTIVATION OF PHTHORIMAEA OPERCULELLA GRANULOVIRUS (POGV) DUE TO NATURAL RADIATION AND THE POTENTIAL OF UV-ADJUVANTS FOR VIRAL PROTECTION**

Marc Sporleder, Jürgen Kroschel, *International Potato Center (CIP), PERU*; Jürg Huber, *Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, GERMANY*; Octavio Zegarra, *International Potato Center (CIP), PERU*; Aziz Lagnaoui, *Environmentally and Socially Sustainable Development, The World Bank, USA*

14:15 **HORIZONTAL AND VERTICAL TRANSMISSION OF WILD-TYPE AND RECOMBINANT HASNPV**

Xiulian Sun, Mingzhe Zhou, *Wuhan Institute of Virology, Chinese Academy of Sciences,Wuhan, 430071, Hubei, CHINA*; Wopke Van der Werf, *Crop and Weed Ecology Group, Wageningen University, THE NETHERLANDS*; Just M. Vlak, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Zhihong Hu, *Wuhan Institute of Virology, Chinese Academy of Sciences,Wuhan, 430071, Hubei, CHINA*

14:30 **THE POLYHEDRIN GENE OF THE AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDRO-VIRUS IS A MOSAIC OF GROUP I AND GROUP II NPV POLYHEDRIN GENES**

Johannes A. Jehle, *Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate, GERMANY*

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 1

Symposium (Division of Nematodes)
Nematodes and cold adaptations

Chair: Patricia Stock

13:30 **COLD TOLERANCE STRATEGIES OF ENTOMOPATHOGENIC NEMATODES**

Ian M. Brown, *Biology, Georgia Southwestern State University, U.S.A.*; Randy Gaugler, *Entomology, Rutgers University, U.S.A.*

13:50 **PHYSIOLOGY OF NEMATODE COLD TOLERANCE**

Parwinder S. Grewal, Ganpati B. Jagdale, *Department of Entomology, Ohio State University, U.S.A.*

14:10 **STU** **SEASONAL DYNAMICS OF ENTOMOPATHOGENIC NEMATODES OF THE GENERA STEINERNEMA AND HETERORHABDITIS AND THEIR INSECT HOSTS, WITH COMMENTS ON THE WINTER PERIOD**

Vladimir Puza, Zdenek Mracek, *Institute of Entomology, Czech Academy of Sciences, CZECH REPUBLIC*

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 12

Contributed Papers (Division of Microbial Control)
microbial control / contributed paper session 1

Chair: Lawrence Lacey; Shawn McLaughlin

13:30 **EVALUATION OF COMMERCIAL FORMULATIONS OF THE CODLING MOTH GRANULOVIRUS AGAINST NATURAL CODLING MOTH INFESTATIONS IN PACIFIC NORTHWEST APPLE AND PEAR ORCHARDS**

Steven Arthurs, Lawrence Lacey, *USDA-ARS, USA*

13:45 **CONTROL OF THE BROWNTAIL MOTH, EUPROCTIS CHRYSORRHOEA, IN THE UNITED STATES WITH A BACULOVIRUS**

James Slavicek, *USDA Forest Service, USA*; Joseph Elkinton, *University of Massachusetts, USA*; John D. Podgwaite, *USDA Forest Service, USA*

14:00 **DEVELOPMENT OF SPODOPTERA EXEMPTA NUCLEOPOLYHEDROVIRUS (SPEXMNPV) FOR THE CONTROL OF AFRICAN ARMYWORM IN EAST AFRICA**

David Grzywacz, Mark Parnell, *Natural Resources Institute, UK*; Wilfred Mushobozi, *Pest Control Services, TANZANIA*; Ken Wilson, *Lancaster University, UK*

14:15 **IMPACT OF DISEASES ON SOFTSHELL CLAM (MYA ARENARIA) POPULATIONS**

Shawn M. McLaughlin, *NOAA National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research/Cooperative Oxford Laboratory, U.S.A.*

14:30 **MICROBIAL CONTROL OF VARROA: FIELD ADVENTURES**

Rosalind James, Craig Huntzinger, Ellen Klinger, *USDA-ARS Bee Biology and Systematics Laboratory, U.S.A.*

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Corridor, II and III levels

Poster Session 1: Posters for fungi and bacteria

B-1 **ACTIVITY OF BACILLUS THURINGIENSIS TOXINS AGAINST COCOA POD BORER LARVAE**

Tetty Chaidamsari, *Plant Research International, NETHERLANDS*; Djoko Santoso, *Biotechnology Research Institute for Estate Crops, INDONESIA*; Soekadar Wiryadiputra, *Indonesian Coffee and Cacao Research Institute, INDONESIA*; Ruud De Maagd, *Plant Research International, NETHERLANDS*

B-2 **INTERACTION BETWEEN P20 AND CYT1AA IN VIVO USING THE TWO-HYBRID SYSTEM OF SACCHAROMYCES CEREVISIAE**

Olga Burgazliev, Robert Manasherob, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

B-3 **AN ATTEMPT TO IMPROVE MOSQUITO LARVICIDAL ACTIVITY OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS**

Nadine Sela-Baranes, Robert Manasherob, Eitan Ben-Dov, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

B-4 **LARVICIDAL ACTIVITY OF TRANSGENIC ESCHERICHIA COLI EXPRESSING TOXIN GENES FROM BACILLUS THURINGIENSIS TO SUSCEPTIBLE LEPIDOPTERA**

Maria Menin, *Ben-Gurion University of the Negev, ISRAEL*; Vadim Khasdan, *Dept. of Entomology, ARO, Gilat Research Center, ISRAEL*; Eitan Ben-Dov, Robert Manasherob, Sammy Boussiba, *Ben-Gurion University of the Negev, ISRAEL*; Rami Horowitz, *Dept. of Entomology, ARO, Gilat Research Center, ISRAEL*; Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

B-5 **PHAGOCYTOSIS BY INSECT MACROPHAGES: A MORPHOLOGICAL AND BIOCHEMICAL STUDY**

Sonia Costa, Carlos Ribeiro, *Departamento de Biologia, Universidade dos Açores, PORTUGAL*; Robert Zumbihl, Fabienne Vigneux, Noel Boemare, Michel Brehélin, *EMIP Unité INRA UMII 1133, Université de Montpellier II, FRANCE*

B-6 **PARTIAL RESISTANCE OF PLUTELLA XYLOSTELLA TO COMMERCIAL FORMULATES OF BACILLUS THURINGIENSIS IN AGRICULTURAL FIELDS IN MXICO***

Artemisa Perea, Magdalena Iracheta-Cardenas, *Facultad de Ciencias Biologicas/UANL, MEXICO*; Rafael Bujanos-Muñiz, *INIFAP-Celaya, MEXICO*; Luis Galan-Wong, Benito Pereyra-Alferez, *Facultad de Ciencias Biologicas/UANL, MEXICO*

B-7 **CLONING AND EXPRESSION OF NOVEL CRY GENES FROM A MOSQUITOCIDAL STRAIN OF BACILLUS THURINGIENSIS SEROVAR SOTTO**

Akira Ohgushi, *Graduate School of Agriculture, Kyushu University, JAPAN*; Hiroyuki Saitoh, Naoya Wasano, *Biotechnology & Food Research Institute, JAPAN*; Michio Ohba, *Graduate School of Agriculture, Kyushu University, JAPAN*

B-8 **CLONING AND CHARACTERIZATION OF A NOVEL GENE, CRY9EC1, ENCODING A LEPIDOPTERA-SPECIFIC CRYSTAL PROTEIN FROM A BACILLUS THURINGIENSIS SEROVAR GALLERIAE STRAIN**

Naoya Wasano, Hiroyuki Saitoh, Eiichi Mizuki, *Fukuoka Industrial Technology Center, JAPAN*; Minoru Maeda, *Kyushu Medical Co. Ltd., JAPAN*; Akira Ohgushi, Michio Ohba, *Kyushu University, JAPAN*

B-9 **EXPRESSION OF A VEGETATIVE INSECTICIDAL PROTEIN GENE UNDER THE CONTROL OF PROMOTER PLUS SD SEQUENCES OF CRY GENES FROM BACILLUS THURINGIENSIS**

Jianwu Chen, Fan Sun, Mujing Tang, Yongxia Shi, Wei Xu, Jianxiu Yu, Yi Pang, *State Key Laboratory for Biocontrol & Institute of Entomology, Zhongshan University, CHINA*

B-10 **EXPRESSION OF VIP1/VIP2 GENES IN ESCHERICHIA COLI AND BACILLUS THURINGIENSIS**

Yongxia Shi, Wei Xu, Meijin Yuan, Mujing Tang, Jianwu Chen, Yi Pang, *State Key Laboratory for Biocontrol & Institute of Entomology, Zhongshan University, CHINA*

B-11 **RECOVERY OF BACILLUS THURINGIENSIS FROM ACTIVATED SLUDGES OF A WASTE WATER TREATMENT PLANT IN A MISO FACTORY**

Tokio Ichimatsu, Kazuhiko Higuchi, *Fukuoka Industrial Technology Center, JAPAN*; Kumiko Kagoshima, *Kyushu University, JAPAN*; Eiichi Mizuki, *Fukuoka Industrial Technology Center, JAPAN*; Michio Ohba, *Kyushu University, JAPAN*

B-12 **CANCER CELL-KILLING ACTIVITY OF PARASPORAL INCLUSION PROTEINS FROM JAPANESE ISOLATES OF BACILLUS THURINGIENSIS**

Eiichi Mizuki, *Fukuoka Industrial Technology Center, JAPAN*; Yoshitaka Murata, Masako Nomaguchi, *Kyurin Corporation, JAPAN*; Hiroyuki Saitoh, Satoko Yamashita, *Fukuoka Industrial Technology Center, JAPAN*; Yasuyuki Sasaguri, *University of Occupational and Environmental Health, JAPAN*; Michio Ohba, *Kyushu University, JAPAN*

B-13 **LYOPHILIZATION OF LEPIDOPTERAN MIDGUTS: A PRESERVING METHOD FOR BACILLUS THURINGIENSIS TOXIN BINDING STUDIES**

Carmen Sara Hernández, Ana Rodrigo, Juan Ferré, *Universitat de València, SPAIN*

B-14 **MOLECULAR STUDIES OF A BACILLUS THURINGIENSIS PUTATIVE VIRULENCE OPERON**

Jinhong Wang, David Ellar, *Biochemistry Department, University of Cambridge, UNITED KINGDOM*

B-15 **A NOVEL TOXIN FROM A BRAZILIAN STRAIN OF BACILLUS THURINGIENSIS REPORTED TO KILL THE COTTON BOLL WEEVIL (ANTHONOMUS GRANDIS)**

Joseilde Silva-Werneck, David Ellar, *University of Cambridge, UK*

B-16 **ROLE OF BACILLUS THURINGIENSIS TOXINS DOMAINS II AND III IN TOXICITY AND BINDING TO MIDGUT RECEPTORS OF SPODOPTERA EXIGUA (HÜBNER).**

Joel González-Cabrera, *Universidad de Valencia, ESPAA*; Salvador Herrero, Petra L. Bakker, Ruud De Maagd, *Plant Research International B.V., THE NETHERLANDS*; Juan Ferré, *Universidad de Valencia, ESPAA*

B-17 **CRY1C-TOLERANCE STUDIES USING SF9 CELLS AS A MODEL SYSTEM**

Dror Avisar, Michal Segal, Baruch Sneh, Aviah Zilberstein, *Tel Aviv University, ISRAEL*

B-18 **POTENTIAL NON-TARGET IMPACTS OF BT-CANOLA**

Peter G. Mason, Lorraine Braun, Suzanne I. Warwick, *Agriculture and Agri-Food Canada, CANADA*; C. Neal Stewart, *University of Tennessee, USA*

B-19 **BIOCHEMICAL CHARACTERIZATION OF FIELD EVOLVED RESISTANCE TO BACILLUS THURINGIENSIS TOXIN CRY1AC IN DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA**

Maria Sales Ibiza-Palacios, *Department of Genetics, Universitat de València, SPAIN*; Ali Sayyed, Ben Raymond, Denis Wright, *Department of Biological Sciences, Imperial College London, UK*; Baltasar Escriche, *Department of Genetics, Universitat de València, SPAIN*

B-20 **PURIFICATION AND CHARACTERIZATIONS OF A NEW BACILLUS THURINGIENSIS VIRULENCE FACTOR : THE INHA2 METALLOPROTEASE**

Myriam Hajaij, *Unité Génétique Microbienne et Environnement, INRA, la Minière, FRANCE*; Michel Gohar, *Unité Génétique Microbienne et Environnement, INRA, Unité Microbiologie et Génétique Microbienne, INRA, FRANCE*; Sinda Fedhila, *Unité Génétique Microbienne et Environnement, INRA, la Minière, FRANCE*; Didier Lereclus, Christina Nielsen-LeRoux, *Unité Génétique Microbienne et Environnement, INRA, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*

B-21 **HOST RANGE EXTENSION OF BACILLUS THURINGIENSIS CRY TOXINS TO THE SPINY BOLLWORM EARIAS INSULANA (BOIS.) (LEPIDOPTERA: NOCTUIDAE)**

María A. Ibargutxi, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*; Anna Estela, Juan Ferré, *Departamento de Genética Universidad de Valencia, SPAIN*; Primitivo Caballero, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*

B-22 **ISOLATION OF A NEW BACILLUS THURINGIENSIS STRAIN CYZ-13 AND CRY-TYPE GENE ANALYSIS BY PCR-RFLP**

Li Changyou, *1. Dept. of Plant Protection, Laiyang Agricultural College, P.R. CHINA*; Lu Xiujun, *2. Dept. of Entomology Agricultural University of Hebei, P.R. CHINA*; Zheng Guiling, *1. Dept. of Plant Protection, Laiyang Agricultural College, P.R. CHINA*; Cheng Linyou, *2. Dept. of Entomology Agricultural University of Hebei, P.R. CHINA*; Li Guoxun, *1. Dept. of Plant Protection, Laiyang Agricultural College, 2. Dept. of Entomology Agricultural University of Hebei, P.R. CHINA*

B-23 **HIGH LEVEL OF CYT1A SYNTHESIS IN BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS IS DUE TO THREE PROMOTERS AND A STRONG 3' MRNA STEM-LOOP STRUCTURE**

Yuko Sakano, *Department of Entomology, University of California, U.S.A.*; Baoxue Ge, *Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, U.S.A.*; Hyun-Woo Park, *Department of Entomology, University of California, U.S.A.*; Brian A. Federici, *Department of Entomology, Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, U.S.A.*

B-24 **THE 20-KDA PROTEIN OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS ENHANCES**

Hyun-Woo Park, Dennis K. Bideshi, *Department of Entomology, University of California, U.S.A.*; Brian A. Federici, *Department of Entomology, Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, U.S.A.*

B-25 **BACILLUS CEREUS SENSU LATO POPULATION FROM SOW BUG: VIRULENCE GENE PROFILES VERSUS CHROMOSOMAL DNA RELATIONSHIP REVEALED BY PFGE**

Izabela Swiecicka, *Department of Microbiology, University of Bialystok, Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, POLAND*; Jacques Mahillon, *Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, BELGIUM*

B-26 **MOLECULAR CLONING OF A NEW GENE ENCODING A CRY PROTEIN EFFECTIVE TOWARDS COTTON BOLL WEEVIL, ANTHONOMUS GRANDIS**

Maria F. Grossi de Sa, Mariana T. Q. De Magalhães, João A. N. Batista, Shirley M. B. Da Silva, Rodrigo R. Fragoso, Osmundo B. Oliveira-Neto, Rose Monnerat, Edson L. Z. Figueira, *Embrapa Recursos Genéticos e Biotecnologia. Parque Estação Biológica, BRAZIL*

B-27 **DIVERSITY OF BACILLUS SPP. POPULATIONS IN THE DIGESTIVE TRACT OF LUCILIA CAESAR AND LUCILIA SERICATA BLOWFLIES (DIPTERA: CALLIPHORIDAE)**

Sophie Buyle, Alan Fauconnier, *Nivelles Laboratories, BELGIUM*; Izabela Swiecicka, Jacques Mahillon, *UCL, BELGIUM*

B-28 **THE DEVELOPMENT OF AN ASPOROGENIC STRAIN OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS BY DISRUPTING THE SIGK GENE AFFECTS CRYSTAL PROTEIN EXPRESSION AND TOXICITY**

Adriana González, Gemma Armengol, *Biotechnology and Biological Control Unit, Corporation for Biological Research, COLOMBIA*; Sergio Orduz, *Biotechnology and Biological Control Unit, Corporation for Biological Research, Universidad de Pamplona, COLOMBIA*; Neil Crickmore, *School of Biological Sciences, University of Sussex, UNITED KINGDOM*

B-29 **GENOMIC SEQUENCE OF A CADHERIN-LIKE GENE FROM THE EUROPEAN CORN BORER (OSTRINIA NUBILALIS, HÜBNER)**

Yolanda Bel, Baltasar Escriche, *University of Valencia, SPAIN*

B-30 **MOLECULAR EPIDEMIOLOGY OF PAENIBACILLUS LARVAE SUBSP. LARVAE**

Jaana Pentikäinen, Eija Kalliainen, Sirpa Heinikainen, Sinikka Pelkonen, *National Veterinary and Food Research Institute, Kuopio Department, FINLAND*

B-31 **GLOBAL ASSESSMENT OF BACILLUS THURINGIENSIS CRY1 GENE CONTENTS USING DNA MICROARRAYS.**

Jaroslaw Letowski, *NRC-Biotechnology Research Inst.,, CANADA*; Alejandra Bravo, *Instituto de Biotecnologia UNAM, MEXICO*; Roland Brousseau, Luke Masson, *NRC-Biotechnology Research Inst.,, CANADA*

B-32 **A VIP NOMENCLATURE?**

Neil Crickmore, *University of Sussex, UK*; Dan Ziegler, *Bacillus Genetic Stock Center, USA*; Alejandra Bravo, *National University, MEXICO*; Ernest Schnepf, *Independent, USA*; Didier Lereclus, *Institiut Pasteur, FRANCE*; Jim Baum, *Monsanto, USA*; Jeroen Van Rie, *Bayer Crop Science, BELGIUM*; Donald Dean, *Ohio State University, USA*

B-33 **INHIBITORY EFFECT OF THE ENTOMOPATHOGENIC BACTERIUM PHOTORHABDUS LUMINESCENS ON MANDUCA SEXTA PHENOLOXIDASE**

Ioannis G. Eleftherianos, Nicholas Waterfield, Richard ffrench-Constant, Stuart E. Reynolds, *Department of Biology & Biochemistry, University of Bath, UNITED KINGDOM*

F-1 **IMPROVEMENT OF MYCOINSECTICIDE BY SIMULTANEOUSLY OVEREXPRESSING A SUBTILISIN-LIKE GENE AND AN ENDOCHITINASE GENE IN BEAUVERIA BASSIANA**

Wei-Guo Fang, Jin-Cheng Ma, Kai Jin, Yong-Jun Zhang, Yan Pei, *Biotechnology Research Center Southwest Agricultural University, P.R.CHINA*

F-2 **MYIOPHAGUS UCRAINICUS, A CHYTRIDIOMYCETE FUNGAL PATHOGEN OF SPODOPTERA FRUGIPERDA IN NON-IRRIGATED RICE IN COLOMBIA**

Richard A. Humber, *USDA-ARS Plant Protection Research, USA*; Ruber J. Delgado C., *Apartado Aero No. 03, Chigorodo Antioquia, COLOMBIA*

F-3 **A NOVEL TECHNIQUE TO INOCULATE CONIDIA OF ENTOMOPATHOGENIC FUNGI AND ITS APPLICATION FOR INVESTIGATION OF SUSCEPTIBILITY OF THE JAPANESE PINE SAWYER TO BEAUVERIA BASSIANA**

Mitsuaki Shimazu, *Forestry and Forest Products Research Institute, JAPAN*

F-4 **MOLECULAR CHARACTERISATION OF BEAUVERIA BASSIANA ISOLATES OBTAINED FROM OVERWINTERING SITES OF SUNN PESTS IN WEST ASIA AND THE MIDDLE EAST**

Marilena Aquino de Muro, Sarah Elliott, *CABI Bioscience, UK*; David Moore, *CABI Biosciene, UK*; Bruce Parker, Margaret Skinner, William Reid, *University of Vermont, USA*; Mustapha El Bouhssini, *ICARDA, SYRIA*

F-5 **BEAUVERIA CALEDONICA AS A NATURALLY OCCURRING PATHOGEN OF HYLASTES ATER AND HYLURGUS LIGNIPERDA IN NEW ZEALAND**

Travis Glare, *Agresearch, NEW ZEALAND*; Stephen Reay, *SBFR, NEW ZEALAND*; Tracey Nelson, *AgResearch, NEW ZEALAND*

F-6 **EFFECTS OF SELECTED PESTICIDES ON THE GROWTH AND GERMINATION OF CONIDIA OF THE APHID PATHOGENIC FUNGUS ERYNIA NEOAPHIDIS REMAUDIERE ET HENNEBERT**

Cezary Tkaczuk, *Department of Plant Protection, University of Podlasie, POLAND*

F-7 **HORIZONTAL AND VERTICAL TRANSMISSION OF ENTOMOPATHOGENIC FUNGI AND ENDOSYMBIONT BACTERIA IN APHID POPULATIONS**

Annette Bruun Jensen, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*; Lise Petersen, *Bioinformatics Centre, University of Copenhagen, DENMARK*; Lars Monrad Hansen, *Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, Department of Crop Protection, DENMARK*; Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*

F-8 **EFFECTS OF DETRIVORES ON A PLANT HERBIVORE ENTOMOPATHOGEN SYSTEM.**

Karsten Dromph, Jakob Magid, Jørgen Eilenberg, Peter Esbjerg, *The Royal Veterinary and Agricultural University, DENMARK*

F-9 **BIOLOGICAL CONTROL OF VARROA DESTRUCTOR DISSEMINATION AND IMPACT OF SPORE INOCULUM**

Caroline Birchall, *Rothamsted Research, UK*; Gillian Davidson, *Warwick HRI, UK*; Brenda Ball, Judith K. Pell, *Rothamsted Research, UK*; David Chandler, *Warwick HRI, UK*

F-10 **THE COST ACTION 842: STATUS OF RESEARCH ON ENTOMOPHTHORALES IN EUROPE**

Siegfried Keller, *Federal Research Station for Agroecology and Agriculture, SWITZERLAND*

F-11 **INFLUENCE OF ZN ON GROWTH AND PRODUCTION OF ORGANIC ACIDS BY PAECILOMYCES FUMOSOROSEUS IN SOLID AND SUBMERGED CULTURE**

Ali Asaff, *UAM-Iztapala, MEXICO*; Octavio Gómez, Carlos Cerda, Mayra De la Torre, *CINVESTAV, MEXICO*; Gustavo Viniegra, *UAM-Iztapalapa, MEXICO*

F-12 **CONIDIAL COLOR IS IMPORTANT FOR SOLAR RADIATION TOLERANCE IN THE ENTOMOPATHOGENIC FUNGUS METARHIZIUM ANISOPLIAE VAR. ANISOPLIAE**

Gilberto Braga, *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, 14040-903,, BRAZIL*; Drauzio Rangel, *Department of Biology, Utah State University, USA*; Stephan Flint, *Dept. of Forest, Range and Wildlife Sciences, Utah State University, USA*; Anne Anderson, Donald Roberts, *Department of Biology, Utah State University, USA*

F-13 **THE EFFECT OF AMMONIA ON CONIDIAL LONGEVITY OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE**

Tove Steenberg, Ole Kilpinen, *Danish Pest Infestation Laboratory, DENMARK*; Dave Moore, *CABI Bioscience, UNITED KINGDOM*

F-14 **INVESTIGATION OF THE SURVIVAL OF CONIDIA OF ENTOMOPATHOGENIC FUNGI WITH POTENTIAL FOR CONTROL OF VARROA DESTRUCTOR IN HONEY BEE COLONIES**

Gillian Davidson, *Warwick HRI, UNITED KINGDOM*; Caroline Birchall, Judith K. Pell, Brenda Ball, *Rothamsted Research, UNITED KINGDOM*; David Chandler, *Warwick HRI, UNITED KINGDOM*

F-15 **PATHOGENS ASSOCIATED WITH THE ANT, MYRMICA RUBRA, IN ITS INTRODUCED AND NATIVE RANGE**

Eleanor Groden, Shicai Yan, Frank Drummond, *Department of Biological Sciences, University of Maine, U.S.A.*

F-16 **PRELIMINARY SURVEY OF ENTOMOPATHOGENIC FUNGI ASSOCIATED WITH THE AFRICAN ROOT AND TUBER SCALE STICTOCOCCUS VAYSSIEREI RICHARD (HEMIPTERA: STICTOCOCCIDAE)**

Leonoor Wijnans, Maurice Tindo, *International Institute of Tropical Agriculture, Humid Forest Center, CAMEROON*; Rachid Hanna, *International Institute of Tropical Agriculture, Biological Control Center for Africa, REPUBLIC OF BENIN*

F-17 **CICADAPEPTINS, NEW AIB-CONTAINING PEPTIDES,**

Stuart B. Krasnoff, *Department of Plant Pathology, Cornell University, U.S.A.*; Donna M. Gibson, *USDA, ARS, Plant Protection Research Unit, U.S.A.*; Melissa Wagenaar, Ricardo Reátegui, James B. Gloer, *Department of Chemistry, University of Iowa, U.S.A.*

F-18 **STUDY OF THE SPORULATION OF PAECILOMYCES FUMOSOROSEUS VARYING CARBON AND NITROGEN SOURCE**

Ana Gabriela Osuna Paez, *Consejo Estatal de Ciencia y Tecnología, MEXICO*; Héctor Manuel Cárdenas Cota, *Centro de Ciencias de Sinaloa, MEXICO*; Rene Castro Montoya, *Facultad de Fisicomatemáticas, Ciudad Universitaria, MEXICO*

F-19 **SUSCEPTIBILITY OF THE CEREAL APHID METOPOLOPHIUM DIRHODUM TO THE ENTOMOPATHOGENIC FUNGUS PANDORA NEOAPHIDIS ON GNA WHEAT**

Paresh A. Shah, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*; A.M.R. Gatehouse, *School of Biology, King George VI Building, University of Newcastle,, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

F-20 **EFFECTIVENESS OF LOCAL FUNGAL ISOLATES FOR COLORADO POTATO BEETLE IN UZBEKISTAN**

Kerim K. Ergashev, Erkin N. Abdullaev, *Samarkand State University, UZBEKISTAN*

F-21 **DIFFERENTIAL SUSCEPTIBILITY BETWEEN DIAPAUSING AND NON-DIAPAUSING COLORADO POTATO BEETLES (LEPTINOTARSA DECEMLINEATA) TREATED WITH BEAUVERIA BASSIANA**

C. Noronha, *Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, CANADA*; Mark Goettel, *Lethbridge Research Centre, Agriculture and Agri-Food Canada, CANADA*

F-22 **METHODS FOR RISK ASSESSMENT OF BIOLOGICAL CONTROL PROGRAMS IN THE SAHELIAN REGION**

Eva Nølke Fisker, Niels Elmegaard, *The Danish National Environmental Research Institute, DENMARK*; Jørgen Eilenberg, *The Royal Veterinary and Agricultural University, DENMARK*; Christiaan Koyman, Jürgen Langewald, *The International Institute of Tropical Agriculture, BENIN*; Zakaria Ouambama, Abdoulaye Tonkoano, *AGRHYMET Regional Centre, NIGER*

F-23 **ISOLATION AND CHARACTERISATION OF NATURALLY OCCURRING BEAUVERIA BASSIANA FROM VEGETATION SHOW HIGH DIVERSITY**

Nicolai Vitt Meyling, Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40 DK-1871 Frederiksberg C, DENMARK*; Mette Lubeck, *Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40 DK-1871 Frederiksberg C, DENMARK*

F-24 **BAUVERIA BASSIANA MUTANTS OVER-PRODUCING PROTEASES SHOW DIFFERENT PROTEASE-PROFILE THAN PARENTAL STRAIN**

Andrea Alcazar-Pizaña, Magdalena Iracheta-Cardenas, Luis Galan-Wong, Hugo Luna-Olvera, Benito Pereyra-Alferez, *Universidad Autonoma de Nuevo Leon, MEXICO*

F-25 **INSECT PATHOGENIC FUNGI AND PARASITOIDS AS NATURAL CONTROL AGENTS OF THE APPLE APHIDS APHIS POMI AND DYSAPHIS PLANTAGINEA**

Karin Westrum, Ingeborg Klingen, *The Norwegian Crop Research Institute, NORWAY*

F-26 **THE EFFECT OF METHOD USED ON OBSERVED INFECTION LEVEL OF NEOZYGITES FLORIDANA IN A TETRANYCHUS URTICAE POPULATION IN STRAWBERRY**

Inger Nordengen, Ingeborg Klingen, *The Norwegian Crop Research Institute, Plant Protection Centre, NORWAY*

F-27 **INTERACTIONS BETWEEN PANDORA BLUNCKII AND ZOOPHTHORA RADICANS ISOLATES IN PLUTELLA XYLOSTELLA POPULATIONS**

A. Guzman-Franco, *Plant and Invertebrate Ecology Division, Rothamsted Research, School of Biosciences, University of Nottingham, UNITED KINGDOM*; S. J. Clark, *Biomathematics Unit, Agriculture and the Environment Division, Rothamsted Research, UNITED KINGDOM*; P. G. Alderson, *School of Biosciences, University of Nottingham, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

F-28 **EFFECT OF FUNGAL INFECTION ON THE REPRODUCTIVE POTENTIAL OF APHIDS AND THEIR PROGENY**

Jason Baverstock, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*; H. E. Roy, *Department of Life Sciences, Anglia Polytechnic University, UNITED KINGDOM*; S. J. Clark, *Agriculture and the Environment Division, Rothamsted Research, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

F-29 **EFFECT OF DIFFERENT CONIDIAL CONCENTRATIONS OF THE FUNGUS, VERTICILLIUM LECANII (ZIMM.)VIEGAS ON THE NET REPRODUCTIVE RATE (R0) OF THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS)**

S.A. Safavi, Aziz Kharazi Pakdel, G.R. Rasoulian, *Department of Plant Protection, Faculty of Agriculture, University of Tehran, IRAN*; H. Askari, *Research Institute of Forests and Rangelands, Tehran, IRAN*

F-30 **COMPARISION OF TWO PROPAGULES TYPES OF BEAUVERIA BASSIANA AGAINST TRIALEURODES VAPORARIORUM.**

Javad Karimi, Aziz Kharazi Pakdel, Ali Mirshekar, *Dept. plant protection,Tehran univ., IRAN*

F-31 **IN VIVO PATHOGENICITY OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE ON CHROTOGONUS TRACHYPTERUS(ORTH.:PYRGOMORPHIDAE).**

Ali Mirshekar, Aziz Kharazi Pakdel, *Dept. plant protection,Tehran univ., IRAN*; Mehran Ghazavi, *Plant Pest & Diseases Research Institute,Tehran, IRAN*; Javad Karimi, *Dept. plant protection,Tehran univ., IRAN*

F-32 **ARE OLIGOPHAGOUS LABOULBENIALES SPECIES ACTUALLY SPATIALLY MONOPHAGOUS SPECIES?**

Larry Huldén, *Finnish Museum of Natural History, FINLAND*

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 6

Contributed Papers (Division of Viruses)

virus / contributed papers session 2

Chair: D. Lynn; J. Burand

15:00 **LEPIDOPTERAN CELL LINES AFTER LONG-TERM CULTURE IN A COMMERCIAL SERUM-FREE MEDIUM: COMPARISON OF GROWTH RATES AND BACULOVIRUS REPLICATION.**

Dwight Lynn, *USDA, Insect Biocontrol Laboratory, USA*

15:15 **ALTERATION OF THE REPRODUCTIVE TISSUES OF HELICOVERPA ZEA FEMALES INFECTED WITH HZ-2V**

John Burand, Weijia Tan, Woojin Kim, *University of Massachusetts, USA*

15:30 **STU** **REPLICATION OF A NOVEL PICORNA-LIKE VIRUS OF THE GENUS IFLAVIRUS**

Juliette Ongus, Dick Peters, Just M. Vlak, *Wageningen University, THE NETHERLANDS*; Eberhard Bengsch, *Centre de Biophysique Moléculaire, CNRS, Orléans,, FRANCE*; Monique M. Van Oers, *Wageningen University, THE NETHERLANDS*

15:45 **THE BIOLOGY AND CHARACTERISATION OF AN ASCOVIRUS (ASCOVIRIDAE) FROM AUSTRALIA**

Ian Newton, *University of Queensland, AUSTRALIA*

16:00 **A STUDY OF SINGLE NUCLEOCAPSID NUCLEOPOLYHEDROVIRUS ENVELOPE PROTEIN P74 REQUIRED TO THE INFECTION OF HOST MIDGUT**

Lun-Guang Yao, Wen-Ke Zhou, Feng Yan, Hua Xu, Yong Zheng, Yi-Peng Qi, *Wuahn University, CHINA*

16:30 **WSSV INTERACTION WITH FRESHWATER CRAYFISH**

Pikul Jiravanichpaisal, Kenneth Söderhäll, Irene Söderhäll, *Department of Comparative Physiology, Evolutionary Biology Centre, Uppsala university, SWEDEN*

16:45 **DENSOVIRUSES (DNVS) WITH AN AMBISENSE GENOME ARE HIGHLY DIVERSIFIED IN THEIR MODE OF EXPRESSION**

Max Bergoin, Yi Li, Adly Abd-Alla, *Laboratoire de Pathologie Comparée, Université Montpellier II, FRANCE*; Gilles Fédière, *Institut de Recherche pour le Développement, Faculty of Agriculture, Cairo University, EGYPT*; François Cousserans, Elizabeth Baquerizo, Françoise-Xavière Jousset, *Laboratoire de Pathologie Comparée, Université Montpellier II, FRANCE*; Peter Tijssen, Mohamed El-Far, *INRS-Institut Armand-Frappier, Université du Québec, CANADA*

17:00 **SPLTMNPV BLOCKS SEMNPV-INDUCED APOPTOSIS IN A SPODOPTERA LITURA CELL LINE**

Mei Yu, Kai Yang, Lei Lv, Lijing Pan, Yi Pang, *State Key Laboratory for Biocontrol & Institute of Entomology,Zhongshan University, CHINA*

17:15 **THE ANTICARSIA GEMMATALIS NUCLEOPOLYHEDROVIRUS (AGMNPV) GENOME**

Jose Luiz Caldas Wolff, *Laboratrio de Virologia Molecular, Nucleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes Mogi das Cruzes, SP, BRAZIL*; Bergmann Morais Ribeiro, *Departamento de Biologia Celular, Universidade de Brasilia, Brasilia DF, BRAZIL*; Alejandra Garcia-Maruniak, James Maruniak, *Entomology & Nematology Department, University of Florida, Gainesville, FL, USA*; Flavio Moscardi, *Embrapa/CNPSO, Londrina, PR, BRAZIL*; Marlinda Lobo de Souza, Maria Elita Batista de Castro, *Embrapa/CENARGEN, Brasilia, DF, BRAZIL*; Paolo M. de A. Zanotto, *Instituto de Ciencias Biomedicas, USP, Av. Lineu Prestes, Sao Paulo, SP, BRAZIL*

17:30 **TOWARDS A COMPREHENSIVE PHYLOGENY OF LEPIDOPTERAN SPECIFIC BACULOVIRUSES**

Martin Lange, *Laboratory of Biotechnological Crop Protection, Agricultural Service Center Palatinate, GERMANY*; Hualin Wang, Zhihong Hu, *Joint Laboratory of Invertebrate Pathology, Wuhan Institute of Virology, P.R. CHINA*; Johannes A. Jehle, *Laboratory of Biotechnological Crop Protection, Agricultural Service Center Palatinate, GERMANY*

17:45 **DETERMINATION OF THE PROMOTER REGION OF THE CHILO IRIDESCENT VIRUS DNA POLYMERASE GENE**

Remziye Nalçacioglu, Just M. Vlak, *Wageningen University, THE NETHERLANDS*; Zihni Demirbag, *Karadeniz Technical University, TURKEY*; Monique M. Van Oers, *Wageningen University, THE NETHERLANDS*

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 1

Symposium (Division of Fungi)

Insect-fungal associations

Chair: Fernando Vega; Meredith Blackwell

15:10 **PHYLOGENETICS OF THE INSECT PATHOGENIC FUNGUS BEAUVERIA**

Stephen Rehner, *USDA, ARS, Insect Biocontrol Laboratory, USA*

15:35 **CRYPTIC SPECIES AND RECOMBINATION IN THE INSECT PATHOGENIC FUNGUS, METARHIZIUM**

Michael Bidochka, Cherrie-Lee Small, *Brock University, CANADA*; Michael Spironello, *University of Toronto, CANADA*

16:00 **INTERACTIONS AMONG INSECT PARASITOIDS, ARTHROPOD PREDATORS AND ENTOMOPATHOGENIC FUNGI**

Michael Furlong, *University of Queensland, AUSTRALIA*; Judith K. Pell, *Rothamsted Research, UK*

16:25 **ECOLOGY AND EVOLUTION OF FUNGAL ENDOPHYTES AND THEIR ROLES AGAINST INSECTS**

A. Elizabeth Arnold, *Duke University, USA*; Leslie Lewis, *USDA Agricultural Research Service, USA*

16:50 **EVOLUTIONARY DYNAMICS OF THE MUTUALISTIC SYMBIOSIS BETWEEN FUNGUS-GROWING TERMITES AND TERMITOMYCES FUNGI.**

Duur K. Aanen, Jacobus J. Boomsma, *Biological Institute, University of Copenhagen, DENMARK*

17:15 **FUNGAL BIOTROPHIC PARASITES OF INSECTS AND OTHER ARTHROPODS**

Alex Weir, *Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York, USA*; Meredith Blackwell, *Department of Biological Sciences, Louisiana State University, USA*

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 12

Symposium (Division of Microbial Control)

Bringing pathogens from the laboratory to the field

Chair: Vince d'Amico

15:00 **THE GYPSY MOTH, LYMANTRIA DISPAR, NUCLEOPOLYHEDROVIRUS PRODUCT GYPCHEK:**

John D. Podgwaite, *USDA Forest Service, Northeastern Research Station,, U.S.A.*; Vincent D'Amico, *USDA Forest Service, Northeastern Research Station, Department of Entomology and Applied Ecology, University of Delaware, U.S.A.*

15:30 **DEVELOPING A MICROBIAL: CHOOSING THE RIGHT FUNGAL STRAIN**

Ann Hajek, Thomas Dubois, Jennifer Lund, Charlotte Nielsen, *Dept. Entomology, Cornell University, USA*; Leah Bauer, *USDA, Forest Service, U.S.*; Michael Smith, *USDA, ARS, U.S.*; Zengzhi Li, *Dept. Forestry, Anhui Agric. Univ., CHINA*

16:00 **ENTOMOPATHOGENIC NEMATODES: FROM LABORATORY STUDIES TO USE IN THE ORCHARD**

Lawrence Lacey, *USDA-ARS-YARL, USA*; David I. Shapiro-Ilan, *USDA-ARS-Byron, USA*; Robin Stuart, *University of Florida, USA*; Joel Siegel, *USDA-ARS-Parlier, USDA*

16:30 **BRINGING SERRATIA ENTOMOPHILA FROM UNKNOWN BACTERIUM TO A COMMERCIAL BIOPESTICIDE**

Trevor Jackson, *AgResearch, NEW ZEALAND*

17:00 **FROM BASIC RESEARCH TO FIELD APPLICATION WITH GENETICALLY ENGINEERED BACTERIAL INSECTICIDES**

Brian A. Federici, Hyun-Woo Park, Dennis K. Bideshi, Yuko Sakano, Margaret Wirth, *Department of Entomology, University of California, UNITED STATES*

Monday, August 2^{nd}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Symposium (Division of Bacteria)

Risk assessment and non-target effects of Cry toxins in sprays and transgenic plants

Chair: Brian Federici; Juan Ferré

16:00 **THE MAMMALIAN SAFETY OF BACILLUS THURINGIENSIS SPRAYS, WITH AN EMPHASIS ON THE HUMAN EXPERIENCE**

Joel Siegel, *USDA/ARS, UNITED STATES OF AMERICA*

16:25 **EMETIC TOXIN AND ENTEROTOXINS A POTENTIAL RISK OF USING B. THURINGIENSIS PRODUCTS?**

Hansen Bjarne Munk, Niels Bohse Hendriksen, *Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, DENMARK*

16:50 **MULTIYEAR FIELD EVALUATIONS OF BT COTTON AND CORN INDICATE NO BIOLOGICALLY SIGNIFICANT IMPACTS ON NONTARGET INSECTS**

William Moar, Micky Eubanks, Barry Freeman, *Auburn University, UNITED STATES*; Sam Turnipseed, *Clemson University, UNITED STATES*; John Ruberson, *University of Georgia, UNITED STATES*; Galen Dively, *University of Maryland, UNITED STATES*; Graham Head, *Monsanto, UNITED STATES*

17:15 **PRECAUTIONARY PRINCIPLE AND THREE YEARS OF FIELD TRIAL EXPERIENCE IN BT-MAIZE MONITORING: IMPLICATIONS FOR A FUTURE RISK ASSESSMENT**

Achim Gathmann, Ingolf Schuphan, *Biology V, RWTH Aachen, GERMANY*

Monday, August 2^{nd}, 2004
Time: 18:30 - 20:30, Main Building, Lehtisali

Helsinki University reception

Chair: Hannele Niemi, *Vice Rector, Helsinki University*

Monday, August 2^{nd}, 2004
Time: 20:00 - 22:00, Lecture Halls 1, 12, 6, 10

Division meetings: V, B, N, Ms

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 10:00, Lecture Room 12

Contributed Papers (Division of Fungi)

fungus / contributed paper session 1

Chair: Cezary Tkaczuk; Richard Meadow

08:00 **CLIMATIC CONSTRAINTS FOR FUNGAL INFECTION OF TRIALEURODES VAPORARIORUM IN MEDITERRANEAN TOMATO GREENHOUSE**

Jacques Fargues, Thierry Boulard, Benoît Jeannequin, *INRA, FRANCE*

08:15 **INFLUENCE OF TEMPERATURE PREFERENCE OF TWO RDNA-ITS LINEAGES OF PAECILOMYCES FUMOSOROSEUS ON THEIR COINFECTION PATTERN**

Jacques Fargues, *INRA, FRANCE*; Marie-Claude Bon, *EBCL/USDA-ARS, FRANCE*

08:30 **EFFECT OF INITIAL HIGH HUMIDITY EXPOSURE ON THE EFFICACY OF LECANICILLIUM LECANII BLASTOSPORES AGAINST THE HEMLOCK WOOLLY ADELGID ADELGES TSUGAE ANNAND (HOMOPTERA: ADELGIDAE).**

William Reid, Vladimir Gouli, Svetlana Gouli, *University of Vermont, USA*

08:45 **GERMINABILITY OF METARHIZIUM ANISOPLIAE AND BEAUVERIA BASSIANA CONIDIA IN THE PRESENCE OF COMMON SOIL AND PHYLLOPLANE FUNGI**

Richard Meadow, *Norwegian Crop Research Institute, NORWAY*; Linda Gordon Hjeljord, *Agricultural University of Norway, NORWAY*

09:00 **DOSE DEPENDENT ACQUISITION OF BEAUVERIA BASSIANA CONIDIA BY WESTERN FLOWER THRIPS, FRANKLINIELLA OCCIDENTALIS (PERGANDE).**

T.A. Ugine, *Cornell University, UNITED STATES*; S. P. Wraight, *USDA-ARS, UNITED STATES*; J.P. Sanderson, *Cornell University, UNITED STATES*

09:15 **STU** **REDUCING ADULT LIFE SPAN OF MALARIA (ANOPHELES GAMBIAE S.L.) AND FILARIASIS (CULEX QUINQUEFASCIATUS) VECTORS USING THE ENTOMOPATHOGENIC FUNGUS METARHIZIUM ANISOPLIAE: A FIELD STUDY IN TANZANIA**

Ernst-Jan Scholte, *Wageningen University and Research, THE NETHERLANDS*; Kija Ng'abi, *Ifakara Health, Research and Development Centre, TANZANIA*; Bart Knols, *International Atomic Energy Agency, AUSTRIA*; Willem Takken, *Wageningen University and Research, THE NETHERLANDS*; Salim Abdulla, Gerry Killeen, *Ifakara Health, Research and Development Centre, TANZANIA*

09:30 **SELECTION OF BEAUVERIA BASSIANA STRAINS FOR CONTROL OF LYGUS POPULATIONS**

Michael McGuire, Jarrod Leland, *USDA-ARS, USA*

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 09:30, Lecture Room 10

Symposium (Division of Microsporidia)

Can microsporidia be seriously considered as biological control agents?

Chair: Rudolf Wegensteiner

08:00 **MICROSPORIDIA IN MOSQUITOES: CONTROL VERSUS MANAGEMENT STRATEGIES**

James Becnel, *USDA/ARS/CMAVE, U.S.*

08:25 **RHYME OR REASON: ISSUES FOR RELEASE OF EUROPEAN GYPSY MOTH MICROSPORIDIA INTO NORTH AMERICAN HOST POPULATIONS**

Leellen F. Solter, *Illinois Natural History Survey, UNITED STATES*; Michael L. McManus, *USDA Forest Service, NERS, UNITED STATES*

08:50 **THE INTRODUCTION AND ESTABLISMENT OF PARANOSEMA (NOSEMA) LOCUSTAE IN GRASSHOPPERS (ORTHOPTERA: ACRIDOIDEA) OF ARGENTINA.**

Carlos Lange, *CEPAVE, CIC-UNLP-CONICET, ARGENTINA*; María Laura De Wysiecki, *CEPAVE, UNLP-CONICET, ARGENTINA*

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 10:00, Lecture Room 1

Symposium (Cross-Divisional)

Oryctes virus - from discovery to classical microbial control agent

Chair: Trevor Jackson; Suzanne Thiem

08:00 **THE ORYCTES BACULOVIRUS: ITS DETECTION, IDENTIFICATION, AND IMPLEMENTATION IN BIOLOGICAL CONTROL OF THE COCONUT PALM RHINOCEROS BEETLE, ORYCTES RHINOCEROS**

Alois M. Huger, *Federal Biological Research Centre for Agriculture and Forestry,, GERMANY*

08:30 **REPLICATION, GENETICS AND MOLECULAR BIOLOGY OF ORYCTES VIRUS**

Allan Crawford, *AgResearch, NEW ZEALAND*

08:55 **THE INCIDENCE AND USE OF ORYCTES VIRUS FOR CONTROL OF RHINOCEROS BEETLE IN OIL PALM PLANTATIONS IN MALAYSIA**

Ramle Molsem, Norman Kamerudin, Wahid Mohd Basri, *MPOB, MALAYSIA*; Travis Glare, Trevor Jackson, *AgResearch, NEW ZEALAND*

09:20 **ORYCTES VIRUS TIME FOR A NEW LOOK AT A USEFUL BIOCONTROL AGENT**

Trevor Jackson, Travis Glare, *AgResearch, NEW ZEALAND*

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 10:00, Lecture Room 6

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 1

Chair: Juan Ferré; P. Caballero

08:00 **CRY1AC INTERACTION WITH THE HELIOTHIS VIRESCENS CADHERIN-LIKE RECEPTOR**

Meibao Zhuang, Ruiyu Xie, Linda Ross, Sarjeet Gill, *Department of Cell Biology and Neuroscience, University of California, USA*

08:20 **RESISTANCE TO CRY2AB IN HELICOVERPA ARMIGERA**

Ray Akhurst, Karen Olsen, Lisa Bird, Rod Mahon, *CSIRO Entomology, AUSTRALIA*

08:40 **INHERITANCE OF CRY-RESISTANCE AND CROSS-RESISTANCE IN CULEX QUINQUEFASCIATUS SELECTED WITH TOXINS FROM BACILLUS THURINGIENSIS ISRAELENSIS**

Margaret Wirth, Jeffrey Johnson, *Dept. of Entomology, University of California, USA*; Brian A. Federici, *Dept. of Entomology & Interdepartmental Graduate Program in Genetics, University of California, USA*; William Walton, *Dept. of Entomology, University of California, USA*

09:00 **RESTORATION OF ANTI-BACTERIAL ACTIVITY OF A CRYPTIC ORF (CYT1CA) FROM B. THURINGIENSIS ISRAELENSIS BY SITE-DIRECTED MUTAGENESIS**

Mark Itsko, Robert Manasherob, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

09:20 **A NOVEL BACILLUS THURINGIENSIS INSECTICIDAL PROTEIN TOXIC TO MEMBERS OF SEVERAL FAMILIES FROM LEPIDOPTERA AND COLEOPTERA.**

Ruiz De Escudero, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*; Anna Estela, *Departamento de Genética, Universidad de Valencia, SPAIN*; M. Porcar, F. J. Pérez-Llarena, J. A. Oguiza, C. Martínez, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*; Baltasar Escriche, Juan Ferré, *Departamento de Genética, Universidad de Valencia, SPAIN*; Primitivo Caballero, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*

09:40 **IFICATION AND CHARACTERIZATIONS OF A NEW BACILLUS THURINGIENSIS VIRULENCE FACTOR : THE INHA2 METALLOPROTEASE**

Myriam Hajaij, *Unité Génétique Microbienne et Environnement, INRA, FRANCE*; Michel Gohar, *Unité Génétique Microbienne et Environnement, INRA, Unité Microbiologie et Génétique Microbienne, INRA, FRANCE*; Sinda Fedhila, *Unité Génétique Microbienne et Environnement, INRA, FRANCE*; Didier Lereclus, Christina Nielsen-LeRoux, *Unité Génétique Microbienne et Environnement, INRA, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*

Tuesday, August 3^{rd}, 2004
Time: 10:15 - 12:00, Lecture Room 1

Society General Meeting

Chair: Harry Kaya

Tuesday, August 3^{rd}, 2004
Time: 12:00 - 14:30, Solvalla

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5 k Fun Run

Note: Departure at 12:15 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 13:00 - 18:00, Nuuksio

Excursion 1: Nuuksio National Park (off-path)

Host: Larry Huldén
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 13:00 - 18:00, Nuuksio

Excursion 2: Nuuksio National Park (easy)

Host: Lena Huldén
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 13:00 - 18:00,

Excursion 3: Marimekko factory outlet

Host: Ingeborg Menzler-Hokkanen
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 19:00 - 24:00, Tolkkinen

BBQ

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 10

Contributed Papers (Division of Microsporidia)

microsporidia / contibuted paper session 1

Chair: Rudolf Wegensteiner; Regina Kleespies

09:00 **THE DIVERSITY OF MICROSPORIDIA IN FRESHWATER AMPHIPODS: HOST-PARASITE INTERACTION DURING INVASIONS**

Johanna Slothouber-Galbreath, Judith Smith, Rebecca Terry, *School of Biology, University of Leeds, UNITED KINGDOM*; James Becnel, *USDA/ARS, Center for Medical, Agricultural and Veterinary Entomology, UNITED STATES*; Alison Dunn, *School of Biology, University of Leeds, UNITED KINGDOM*

09:20 **MICROSPORIDIAN PARASITES OF AUSTRALIAN FRESHWATER CRAYFISH, CHERAX DESTRUCTOR AND CHERAX SETOSUS (DECAPODA: PARASTACIDAE)**

Elizabeth Moodie, *University of New England, AUSTRALIA*

09:40 **MICROSPORIDIA SUPPRESS MELANIZATION REACTION AND PHENOLOXIDASE ACTIVITY OF THE HAEMOLYMPH OF THEIR INSECT HOSTS**

Yuriy Tokarev, *All-Russ. Inst. Plant Protection, Dept. Microb. Control, RUSSIA*; Ya.L Vorontsova, *Inst. Anim. Syst. Ecol., Lab. Insect Pathol, RUSSIA*; Yulia Sokolova, *Inst. Cytol., Lab. Cytol. Unicell. Org., RUSSIA*; V.V. Glupov, *Inst. Anim. Syst. Ecol., Lab. Insect Pathol, RUSSIA*; R. Entzeroth, *Dresden Techn. Uni., Lab Parasitol, GERMANY*

10:00 **TRANSMISSION OF THE MICROSPORIDIAN, NOSEMA FUMIFERANAE, IN SPRUCE BUDWORM POPULATIONS**

Christina Campbell, Sandy Smith, *University of Toronto, CANADA*; Kees Van Frankenhuyzen, *Canadian Forest Service, Natural Resources Canada, CANADA*

10:20 **STRUCTURE AND DEVELOPMENT OF THELOHANIA SOLENOPSAE IN FIRE ANTS**

Yulia Sokolova, James Fuxa, *Louisiana State University Ag.Center, USA*

10:40 **UNIKARYON DUPLICATI AS COMMON PATHOGEN OF IPS DUPLICATUS ATTACKING SPRUCE**

J. Holusa, *Forestry and Game Management Research Institute, CZECH REPUBLIC*; Jaroslaw Weiser, *Heralecka 964, 140 00 Praha 4, CZECH REPUBLIC*; Z. Zizka, *Electron Microscopy, Inst. of Microbiology, Acad. Sci., CZECH REPUBLIC*

11:00 **THE CYST LIKE SPOROPHOROUS VESICLE OF CHYTRIDIOPSIS TYPOGRAPHI**

Rudolf Wegensteiner, *Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU - University of Natural Resources and Applied Life Science, AUSTRIA*; Jaroslaw Weiser, *Emeritus, Institute of Entomology, Academy of Sciences of the Czech Republic, CZECH REPUBLIC*

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 12

Workshops (Division of Viruses)

Genome analysis methodology -workshop

Chair: Johannes Jehle

09:00 **GENOME SEQUENCING AND ANALYSIS**

Claudio L Afonso, Gerald F. Kutish, *Plum Island Animal Disease Center, Agricultural Research Service, U.S.A.*

09:30 **A FEW SIMPLE AND QUICK STRATEGIES FOR USING WHOLE GENOME SEQUENCE INFORMATION FOR SIMILARITY-BASED CLUSTERING**

Paolo M. de A. Zanotto, *Instituto de Ciencias Biomedicas II, Universidade de São Paulo, USP, Sao Paulo, SP, BRAZIL*; Ricardo Pereira, *Instituto de Matemática e Estatística - IME, Universidade de São Paulo USP, Sao Paulo, SP, BRAZIL*

10:00 **GENOME PHYLOGENIES**

Elisabeth Herniou, *Imperial College London, UK*

10:30 **APPLICATIONS OF DNA MICROARRAYS FOR THE STUDY OF BACULOVIRUS TRANSCRIPTIONAL REGULATION**

Gary W. Blissard, *Boyce Thompson Institute, Cornell University, U.S.A.*; Erik D. Burnett, *Boyce Thompson Institute, Cornell University, Lawrence Livermore National Laboratory, U.S.A.*; Warren F. Lamboy, *Center for Agricultural Bioinformatics, USDA-ARS, Cornell Univ.,, U.S.A.*

11:00 **USE OF GENOME DATA FOR TAXONOMY AND CLASSIFICATION**

David Theilmann, *Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, CANADA*

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 1

Symposium (Cross-Divisional)

Fungi and nematodes under unfavorable conditions

Chair: Solveig Haukeland-Salinas; Ingeborg Klingen

09:00 **IMPROVEMENT OF THE DESSICATION AND TEMPERATURE TOLERANCE OF HETERORHABDITIS BACTERIOPHORA**

Ralf-Udo Ehlers, Olaf Strauch, Jesko Oestergaard, *Institute for Phytopathology, Department for Biotechnology and Biological Control, Christian-Albrechts-University Kiel, GERMANY*

09:25 **EFFICACY OF ENTOMOPATHOGENIC NEMATODES UNDER COLD CONDITIONS**

Haukeland Salinas Solveig, *Norwegian Crop research Institute, NORWAY*

09:50 **PHASMARHABDITIS HERMAPHRODITA TO CONTROL SLUGS UNDER COLD CONDITIONS**

M. J. Wilson, *University of Aberdeen, UNITED KINGDOM*

10:15 **HOW TO FIND FUNGI IN EXTREME ENVIRONMENTS**

Marilena Aquino de Muro, Julian Smith, Paul Cannon, *CABI Bioscience, UNITED KINGDOM*

10:40 **INSECT PATHOGENIC FUNGI COPING WITH THE COLD**

Charlotte Nielsen, Susanne Harding, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Edda Sigurdís Oddsdóttir, Gudmundur Halldórsson, *Iceland Forest Research, Mogilsa, IS 116, Reykjavik, ICELAND*; Tróndur Leivsson, *Forestry Service of the Faroe Islands, Hvítanesvegur 3, P.O Box 1174, FO-110, FAROE ISLANDS*; Niels M. Schmidt, Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*

11:05 **EFFECTIVENESS OF ENTOMOPATHOGENIC FUNGI AS BIOLOGICAL CONTROL AGENTS UNDER DRY CONDITIONS**

Italo Delalibera Jr, *Department of Entomology, Plant Pathology and Zoology, ESALQ-University of São Paulo, BRAZIL*; Ann Hajek, *Department of Entomology, Cornell University, USA*

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Fungi)

fungus / contributed paper session 2

Chair: Ann Hajek; John Vandenberg

13:30 **PCR-BASED STRATEGY FOR THE IDENTIFICATION OF BEAUVERIA BASSIANA ISOLATES**

Emma Ormond, Fiona Kussy, Helen Roy, *Anglia Polytechnic University, UK*; Judith K. Pell, *Rothamsted Research, UK*; Alison Thomas, *Anglia Polytechnic University, UK*

13:45 **STU** **BIOCHEMICAL, MORPHOLOGICAL AND PATHOGENICITY VARIATIONS IN BEAUVERIA BASSIANA ISOLATES**

Reza Talaei Hassanloui, Aziz Kharazi Pakdel, *Dep. Plant Protection, College of Agriculture, University of Tehran, IRAN*; Mark Goettel, *Lethbridge Research Centre, CANADA*; Javad Mozaffari, *Genetic Dep., Seed and Plant Improvement Institute, IRAN*

14:00 **VIRULENCE TO COLORADO POTATO BEETLES AND GENETIC STABILITY OF BEAUVERIA BASSIANA PARASEXUAL RECOMBINANTS**

L. A. Castrillo, *Department of Entomology, Cornell University, UNITED STATES*; Michael H. Griggs, John D. Vandenberg, *USDA-ARS, US Plant, Soil & Nutrition Laboratory, UNITED STATES*

14:15 **GENETIC VARIATION IN THE GYPSY MOTH FUNGAL PATHOGEN ENTOMOPHAGA MAIMAIGA FROM NORTH AMERICA AND ASIA**

Charlotte Nielsen, Michael G. Milgroom, Ann Hajek, *Cornell University, USA*

14:30 **A SINGLE GENE MUTATION IN THE OPPORTUNISTIC FUNGUS ASPERGILLUS FLAVUS RESULTS IN INSECT-HOST SPECIALIZATION**

Lisa Scully, Michael Bidochka, *Brock University, CANADA*

14:45 **BIOLOGICAL PROPERTIES OF A NEW ENTOMOPATHOGENIC FUNGUS ASCHERSONIA MARGINATA**

Svetlana Gouli, Bruce Parker, Vladimir Gouli, *University of Vermont, USA*

15:00 **ADHESION OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA TO SUBSTRATA**

Diane Holder, Nemat Keyhani, *University of Florida, U.S.*

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 6

Contributed Papers (Division of Microbial Control)

microbial control / contributed paper session 2

Chair: Vladimir Gouli; Justin Hatting

13:30 **DIVERSE ENVIRONMENT-DEPENDENT COSTS OF RESISTANCE TO CRY1AC IN DIFFERENT STRAINS OF THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA**

Ben Raymond, Ali Sayyed, Denis Wright, *Imperial College London, UK*

13:50 **RELATIONSHIP BETWEEN BT FORMULATION, TORTRIX VIRIDANA L. (LEPIDOPTERA, TORTRICIDAE), AND PUPAL PARASITOIDS IN OAK CONSORTIONS**

Anatoly Ivashov, Andrei Simchuk, Irina Peletskaya, *V.I. Vernadsky National University, UKRAINE*; Vladimir Gouli, *University of Vermont, USA*

14:10 **COMPARATIVE EFFECTIVENESS OF BASIC METHODS FOR MASS-PRODUCTION OF ENTOMOPATHOGENIC FUNGI**

Vladimir Gouli, Svetlana Gouli, *University of Vermont, USA*

14:30 **THE PERFORMANCE OF METARHIZIUM ANISOPLIAE VAR. ACRIDUM (GREEN MUSCLE) AGAINST MIXED GRASSHOPPER POPULATIONS IN ETHIOPIA UNDER FIELD CONDITION**

Emiru Seyoum, Merid Negash, *Addis Ababa University Department of Biology, ETHIOPIA*

14:50 **EMPLOYING A NOVEL BIOASSAY METHODOLOGY FOR COMPARISON OF THE RELATIVE SUSCEPTIBILITY OF TWO RUSSIAN WHEAT APHID CLONES TO BEAUVERIA BASSIANA (HYPHOMYCETES)**

Justin Hatting, *ARC-Small Grain Institute, SOUTH AFRICA*; Stephen P. Wraight, *ARS-USDA, USA*

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 1

Symposium (Division of Bacteria)

Genomics and pathogenesis of invertebrate pathogens

Chair: R. Aroian; D. Ellar

13:30 **IDENTIFICATION OF NOVEL BACILLUS CEREUS VIRULENCE GENES BY APPLICATION OF IN VIVO EXPRESSION TECHNOLOGY IN AN INSECT INFECTION MODEL**

Sinda Fedhila, Didier Lereclus, *Unité Génétique Microbienne et Environnement, Institut National de la Recherche Agronomique, Groupe Génétique et Physiologie des Bacillus Pathogènes, FRANCE*

13:55 **GENOME ANALYSIS OF PHOTORHABDUS LUMINESCENS, AN ENDOSYMBIONT OF ENTOMOPATHOGENIC NEMATODES**

Eric Duchaud, *Atelier de Bioinformatique, 12 rue Cuvier, 75252 Paris Cedex 05, FRANCE*; Alain Givaudan, Noël Boemare, *Laboratoire de Pathologie Comparée,r II, 34095 Montpellier Cedex 05, FRANCE*; Frank Kunst, *Laboratoire GMP, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, FRANCE*

14:20 **THE TOXIN-CODING PLASMIDS OF BACILLUS THURINGIENSIS AND THEIR HOST BACTERIA: PHENOTYPIC REGULATION AND STRAIN IMPROVEMENT**

Colin Berry, Katherine Gammon, Brian Dancer, *Cardiff School of Biosciences, Cardiff University, UNITED KINGDOM*

14:45 **DISTINCT MAP KINASE PATHWAYS ARE IMPORTANT FOR DEFENSE AGAINST CRYSTAL TOXINS**

Danielle Huffman, *University of California at San Diego, Division of Biological Sciences, USA*; Roman Sasik, *University of California at San Diego, School of Medicine, USA*; Wayne Hsu, *University of California at San Diego, Division of Biological Sciences, USA*; Jacques Corbeil, *University of California at San Diego, School of Medicine, USA*; Raffi Aroian, *University of California at San Diego, Division of Biological Sciences, USA*

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Corridor leves II and III

Poster Session 2: ALL other than fungi and bacteria

V-1 **DEVELOPMENT OF NOVEL AND EFFECTIVE SUBUNIT VACCINES AGAINST EAST COAST FEVER BASED ON INSECT CELL-DERIVED T. PARVA SPOROZOITE SURFACE PROTEIN P67**

Stephen A. Kaba, *Laboratory of Virology, Wageningen University,, THE NETHERLANDS*; Anthony J. Musoke, *International Livestock Research Institute, Nairobi,, KENYA*; Dick Schaap, *Intervet International BV, THE NETHERLANDS*; Vish Nene, *The Institute for Genomic Research, USA, USA*; Just M. Vlak, Monique M. Van Oers, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*

V-2 **COMPETITIVE INTERACTION BETWEEN WILD TYPE AND RECOMBINANT HELICOVERPA ARMIGERA SNPV IN MIXED INFECTIONS IN INSECT LARVAE**

Liljana Georgievska, Monique M. Van Oers, Wopke Van der Werf, Just M. Vlak, *Wageningen University, THE NETHERLANDS*

V-3 **LIGHT AND ELECTRON MICROSCOPICAL INVESTIGATIONS ON A VIRAL DISEASE OF THE COMMON GREEN LACEWING, CHRYSOPERLA CARNEA**

Regina G. Kleespies, *Federal Biological Research Centre for Agriculture and Foresty, Institute for Biological Control, GERMANY*

V-4 **USING PHYLOGENIES TO DELIMITATE SPECIES**

Elisabeth Herniou, *Imperial College London, UK*; Jenny Cory, *Wageningen University, THE NETHERLANDS*; Julie Olzewski, *Shippensburg University, USA*; David O'Reilly, *Syngenta, UK*; Tim Barraclough, *Kew Gardens, UK*

V-5 **SEQUENCING AND GENE ORGANIZATION OF THE OF CHORISTONEURA OCCIDENTALIS GRANULOVIRUS GENOME**

Shannon Coppens, Hilary Lauzon, *Great Lakes Forestry Centre, CANADA*; Peter Krell, *Microbiology, University of Guelph, CANADA*; Basil Arif, *Great Lakes Forestry Centre, CANADA*

V-6 **DISRUPTION OF SYSTEMIC VIRAL RESISTANCE IN GYPSY MOTH (LYMANTRIA DISPAR) BY CO-INFECTION WITH A BACULOVIRUS AND A POLYDNAVIRUS**

James McNeil, Diana Cox-Foster, Mike Grove, Kelli Hoover, *Penn State University, US*

V-7 **NEODIPRION SERTIFER AND NEODIPRION LECONTEI NUCLEOPOLYHEDROVIRUSES: COMPARATIVE GENOMICS AND EVOLUTION**

Hilary Lauzon, *Canadian Forest Service, CANADA*; Paolo M. de A. Zanotto, *Instituto de Ciencias Biomedicas, BRAZIL*; Alejandra Garcia-Maruniak, *University of Florida, USA*; Basil Arif, *Canadian Forest Service, CANADA*; James Maruniak, *University of Florida, USA*

V-8 **BACULOVIRUSES ISOLATED FROM FOREST AND ORCHARD PESTS AND THEIR POTENTIAL AS PEST CONTROL AGENTS IN LATVIA**

Liga Jankevica, Ivars Zarins, *Department of Experimental Entomology, Institute of Biology, University of Latvia, LATVIA*

V-9 **DISRUPTION OF NEGATIVE GEOTAXIS IN GYPSY MOTH (LYMANTRIA DISPAR) LARVAE INFECTED WITH TRANSGENIC BACULOVIRUSES**

Mike Grove, Brianna Reed, Ryan Bissot, *Penn State University, US*; Nancy Hayes-Plazolles, James Slavicek, *USDA Forest Service, USA*; Kelli Hoover, *Penn State University, USA*

V-10 **EFFICACY OF PERITROPHIC MEMBRANE FOR PREVENTING NUCLEOPOLYHEDROVIRUS INFECTION IN ADOXOPHYES HONMAI AND SPODOPTERA LITURA**

Shohei Okuno, Jun Takatsuka, Takayoshi Ishii, Shigeyuki Mukawa, Madoka Nakai, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

V-11 **BLOCKAGE OF ADOXOPHYES HONMAI NUCLEOPOLYHEDROVIRUS INFECTION IN THE MIDGUT OF A NON-PERMISSIVE INSECT, HOMONA MAGNANIMA**

Ayako Hirao, Shohei Okuno, Jun Takatsuka, Takayoshi Ishii, Madoka Nakai, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

V-12 **DIVERSITY OF ADOXOPHYES HONMAI ENTOMOPOXVIRUS FIELD ISOLATES FROM JAPAN**

Jun Takatsuka, Shohei Okuno, Takayoshi Ishii, Yuko Takahashi, Madoka Nakai, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

V-13 **ENHANCEMENT OF NUCLEOPOLYHEDROVIRUS INFECTIVITY AGAINST MAMESTRA BRASSICAE (LEPIDOPTERA: NOCTUIDAE) BY GRANULOVIRUS PROTEINS AND A FLUORESCENT BRIGHTENER**

Chie Goto, Shigeyuki Mukawa, *National Agricultural Research Center, JAPAN*

V-14 **GROWTH AND SURVIVAL OF METEORUS PULCHRICORNIS IN MYTHIMNA SEPARATA INFECTED WITH ENTOMOPOXVIRUS**

Aki Fujimoto, *Tokyo Univercity of Agriculture and Technology, JAPAN*; Shohei Okuno, Takayoshi Ishii, Jun Takatsuka, Kazuko Nakanishi, Madoka Nakai, Yasuhisa Kunimi, *Univercity of Agriculture and Technology, JAPAN*

V-15 **PRIMARY INFECTION IN ADOXOPHYES HONMAI LARVAE THAT ARE RESISTANT TO A NUCLEOPOLYHEDROVIRUS**

Hirokazu Shikata, Madoka Nakai, Jun Takatsuka, Shohei Okuno, Takayoshi Ishii, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

V-16 **CHARACTERIZATION OF THE FP25K GENE OF HELICOVERPA ARMEGIRA SINGLE-NUCLEOCAPSID NUCLEOPOLYHEDROVIRUS**

Dong Wu, Fei Deng, Xiulian Sun, Li Yuan, *Joint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, CHINA*; Just M. Vlak, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Zhihong Hu, *Joint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, CHINA*

V-17 **HAS ACMNPV PIF THE SAME ROLE AS SPLINPV PIF?**

Serafín Gutiérrez, Miguel López-Ferber, *Laboratoire de Pathologie Comparée INRA/CNRS/Universtié de Montpellier II, FRANCE*

V-18 **ORF107 OF HASNPV ENCODES A STRUCTURE PROTEIN OF BOTH BV AND ODV**

Xiaoyu Pan, Gang Long, Zhihong Hu, *Joint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*

V-19 **TRANSLATION ARREST MECHANISM IN ACMNPV-INFECTED LD652Y CELLS**

Christy Mecey, Wade Williams, *Michigan State University, USA*; Monique M. Van Oers, *Wageningen University and Research Centre, THE NETHERLANDS*; Suzanne Thiem, *Michigan State University, USA*

V-20 **PRELIMINARY CHARACTERIZATION OF GENOME ORGANIZATION FOR TNSNPV ISOLATES FROM GREENHOUSE POPULATIONS OF TRICHOPLUSIA NI**

Martin Erlandson, Amanda Neudorf, *1Agriculture and Agri-Food Canada, Saskatoon Research Centre, CANADA*; David Theilmann, *Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, CANADA*

V-21 **LOW VARIATION IN SUSCEPTIBILITY OF SPODOPTERA LITTORALIS STRAINS TO SLNPV AND IN VIRULENCE VARIABILITY OF FOUR EGYPTIAN S. LITTORALIS NUCLEOPOLYHEDROVIRUS ISOLATES**

Luc-Olivier Brun, *Virology Center, IRD- Faculty of Agriculture, Cairo University, EGYPT*; Said El-Salamouny, *Department of Economic Entomology and pesticides, Faculty of Agriculture, Cairo University, EGYPT*; Wael Kamal, *Virology Center, IRD- Faculty of Agriculture, Cairo University, EGYPT*

V-22 **HOST SPECIFICITY OF SPODOPTERA SPP. NUCLEOPOLYHEDROVIRUSES IS NOT DETERMINED BY VIRUS ENTRY OR THE PRIMARY INFECTION CYCLE**

Oihane Simón, Trevor Williams, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Miguel López-Ferber, *2Laboratoire de Patologie Comparée, UMR 5087, INRA-CNRS-Université de Montpellier II, FRANCE*; Primitivo Caballero, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

V-23 **QUANTIFYING THE GENETIC DIVERSITY OF SPODOPTERA EXIGUA MNPV POPULATIONS IN SOIL RESERVOIRS IN SOUTHERN SPAIN**

Rosa Murillo, Delia Muñoz, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Carmen Ruíz-Portero, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, SPAIN*; M. Dolores Alcázar, *3Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; José E. Belda, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; Trevor Williams, Primitivo Caballero, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

V-24 **NUCLEOPOLYHEDROVIRUS (SEMNPV) AND OPTICAL BRIGHTENER FORMULATIONS FOR CONTROL OF SPODOPTERA EXIGUA IN GREENHOUSES IN SOUTHERN SPAIN.**

Rodrigo Lasa, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Carmen Ruíz-Portero, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, SPAIN*; M. Dolores Alcázar, *Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; José E. Belda, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; Primitivo Caballero, Trevor Williams, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

V-25 **A NEW ASCOVIRUS (SEAV6A) ISOLATED FROM SPODOPTERA EXIGUA LARVAE IN CALIFORNIA**

Yeping Tan, Francis Tan, Dennis K. Bideshi, *Department of Entomology, University of California, UNITED STATES*; Yves Bigot, *Unit of Insect Parasite Genetics, University of Tours, FRANCE*; Brian A. Federici, *Department of Entomology, University of California, UNITED STATES*

V-26 **IDENTIFICATION OF A NOVEL SHRIMP PROTEIN PHOSPHATASE AS THE INTERACTING PARTNER FOR LATENCEY-ASSOCIATED PROTEIN ORF427 OF WHITE SPOT SYNDROME VIRUS**

Liqun Lu, Jimmy Kwang, *Temasek Life Sciences Laboratory, SINGAPORE*

V-27 **SUPPRESSION OF FIELD POPULATIONS OF BALSAM FIR SAWFLY WITH ITS NUCLEOPOLYHEDROVIRUS**

G. Moreau, E.G. Kettela, G.S. Thurston, S. Holmes, C. Weaver, B. Morin, *Canadian Forest Service, CANADA*; D.B. Levin, *Department of Biology, University of Victoria, CANADA*; C.J. Lucarotti, *Canadian Forest Service, CANADA*

V-28 **EUROPEAN LEUCOMA SALICIS MNPV IS CLOSELY RELATED TO ORGYIA PSEUDOTSUGATA MNPV OF NORTH AMERICA**

Agata Jakubowska, Monique M. Van Oers, *Laboratory of Virology Wageningen University, NETHERLANDS*; Jadwiga Ziemnicka, *Department of Biocontrol and Quarantine Institute of Plant Protection, POLAND*; Just M. Vlak, *Laboratory of Virology Wageningen University, NETHERLANDS*

MC-1 **RESISTANCE MANAGEMENT FOR BACILLUS THURINGIENSIS SPRAYS AND TOXINS; IS IT COMPATIBLE WITH THE USE OF BACULOVIRUSES AS ADDITIONAL BIOCONTROL AGENTS?**

Ben Raymond, Ali Sayyed, *Imperial College London, UK*; Denis Wright, *Imperial College London, UK*

MC-2 **A NOVEL MECHANISM FOR BACILLUS THURINGIENSIS CRY1AC RESISTANCE IN A FIELD-DERIVED POPULATION OF THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA**

Ali Sayyed, *Imperial College London, UK*; Graham Moores, *Rothamsted Research, UK*; Fred Kemp, *University of Reading, UK*; Robin Gunning, *NSW Ag, AUSTRALIA*; Denis Wright, *Imperial College London, UK*

MC-3 **DANISH CENTRE FOR BIOLOGICAL CONTROL**

Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Annie Enkegaard, *The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, 4600 Slagelse, DENMARK*; Niels Bohse Hendriksen, *The National Environmental Research Institute, Department of Environmental Chemistry and Microbiology, Frederiksborgvej 399, 4000 Roskilde, DENMARK*; Dan Funck Jensen, *Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Jørgen Brøchner Jespersen, *The Danish Institute of Agricultural Sciences, Danish Pest Infestation Laboratory, Skovbrynet 14, 2800 Lyngby, DENMARK*; John Larsen, *The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, 4600 Slagelse, DENMARK*; Anne Mette Madsen, *The National Institute of Environmental Health, Department of Chemical Working Environments, Lersø Parkallé 105, 2100 Kbh. ., DENMARK*; Hans-Peter Ravn, *Forest and Landscape, Department of Applied Ecology, Hørsholm Kongevej 11, 2970 Hørsholm, DENMARK*

MC-4 **QUALIFICATION AND QUANTIFICATION OF CULTURABLE MICROORGANISMS IN MARKETED MICROBIAL PEST CONTROL AGENTS**

Anne Winding, Bjarne Munk Hansen, *Dept. Env. Chem. Microbiol., NERI, DENMARK*; Anita Fjelsted, *Danish EPA, DENMARK*

MC-5 **EFFECTS OF SEVERAL ABIOTIC FACTORS ON THE VIRAL ENHANCING ABILITY OF THE SPINDLE OF ANOMALA CUPREA ENTOMOPOXVIRUS**

Wataru Mitsuhashi, Ritsuko Murakami, Kazuhisa Miyamoto, *National Institute of Agrobiological Sciences, JAPAN*

MC-6 **EFFECT OF BEAUVERIA BASSIANA, VERTICILLIUM LECANII, BACILLUS THURINGIENSIS SUBSP. TENEBRIONIS AND AZADIRACTIN COMPOUNDS ON SITOPHILUS ORYZAE (L.) AND TRIBOLIUM CONFUSUM DU VAL IN STORED RYE**

Dimitris Kontodimas, Nickolas Kavallieratos, Spyridon Mantzoukas, *Benaki Phytopathological Institute, GREECE*; Christos Athanassiou, *Agricultural University of Athens, GREECE*; Maria Anagnou-Veroniki, *Benaki Phytopathological Institute, GREECE*

MC-7 **ARE NOMURAEA RILEYI EPIZOOTICS TRIGGERED BY THE MICROENVIRONMENT OF SOYBEAN PLANT AREA OR FAVORED BY SELECTIVE FUNGICIDES?**

Daniel R. Sosa-Gómez, Jose J. Da Silva, *Embrapa Soja, BRAZIL*; Francislene Angelotti, *Universidade Estadual de Maringá, BRAZIL*; Ivan T.V. Licursi, *Fundação Dalmo Giacometti, BRAZIL*; Eduardo Polloto, *Universidade Estadual de Maringá, BRAZIL*

MC-8 **TRANSGENIC RISK ASSESSMENT: POTENTIAL EFFECTS OF TRANSGENIC CHITINASE AND 1,3-GLUCANASE EXPRESSION ON GRAPE VINE ARTHROPODS**

Hugo M. Arends, Claudia Vogel, Johannes A. Jehle, *Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate, GERMANY*

MC-9 **ISOLATION OF ENTOMOPATHOGENS FROM SOUTH AFRICAN SOILS USING THE GALLERIA MELLONELLA-BAIT TECHNIQUE**

Justin Hatting, *ARC-Small Grain Institute, SOUTH AFRICA*; Selcuk Hazir, *Hacettepe University, TURKEY*; Gloria Macucwa, Hannetjie Jooste, Astrid Jankielsohn, *ARC-Small Grain Institute, SOUTH AFRICA*

MC-10 **ENTOMOPATHOGENIC FUNGI FOR WHITE GRUB CONTROL IN NEPAL**

Yubak Dhoj Gc, *Institute of Agriculture and Animal Sciences, Rampur, Chitwan,, NEPAL*; Siegfried Keller, *Swiss Federal Research Station for Agroecology and Agriculture, SWITZERLAND*

MC-11 **LABORATORY BIOASSAYS OF PAECILOMYCES FUMOSOROSEUS ON COPTOTERMES FORMOSANUS: THE EFFECTS OF TERMITE SEPARATION AND SPORE CONCENTRATIONS ON TERMITE SURVIVAL**

William Meikle, Guy Mercadier, Alan Kirk, Franck Derouane, *European Biological Control Laboratory, FRANCE*; Rebecca Rosengaus, *Northeastern University, USA*; Yurong He, *Laboratory of Insect Ecology, CHINA*; Chuck Quimby, *European Biological Control Laboratory, FRANCE*

MC-12 **VIRULENCE OF NEW STRAINS OF ENTOMOPATHOGENIC HYPHOMYCETES (DEUTEROMYCOTA, HYPHOMYCETES) TO ORTHOPTERAN INSECTS**

Yuriy Tokarev, Maxim Levchenko, Anton Naumov, George Lednev, *All-Russian Insitute for Plant Protection, RUSSIA*

MC-13 **CONJUGATIVE TRANSFER, STABILITY AND EXPRESSION OF A PLASMID ENCODING A CRY1AC GENE IN BACILLUS CEREUS GROUP STRAINS**

Xiaomin Hu, *Wuhan Institute of Virology, CHINA*; Bjarne Munk Hansen, *National Environmental Research Institute, DENMARK*; Jørgen Eilenberg, *Agricultural University, DENMARK*; Niels Bohse Hendriksen, *National Environmental Research Institute, DENMARK*; Lasse Smidt, *National Institute of Occupational Health, DENMARK*; Zhiming Yuan, *Wuhan Institute of Virology, CHINA*; Gert Bolander Jensen, *National Institute of Occupational Health, DENMARK*

MC-14 **OCCURRENCE OF BACILLUS CEREUS AND B. THURINGIENSIS IN FIELD PLOTS WITH CURLY KALE (BRASSICA OLEARACEA ACEPHALA)**

Niels Bohse Hendriksen, Bjarne Munk Hansen, *National Environmental Research Institute, DENMARK*

MC-15 **BIOASSAY WITH MOSQUITOS FOR EVALUATION OF TRANSCONJUGANT BACILLUS SPP. CONTAINING THE PBTOXIS PLASMID**

Jens Efsen Johansen, *The Agricultural University, DENMARK*; Xiaomin Hu, *Wuhan Institute of Virology, CHINA*; Bjarne Munk Hansen, *National Environmental Research Institute, DENMARK*; Zhiming Yuan, *Wuhan Institute of Virology, CHINA*; Jørgen Eilenberg, *The Agricultural University, DENMARK*

MC-16 **FURTHER DEVELOPMENTS IN THE COMMERCIAL LABORATORY PRODUCTION OF THE NUCLEOPOLYHEDROVIRUS OF ANTICARSIA GEMMATALIS IN BRAZIL**

Flavio Moscardi, *Embrapa Soja, BRAZIL*; Braulio Santos, *Universidade Federal do Paraná, BRAZIL*

MC-17 **SELECTION OF ENTOMOPATHOGENIC FUNGI FOR MICROBIAL CONTROL OF APHID PESTS IN US GREENHOUSES**

Melanie Filotas, *Department of Entomology, Cornell University, U.S.A.*; Stephen P. Wraight, *USDA Agriculture Research Service, US Plant, Soil and Nutrition Laboratory, U.S.A.*; John Sanderson, *Department of Entomology, Cornell University, U.S.A.*

MC-18 **SCREENING OF SHUFFLED ALPHA-AMYLASE INHIBITORS TO COTTON BOLL WEEVIL ALPHA-AMYLASES**

Maria F. Grossi de Sa, Maria Cristina Mattar da Silva, Rafael Perseghini Del Sarto, Marise Ventura Coutinho, Edson Luiz Zangrando Figueira, *Embrapa Recursos Genéticos e Biotecnologia. Parque Estacão Biológica, BRAZIL*

MC-19 **TARGETED DISSEMINATION OF BIOCONTROL AGENTS BY USING THE HONEY BEE:**

Heikki M. T. Hokkanen, *Applied Zoology, University of Helsinki, FINLAND*; Ingeborg Menzler-Hokkanen, *Rural Research and Training Centre, University of Helsinki, FINLAND*

MC-20 **THE INTERACTION BETWEEN ROOT HERBIOVOURUS LARVAE AND BENIFICIAL SOIL ORGANISMS IN NURSERY PEAT VS. FOREST SOIL - A POT EXPERIMENT.**

Edda Sigurdís Oddsdóttir, *Icelandic Forestry Research, ICELAND*; Jørgen Eilenberg, *The Royal Veterinary and Agricultural University, DENMARK*; Robin Sen, *Department of Biosciences, University of Helsinki, FINLAND*; Gudmundur Halldórsson, *Icelandic Forestry Research, ICELAND*

MC-21 **NATURALLY OCCURRING INSECT PATHOGENIC FUNGI ON KEY COFFEE PESTS, AND THE INFLUENCE OF MANAGEMENT PRACTICES**

Arnulfo Monzòn, *UNA, NICARAGUA*; Ingeborg Klingen, *Planteforsk, NORWAY*; Falguni Guharay, *CATIE, NICARAGUA*

MC-22 **BRASSICA HOST PLANT AND FERTILIZER IMPACTS ON STEINERNEMA FELTIAE EFFICIENCY**

Melita Zec-Vojinovic, Heikki M. T. Hokkanen, *Laboratory of Applied Zoology, FINLAND*

N-1 **ECOLOGICAL CHARACTERIZATION OF HETERORHABDITIS SP. (CABORCA STRAIN) (NEMATODA: HETERORHABDITIDAE), A NATURAL PATHOGEN OF DICEROPROCTA ORNEA (HOMOPTERA: CICADIDAE) FROM SONORA, MEXICO**

Benjamin Rivera-Orduño, *División de Ciencias Administrativas, Contables y Agrarias, MEXICO*; S. Patricia Stock, *Department of Plant Sciences, University of Arizona, Tucson AZ 85721-0036, USA*

N-2 **EVALUATING EFFICACY OF APPLICATION OF ENTOMOPATHOGENIC NEMATODES VIA A DRIP LINE IRRIGATION SYSTEM**

Andrew Brown, *Imperial College London, UK*; Simon Piggott, Jeremy Pearce, *Becker Underwood, UK*; Denis Wright, *Imperial College London, UK*

N-3 **NON-TARGET EFFECTS OF ENTOMOPATHOGENIC NEMATODES ON SOIL MICROBIAL COMMUNITY AND NUTRIENT CYCLING PROCESSES: A MICROCOSM STUDY**

E. A. B. De Nardo, *Department of Entomology, Ohio State University, EMBRAPA Meio Ambiente, U.S.A.*; Parwinder S. Grewal, D. McCartney, B. R. Stinner, *Department of Entomology, Ohio State University, U.S.A.*

M-1 **COMPARATIVE ULTRASTRUCTURAL ANALYSIS OF THREE SPECIES OF THE GENUS PARANOSEMA FROM ORTHOPTERA AND COLEOPTERA**

Yulia Sokolova, *Instirute of Cytology Russian Academy of Sciences, St.Petersburg, RUSSIA*; Irma Issi, Yuriy Tokarev, *Institute for Plant Protection, St.Petersburg, RUSSIA*; Elena Morzhina, *Instirute of Cytology Russian Academy of Sciences, St.Petersburg, RUSSIA*; Carlos Lange, *Center for Parasitological Studies, La Plata National University, ARGENTINA*

M-2 **HYPERTROPHY OF SPODOPTERA FRUGIPERDA CELLS INDUCED BY MICROSPORIDIAN INFECTION**

Hidetoshi Iwano, Hideki Tanaka, Tetsufumi Yazu, Kouji Iyama, Tosihiko Hukuhara, *Nihon University, JAPAN*

CA-1 **TEMPERATURE AND THE NORTHERN RANGE OF PLASMODIUM VIVAX IN EUROPE**

Lena Huldén, *University of Helsinki, FINLAND*

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 10

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 2

Chair: R. de Maagd; D. Pauron

16:00 **STU** **PHAGE-DISPLAY PEPTIDES THAT BIND TO THE CRY11A TOXIN OR TO THE RECEPTOR, REVEALED AN IMPORTANT ROLE OF DOMAIN II REGIONS IN RECEPTOR INTERACTION AND TOXICITY TO AE. AEGYPTI**

Luisa Elena Fernández-Altuna, *Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO*; Lorenzo Segovia, *Cellular Biology and Biocatalisis Department of the Instituto de Biotecnología, UNAM, MEXICO*; Oswaldo Lopez, *Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO*; Sarjeet Gill, *Cell Biology & Neuroscience of University of California-Riverside, USA*; Alejandra Bravo, Mario Soberón, *Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO*

16:15 **STU** **ANALYSIS OF THE INTERACTION BETWEEN CRY11A AND CYT1A OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS: BIOLOGICAL ROLE IN SYNERGISM**

Claudia Pérez, Luisa Fernández, *IBT-UNAM, MEXICO*; Sarjeet Gill, *University of Riverside, California, UNITED STATES*; Mario Soberón, Alejandra Bravo, *IBT-UNAM, MEXICO*

16:30 **CHARACTERIZATION OF THE CELLULAR MODE OF ACTION OF THE BACILLUS SPHAERICUS BINARY TOXIN IN AN EPITHELIAL CELL LINE.**

Yannick Pauchet, *INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux , FRANCE*; Frédéric Luton, *IPMC, CNRS-UMR 6097, FRANCE*; Claude Castella, *INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux , FRANCE*; Jean-François Charles, *Institut Pasteur, Unité de génétique des génomes bactériens, FRANCE*; David Pauron, *INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux , FRANCE*

16:45 **UNFOLDING EVENTS IN THE MONOMERIC CRY1AB TOXIN DURING TRANSITION TO MEMBRANE INSERTED OLIGOMERIC PORE: DOMAIN I IS THE ONLY INTEGRAL MEMBRANE DOMAIN**

Carolina Rausell, *Instituto de Biotecnología. Universidad Nacional Autónoma de Mexico, MEXICO*; Jorge Sanchez, Carlos Munoz-Garay, Claudia Morera, Mario Soberón, Alejandra Bravo, *Instituto de Biotecnología. Universidad Nacional Autónoma de Mexico., MEXICO*

17:00 **CLONING AND EXPRESSION ANALYSIS OF GENES INVOLVED IN INSECT RESPONSE TO BACILLUS THURINGIENSIS TOXINS**

Salvador Herrero, *Laboratory of Virology, Wageningen University and Plant Research International, Wageningen, THE NETHERLANDS*; Tsanko Gechev, Petra L. Bakker, *Plant Research International, Wageningen, THE NETHERLANDS*; William Moar, *Department of Entomology and Plant Pathology, Auburn University, USA*; Just M. Vlak, Monique M. Van Oers, *2Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Ruud De Maagd, *Plant Research International, Wageningen, THE NETHERLANDS*

17:15 **GALLERIA MELLONELLA LARVAE AS A MODEL FOR INTESTINAL INFECTIONS: BACTERIAL LOCALIZATION AND VIRULENCE GENE EXPRESSION**

Christina Nielsen-LeRoux, *Unité Génétique Mirobienne et Environnement, INRA, la Minière, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*; Myriam Hajaij, Christophe Buisson, Patricia Nel, Elisabeth Guillemet, *Unité Génétique Mirobienne et Environnement, INRA, la Minière, FRANCE*; Laurence Fiette, *Unité d'Histotechnologie et Pathologie, Institut Pasteur, FRANCE*; Didier Lereclus, *Unité Génétique Mirobienne et Environnement, INRA, la Minière, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*

17:30 **IN VIVO INDUCTION OF APOPTOSIS BY XENORHABDUS NEMATOPHILA IN INSECT PHAGOCYTES**

Fabienne Vigneux, Carlos Ribeiro, *Laboratoire d'Ecologie Microbienne des Insectes Interactions Hôtes-Pathogènes UMR 1133 INRA-Université de Montpellier II, 34090 Montpellier, FRANCE*; Stephen Baghdiguian, *Institut des Sciences de l'Evolution, UMR 5554, Université de Montpellier II, 34090 Montpellier, FRANCE*; Michel Brehélin, *Laboratoire d'Ecologie Microbienne des Insectes Interactions Hôtes-Pathogènes UMR 1133 INRA-Université de Montpellier II, 34090 Montpellier, FRANCE*

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Contributed Papers (Division of Fungi)

fungi / contributed paper session 3

Chair: Judith Pell; Ingeborg Klingen

16:00 **THE ABILITY OF FUNGAL INFECTED APHIDS TO PRODUCE AND RESPOND TO ALARM PHEROMONE**

Helen Roy, *Anglia Polytechnic University, UK*; Jason Baverstock, Keith Chamberlain, Judith K. Pell, *Rothamsted Research, UK*

16:15 **PREVALENCE OF INSECT PATHOGENIC FUNGI AND PARASITOIDS ON THE BLACK CHERRY APHID, MYZUS CERASI**

Ingeborg Klingen, Karin Westrum, *The Norwegian Crop Research Institute, Plant Protection Centre, NORWAY*; Gunnhild Jaastad, *The Norwegian Crop Research Institute, Ullensvang Research Centre, NORWAY*

16:30 **STU** **INTRAGUILD INTERACTIONS BETWEEN THE APHID PATHOGEN PANDORA NEOAPHIDIS AND THE PARASITOID APHIDIUS ERVI: IMPLICATIONS FOR MULTI-SPECIES BIOCONTROL**

Jason Baverstock, *Plant and Invertebrate Ecology Division, Rothamsted Research, Division of Agricultural Sciences, The University of Nottingham, UNITED KINGDOM*; P. G. Alderson, *Division of Agricultural Sciences, The University of Nottingham, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

16:45 **THE RELATIONSHIP OF NUMBER OF CONIDIA, MOLTING AND INSECT DEVELOPMENTAL STAGE TO SUSCEPTIBILITY OF COTTON APHID, APHIS GOSSYPII, TO THE FUNGUS VERTICILLIUM LECANII**

Jeong Jun Kim, Dae Joon Im, *Division of Entomology, NIAST, RDA, KOREA*; Kyu Chin Kim, *Dept. Agrobiology, Chonnam National University, KOREA*; Dong Ro Choi, *Division of Entomology, NIAST, RDA, KOREA*; Donald Roberts, *Dept. Biology, Utah State University, USA*

17:00 **RECENT RESEARCH ON FUNGOUS PATHOGENS OF MITES (ACARI) IN POLAND**

Cezary Tkaczuk, Ryszard Miêtkiewski, *University of Podlasie, POLAND*; Stanislaw Balazy, *Research Centre for Agricultural and Forest Environment, POLAND*

17:15 **NEOZYGITES FLORIDANA KILLING TETRANYCHUS URTICAE IN STRAWBERRIES AND THE INFLUENCE OF MANAGEMENT SYSTEM**

Ingeborg Klingen, Nina Trandem, *The Norwegian Crop Research Institute, Plant Protection Centre, NORWAY*

17:30 **USE OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA FOR BIOCONTROL OF IXODIDAE TICK SPECIES**

Greg Westwood, Brett Kirkland, Eun-Min Cho, Nemat Keyhani, *University of Florida, U.S.*

17:45 **INVESTIGATIONS OF COLORADO POTATO BEETLE MORTALITY FOLLOWING FOLIAR**

Stephen P. Wraight, Mark E. Ramos, *USDA-ARS, U.S. Plant, Soil and Nutrition Laboratory, U.S.A.*

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Contributed Papers (Division of Nematodes)

nematodes / contributed paper session 1

Chair: Holger Philipsen; Rob van Tol

16:00 **GENETIC IMPROVEMENT FOR PREVENTION OF BENEFICIAL TRAIT DETERIORATION IN HETERORHABDITIS BACTERIOPHORA THROUGH CREATION OF INBRED LINES**

Cheng Bai, David I. Shapiro-Ilan, *USDA-ARS, SAA, U.S.A.*; Randy Gaugler, *Dept. Entomology, Rutgers University, U.S.A.*; Keith R. Hopper, *USDA-ARS, U.S.A.*

16:15 **PLANTS PROTECT THEIR ROOTS BY ALERTING THE ENEMIES OF GRUBS**

Rob Van Tol, Marleen Riemens, Frans Zoon, *Plant Research International, P.O. Box 16, 6700 AA Wageningen, NETHERLANDS*

16:30 **CROP INFLUENCE ON THE ABUNDANCE OF STEINERNEMA FELTIAE**

Holger Philipsen, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*; Otto Nielsen, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*

16:45 **ESTABLISHMENT AND PERSISTENCE OF ENTOMOPATHOGENIC NEMATODES IN CONVENTIONAL AND ORGANIC AGRICULTURE**

Alper Susurluk, Ralf-Udo Ehlers, *Institute for Phytopathology, Department of Biotechnology and Biological Control, Christian-Albrechts-University Kiel, GERMANY*

17:00 **INTERACTIONS BETWEEN FUSARIUM OXYSPORUM F. SP. ASPARAGI (ASCOMYCOTA: PYRENOMYCETES) AND HETERORHABDITIS CABORCA STRAIN (HETERORHABDITIDAE) IN GALLERIA MELLONELLA LARVAE**

Jennifer Bauman, *Department of Plant Sciences. University of Arizona. Tucson AZ 85721-0036, USA, USA*; Benjamin Rivera-Orduño, *División de Ciencias Administrativas, Contables y Agrarias, Universidad de Sonora, Santa Ana, Sonora, MEXICO*; S. Patricia Stock, *Department of Plant Sciences. University of Arizona. Tucson AZ 85721-0036, USA, USA*

17:15 **CONTROL OF PLUTELLA XYLOSTELLA USING NOVEL FORMULATION TECHNIQUES TO IMPROVE PERFORMANCE OF ENTOMOPATHOGENIC NEMATODES ON THE FOLIAGE**

Sibylle Schroer, Ralf-Udo Ehlers, *Institute for Phytopathology, Dept. Biotechnology & Biol. Control, Christian-Albrechts-University Kiel, GERMANY*

17:30 **CONTROLLING THE QUALITY OF ENTOMOPATHOGENIC NEMATODE PRODUCTS**

Arne Peters, *E-nema GmbH, GERMANY*; Ursula Koelzer, *GAB Biotechnologie GmbH, GERMANY*; Klaus Iwahn, *Öre Bio-Protect GmbH, GERMANY*; Frank Stepper, *Sautter & Stepper GmbH, GERMANY*

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 6

Symposium (Division of Viruses)

Role of native immune systems/molecular host response

Chair: Diana Cox-Foster; John Burand

16:00 **IMMUNE SYSTEMS (D. HULTMARK)**

D. Hultmark, -, -

16:25 **IMMUNE SYSTEMS (I. FAYE)**

I. Faye, -, -

16:50 **STU** **LECTIN-INDUCED HEMOCYTE INACTIVATION: A PARADIGM FOR PARASITOPID-MEDIATED IMMUNE-SUPPRESSION?**

Richard Glatz, *University of Adelaide, AUSTRALIA*; Sassan Asgari, *University of Queensland, AUSTRALIA*; Otto Schmidt, *University of Adelaide, AUSTRALIA*

17:15 **A RECIPE FOR DEATH: THE INTERPLAY BETWEEN HONEYBEE IMMUNITY, IMMUNOSUPPRESSION BY MITES, AND PICORNA-LIKE VIRUSES**

Diana Cox-Foster, Xiaolong Yang, Miaoqing Shen, Liwang Cui, Nancy Ostiguy, *Penn State University, USA*

Wednesday, August 4^{th}, 2004
Time: 20:00 - 22:00, Lecture Rooms 1, 12

Division meetings: MC, F

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 12

Symposium (Division of Bacteria)

New advances in research and development of insecticidal proteins

Chair: James Baum; Trevor Jackson

08:30 **CRY TOXIN DISPLAY: ITS JUST A PHAGE WE'RE GOING THROUGH**

Susana Vilchez, Craig Pigott, *Department of Biochemistry, Cambridge University, UNITED KINGDOM*; Juliette Jacoby, *Dept. of Medicine, Cambridge University, UNITED KINGDOM*; David Ellar, *Department of Biochemistry, Cambridge University, UNITED KINGDOM*

09:00 **GENESIS OF MON 863, A TRANSGENIC CORN HYBRID RESISTANT TO CORN ROOTWORM FEEDING DAMAGE**

Ty Vaughn, James Baum, *Monsanto, USA*

09:30 **INSECTICIDAL PROTEINS FROM PAENIBACILLUS STR IDAS1529**

Scott Bintrim, Scott Bevan, Baolong Zhu, Weiting Ni, Don Merlo, Ernie Schnepf, *Dow AgroSciences LLC, USA*

10:30 **PHOTORHABDUS: A NATURAL BORN KILLER.**

Nick Waterfield, Andrea Dowling, Michelle Hares, Phil Daborn, Richard ffrench-Constant, *Biology and Biochemistry, University of Bath, UNITED KINGDOM*

11:00 **NOVEL SERRATIA ENTOMOPHILA ANTIFEEDING GENES CONTAIN A PUTATIVE DEFECTIVE PROPHAGE ACTIVE AGAINST THE GRASS GRUB COSTELYTRA ZEALANDICA**

Mark Hurst, Trevor Jackson, Travis Glare, *AgResearch, NEW ZEALAND*

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 1

Workshop (Cross-Divisional)

Risk assessment

Chair: Tariq Butt; Ralf Ehlers

08:45 **RISK ASSESSMENT AND REGISTRATION (G. STERK AND W. RAVENSBERG)**

G. Sterk, *-, BELGIUM*; W. Ravensberg, *-, NETHERLANDS*

09:15 **REGISTRATION OF MICROBIAL PLANT PROTECTION PRODUCTS AND ACTIVE MICROORGANISMS IN EU**

Anita Fjelsted, *Ministry of Environment, Danish Environmental Protection Agency, DENMARK*

10:15 **NONTARGET EFFECTS OF ENTOMOPATHOGENIC FUNGI: ARE WE FINALLY ABLE TO GENERALIZE?**

Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Siegfried Keller, *Federal Research Station for Agroecology and Agriculture, 8046 Zürich, SWITZERLAND*; John D. Vandenberg, *USDA Agricultural Research Service, U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA*

10:45 **DO COMMERCIALISED FUNGAL BIOCONTROL AGENTS PRODUCE RELEVANT METABOLITES WHICH HARM HUMANS AND THE ENVIRONMENT?**

Hermann Strasser, *Institute of Microbiology, Leopold-Franzens University Innsbruck, AUSTRIA*; Claudio Altomare, *Institute of Sciences of Food Productions, Bari, ITALY*; Tariq Butt, *School of Biological Sciences, University of Wales Swansea, WALES*

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 6

Contributed Papers (Division of Viruses)

virus / contributed paper session 3

Chair: P. J. Krell; M. M. van Oers

08:30 **CHARACTERIZATION OF HEPTAD REPEATS OF THE F PROTEIN OF HASNPV: SIMILARITY VERSUS NOVELTY**

Gang Long, Xiaoyu Pan, *The Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*; Zihe Rao, *Laboratory of Structure Biology and MOE Laboratory of Protein Sciences, Tsinghua University, CHINA*; Just M. Vlak, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Zhihong Hu, *The Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*

08:50 **STU** **HA-VP39 BINDING TO ACTIN AND MOLECULAR MECHANISM FOR HANPV TRANSPORTING TO THE HOST NUCLEUS**

Songya Lu, Guoqiong Ge, Yipeng Qi, *Wuhan University, CHINA*

09:10 **IE1 AND IE0 HAVE SEPARATE ROLES IN THE REPLICATION OF AUTOGRAPHA CALIFORNICA MULTIPLE NUCLEOPOLYHEDROVIRUS IN SPODOPTERA FRUGIPERDA CELLS**

Taryn Stewart, Ilse Huijskens, *Faculty of Agricultural Sciences, University of British Columbia, CANADA*; Leslie Willis, David Theilmann, *Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, CANADA*

09:30 **INVOLVEMENT OF THE RING FINGER MOTIF OF ACMNPV EXON0 IN BUDDED VIRUS PRODUCTION**

Xiaojiang Dai, David Theilmann, *Pacific Agri-Food Research Centre, CANADA*

10:20 **ANALYSIS OF CF103, A ZINC-FINGER ORF FROM THE BACULOVIRUS CFMNPV**

Jondavid De Jong, *Department of Microbiology, University of Guelph, CANADA*; Basil Arif, *Canadian Forest Service, Sault Ste, CANADA*; Peter Krell, *Department of Microbiology, University of Guelph, CANADA*

10:40 **IDENTIFICATION AND CHARACTERIZATION OF A DNA PHOTOLYASE-CONTAINING BACULOVIRUS FROM CHRYSODEIXIS CHALCITES**

Monique M. Van Oers, *Laboratory of Virology, Wageningen University, NETHERLANDS*; Elisabeth Herniou, *Department of Biological Sciences, Imperial College London, UNITED KINGDOM*; Awaluddin Junaid, Magda Usmany, *Laboratory of Virology, Wageningen University, NETHERLANDS*; Gerben J. Messelink, *Applied Plant Research, Naaaldwijk, NETHERLANDS*; Just M. Vlak, *Laboratory of Virology, Wageningen University, NETHERLANDS*

11:00 **BACULOVIRUS INDUCTION AND SUPPRESSION OF APOPTOSIS OF SPODOPTERA LITTORALIS SL2 CELLS**

Qinghzen Liu, Nor Chejanovsky, *The Volcani Center, ISRAEL*

11:20 **MAPPING THE POLYPEPTIDE REGIONS OF P10 OF HASNPV THAT ARE REQUIRED FOR FILAMENT FORMATION**

Chunsheng Dong, Dan Li, Gang Long, Fei Deng, Hualin Wang, Zhihong Hu, *Oint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*

11:40 **ANALYSIS OF THE CHITINASE GENE HOMOLOGUE OF THE BACULOVIRUS PLODIA INTERPUNCTELLA GRANULOVIRUS, PIGV**

Caroline Griffiths, Sukvinder Bharya, *Oxford Brookes University, UNITED KINGDOM*; John Burden, *NERC CEH-Oxford, UNITED KINGDOM*; Linda King, *Oxford Brookes University, UNITED KINGDOM*

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 3

Chair: C. Nielsen-LeRoux; A. Bravo

13:30 **BACILLUS THURINGIENSIS EXTRACHROMOSOMAL MOLECULES: FROM SMALL LINEAR PROPHAGES TO LARGE CONJUGATIVE PLASMIDS**

Géraldine Van der Auwera, Delphine Forget-Hanus, Céline Verheust, Jacques Mahillon, *UCL, BELGIUM*

13:45 **INTRACELLULAR EFFECTS OF CYT1AA FROM BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS ON ESCHERICHIA COLI EXPRESSING CYT1AA**

Robert Manasherob, Mark Itsko, Olga Burgazliev, Eitan Ben-Dov, Sammy Boussiba, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

14:00 **PATHOGENICITY OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS AND ENTOMOPATHOGENIC NEMATODES OF THE GENUS STEINERNEMA AGAINST TIPULA PALUDOSA**

Jesko Oestergaard, Ralf-Udo Ehlers, *Institute for Phytopathology, Christian-Albrechts-University Kiel, GERMANY*

14:15 **NEW ENTOMOPATHOGENIC BACTERIA FOR THE CONTROL OF WHITE GRUBS (COLEOPTERA:SCARABAEIDAE)**

Zitlhally Rodríguez Segura, Francisco Javier Villalobos, *Facultad de Ciencias Agropecuarias, Universidad Nacional Autónoma de México, MEXICO*; Luciano Hernández, *Universidad Autónoma del Estado de Morelos, Facultad de Química, Universidad Nacional Autónoma de México, MEXICO*; Eduardo Aranda, *Centro de Investigación en Biotecnología, Universidad Nacional Autónoma de México, MEXICO*; Maria Eugenia Núñez-Valdez, *Facultad de Ciencias Agropecuarias, Universidad Nacional Autónoma de México, MEXICO*

14:30 **THE RISK EVALUATION OF THE GENETICALLY ENGINEERING BACILLUS THURINGIENSIS WG-001 IN SOUTH CHINA VEGETABLE FIELDS**

Zhang Zhenyu, Li Lin, Sun Ming, Yu Ziniu, *State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Huazhong Agriculture University, P.R. CHINA*

14:45 **THE ASSOCIATION OF CHIRONOMIDS AND VIBRIO CHOLERAE**

Meir Broza, Malka Halpern, *Faculty of Science and Science Education, University of Haifa, ISRAEL*; Hanan Gancz, Yechezkel Kashi, *Faculty of Biotechnology, Israel Institute of Technology, ISRAEL*

15:00 **TARGETED DRUG DELIVERY OF CYT1AA PROTEIN FROM BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS**

Shmuel Cohen, *Department of Life Sciences, Ben-Gurion University of the Negev, 2Department of Chemical Engineering and Biotechnology, College Judea and Samaria, ISRAEL*; Eitan Ben-Dov, *Department of Life Sciences, Ben-Gurion University of the Negev, ISRAEL*; Marina Nisnevitch, Rivka Cahan, Michael Firer, *2Department of Chemical Engineering and Biotechnology, College Judea and Samaria, ISRAEL*; Arieh Zaritsky, *Department of Life Sciences, Ben-Gurion University of the Negev, ISRAEL*

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 6

Contributed Papers (Division of Fungi)

fungi / contributed paper session 4

Chair: Jorgen Eilenberg; F. Vega

13:30 **BEAUVERIA BASSIANA AS A KEYSTONE SPECIES IN PINE ECOSYSTEM**

Zengzhi Li, Meizhen Fan, Bin Wang, Degui Ding, *Department of Forestry, Anhui Agricultural University, CHINA*

13:45 **FIELD RELEASES OF BEAUVERIA BASSIANA STRAIN GHA AFFECT GENETIC DIVERSITY OF INDIGENOUS CONSPECIFIC POPULATIONS**

L. A. Castrillo, *Department of Entomology, Cornell University, UNITED STATES*; P. Mishra, L. Annis, Eleanor Groden, *Department of Biological Sciences, University of Maine,, UNITED STATES*; John D. Vandenberg, *USDA-ARS, US Plant, Soil & Nutrition Laboratory, UNITED STATES*

14:00 **STU** **DISTRIBUTION AND OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN THE SOIL IN A SINGLE AGROECOSYSTEM IN DENMARK**

Nicolai Vitt Meyling, Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40 DK-1871 Frederiksberg C, DENMARK*

14:15 **PROTECTION OF ENTOMOPATHOGENIC FUNGI AT THE LANDSCAPE SCALE**

Stanislaw Balazy, *Research Centre for Agricultural and Forest Environment PAS, POLAND*

14:30 **THE ABILITY OF COLLEMBOLANS TO ACT AS NON-HOST VECTORS OF ENTOMOPATHOGENIC HYPHOMYCETE FUNGI.**

Karsten Dromph, *The Royal Veterinary and Agricultural University, DENMARK*

14:45 **SENSITIVITY OF FOLSOMIA CANDIDA (COLLEMBOLA) TO BEAUVERIA BASSIANA GHA STRAIN AND METARHIZIUM ANISOPLIAE VAR. ACRIDUM IMI 330189**

Michael Brownbridge, *University of Vermont, Entomology Research Laboratory, U.S.A.*

15:00 **BEAUVERIA BASSIANA AS A COFFEE ENDOPHYTE.**

Francisco Posada, Fernando Vega, *Insect Biocontrol Lab., USDA, ARS, Bldg. 011A, Beltsville, MD 20705, USA*

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 10

Contributed Papers (Division of Nematodes)

nematodes / contributed paper session 2

Chair: Arne Peters; David Shapiro-Ilan

13:30 **ORAL TOXICITY OF PHOTORHABDUS TEMPERATA AGAINST THRIPS SPECIES**

Lonne Gerritsen, *Plant Research International, P.O. Box 16, 6700 AA Wageningen, NETHERLANDS*; Jana Georgieva, *University of Sofia, Dept. of Biology, Dragan Tzankov Boulevard, Sofia, BULGARIA*; Rob Van Tol, Gerrie Wiegers, *Plant Research International, P.O. Box 16, 6700 AA Wageningen, NETHERLANDS*

13:45 **ENTOMOPATHOGENIC NEMATODES FOR CONTROL OF THE PINE WEEVIL**

Haukeland Salinas Solveig, *Norwegian Crop Research Institute, NORWAY*

14:00 **USE OF STEINERNEMA CARPOCAPSAE FOR POST HARVEST CONTROL OF NAVEL ORANGEWORM (AMYELOIS TRANSITELLA) IN FALLEN PISTACHIOS**

Joel Siegel, Lawrence Lacey, *USDA/ARS, USA*; Bradley Higbee, *Paramount Farming Company, USA*; Robert , Jr. Fritts, *CertisUSA, USA*

14:15 **CAN FOLIAR APPLICATIONS OF ENTOMOPATHOGENIC NEMATODES BE ADOPTED FOR COMBATING THRIPS?**

Nasser Halaweh, Christian Borgemeister, Lemma Ebssa, Hans-Michael Poehling, *Hannover University, GERMANY*

14:30 **STU** **DOES IT MATTER FOR ENTOMOPATHOGENIC NEMATODES IF THRIPS PUPATE AT DIFFERENT SOIL DEPTHS, AND FOR THE THRIPS TO DECIDE WHERE TO PUPATE IF NEMATODES ARE AROUND?**

Lemma Ebssa, Christian Borgemeister, Jörg Semrau, Hans-Michael Poehling, *Hannover University, GERMANY*

14:45 **SCREENING AMONG ENTOMOPATHOGENIC NEMATODE STRAINS FOR VIRULENCE AGAINST DIABROTICA VIRGIFERA VIRGIFERA**

Stefan Toepfer, Ulrich Kuhlmann, Christine Gueldenzoph, *CABI Bioscience Switzerland Centre, SWITZERLAND*; Ralf-Udo Ehlers, *2 Institute for Phytopathology, Department of Phytopathology, Christian-Albrechts-University Kiel, GERMANY*

15:00 **SURVIVAL PATTERNS OF HETERORHABDITIS BACTERIOPHORA IN WATER AND IN FORMULATED PACKAGES**

Arne Peters, *E-nema GmbH, GERMANY*

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 1

Symposium (Cross-Divisional)

Microbial control in greenhouses and nurseries

Chair: Jean-Louis Schwartz; Patricia Stock

13:30 **USE OF ENTOMOPATHOGENIC NEMATODES IN THE NORDIC COUNTRIES**

Haukeland Salinas Solveig, *Norwegian Crop Research Institute, NORWAY*

13:50 **THE EFFECT OF HOST PLANT ON THE EVOLUTION OF BT RESISTANCE IN GREENHOUSE TRICHOPLUSIA NI POPULATIONS**

Alida Janmaat, Judith Myers, *University of British Columbia, CANADA*

14:10 **FIELD EFFICACY OF EPNS IN NURSERY AND TREE APPLICATIONS**

Rob Van Tol, *Plant Research International, Wageningen-UR, NETHERLANDS*; Michael Raupp, *University of Maryland, Central Maryland Research and Education Center, USA*

14:30 **DOES BEAUVERIA BASSIANA DISRUPT GREENHOUSE BIOLOGICAL CONTROL?** STU

Roselyne Labbé, Jacques Brodeur, Conrad Cloutier, *Université Laval, CANADA*; David Gillespie, *Pacific Agriculture and Agri-Food Canada Research Centre, CANADA*

14:50 **SUSCEPTIBILITY OF VARIOUS DEVELOPMENT STAGES OF GLASSHOUSE WHITEFLY TO INFECTION BY ENTOMOPATHOGENIC FUNGUS PAECILOMYCES FUMOSOROSEUS**

Ayhan Gökçe, *University of Gaziosmanpaa, TURKEY*; Mehmet Kubilay Er, *University of Sütçü Ýmam, TURKEY*

15:10 **VARIABILITY IN RESPONSES OF DISCRETE LABORATORY POPULATIONS OF WESTERN FLOWER THRIPS, FRANKLINIELLA OCCIDENTALIS (PERGANDE) TO ENTOMOPATHOGENIC FUNGI**

Michael Brownbridge, *Entomology Research Laboratory, Univ. of Vermont, U.S.A.*; Stephen Goodwin, W.G. Liang, Marilyn Y. Steiner, *NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford, AUSTRALIA*; Ken Fry, *Alberta Research Council, Vegreville, CANADA*

Thursday, August 5^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Workshops (Division of Microbial Control)

Status of microbial control products

Chair: Wendy Gelernter; Jeff Lord

Thursday, August 5^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Workshop (Cross-Divisional)

SIP education workshop

Chair: Helen Roy; Jorgen Eilenberg

16:00 **TEACHING ASPECTS OF MICROBIAL CONTROL AS A COMPONENT OF UNDERGRADUATE COURSES**

Helen Roy, *Department of Life Sciences, APU, UK*

16:20 **EXPERIENCE WITH A LECTURE COURSE AND TWO EXPERIMENTAL LABORATORY COURSES IN BIOLOGICAL CONTROL**

Jørgen Eilenberg, *Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Dan Funck Jensen, John Hockenhull, *Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871 Frb. C, DENMARK*; Holger Philipsen, *Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*

16:40 **MICROSPORIDIA AND BIOLOGICAL INVASIONS**

Alison Dunn, *University of Leeds, UK*; Calum MacNeil, *Queens University, Belfast, UK*; Jolene Slothouber-Galbreath, *University of Leeds, UK*; Jaimie Dick, *Queens University, Belfast, UK*

17:00 **THE ROLE OF ROTHAMSTED RESEARCH IN EDUCATION AND TRAINING IN MICROBIAL CONTROL**

Judith K. Pell, Paresh A. Shah, Judy Mann, Brian R. Kerry, Brenda Ball, *Rothamsted Research, UK*

17:20 **TEACHING PEST MANAGEMENT AND BIOLOGICAL CONTROL TO THE END-USER**

Wendy Gelernter, *PACE Consulting, USA*

Thursday, August 5^{th}, 2004
Time: 19:00 - 24:00, Marina Congress Center

Banquet

Book of Abstracts

SIP 2004

STU indicates papers being judged for
graduate student presentation awards

Saturday, July 31st, 2004
Time: 08:30 - 17:00, Hotel Grand Marina

SIP Council Meeting

Saturday, July 31st, 2004
Time: 13:00 - 18:00, Lecture Room 4

Registration

Sunday, August 1st, 2004
Time: 09:00 - 09:20, State Room

Opening plenary

Presenter: Harry Kaya

09:05 **EARLY NORDIC CONTRIBUTIONS TO INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL**

Jørgen Eilenberg, *Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*

Abstract: People in the Nordic countries have since ancient times been fighting with pest insects. Did they note the presence of insect pathogens? No sign on this is seen in the old Nordic literature.
Later, however, we find examples of insect diseases in the literature, both in a fairy tale by Hans Christian Andersen and in a novel by the Noble price winner Selma Lagerlöf.
Also, the Nordic countries contributed to the early scientific development of invertebrate pathology and microbial control. New species were described since the late 18th century, some of these are among the well-known species considered for microbial control. Also, microbial control experiments have been conducted since the late 19th century, the first experiments were against Melolontha melolontha using fungi.

Sunday, August 1st, 2004
Time: 09:20 - 10:15, State Room

Founder's Memorial Lecture

Presenter: Dudley Pinnock, *Founder's Lecture Committee*
Honoree: Hans Boman
Lecturer: Kenneth Söderhäll

Sunday, August 1st, 2004
Time: 10:30 - 13:30, State Room

Plenary (Cross-Divisional)
SIP - the past, present and future

Presenter: Just Vlak; Harry Kaya

10:40 **HISTORY OF THE SOCIETY FOR INVERTEBRATE PATHOLOGY**

Elizabeth W. Davidson, *School of Life Sciences, Arizona State University, U.S.A.*

Abstract: Early in the 20th century, seminars on invertebrate diseases were held at several national and international scientific meetings, but the critical spark to the building of an international association came from the First International Conference on Insect Pathology and Biological Control, held in Prague, Czechoslovakia in 1958, and the International Colloquium on Insect Pathology and Microbial Control at Wageningen, Netherlands in 1966. Simultaneously, oyster pathologists were also beginning to discuss their common interests in an association. A telephone conversation between Edward Steinhaus, professor at the University of California, Berkeley, and Albert Sparks, scientist at the US National Marine Fisheries Service in Seattle, Washington, led to mailing a questionnaire to more than 500 individuals known to be working in the area. The results led Steinhaus and Sparks to convene an Organizing Committee in Seattle, Washington on May 9, 1967, consisting of Edward Steinhaus, Thomas A. Angus, Arthur M. Heimpel, Mauro E. Martignoni, Carl J. Sindermann, Albert K. Sparks and Victor Sprague. At this meeting it was decided that the Society for Invertebrate Pathology should be established. Steinhaus was elected the first President, Sparks the first Vice President, and Heimpel the first Secretary-Treasurer. The first annual meeting was held in 1968 at Ohio State University.

Annual meetings of SIP are memorable events for most of us. We have met in 13 different countries, enjoying not only intense scientific discussions, but also river trips, hikes up desert mountains, train rides, dinner in a castle, walking over a Roman bridge, flamenco dancers, bullfights, wine tasting, and the list goes on and on. Above all, we enjoy the company of our colleagues from over 50 countries. When we meet again in Helsinki, we will hear much more about the past and future of invertebrate pathology.

11:00 PAST, PRESENT AND FUTURE OF MICROSPORIDIA IN THE SIP

Jaroslaw Weiser, *Praha 4, Heralecka 964, CZECH REPUBLIC*

Abstract: For Microsporidiologists the new Society was a good opportunity to exchange ideas and present articles. Of some priorities, published with the sip we mention, that in 1960 Huger published in the J. Insect Pathology his study of electron microscopy of the microsporidian spore with first perfect illustration of the localization of the polar filament, the nucleus and polaroplast. Another important observation with great impact on the taxonomy of microsporidia was the contribution of Ann Cali at the 1970 Int. Colloq. Insect Pathology, College Park, describing the morphogenesis in the genus Nosema where she demonstrated the diplokaryon and the double nucleus as typical for the genus Nosema. This observation initiated a deep revision of all former descriptions of Nosema's. In 1968 Ishihara and Hayashi presented in JIP the ribosomes of microsporidia as typical for prokaryotes. In 1968 Hazard and Weiser published the polymorphic development of Amblyosporidae. From the beginning the SIP published abstracts of its international and later of its annual meetings for all members of the Society and in this way it brought members together and all had the information about progress in their special field inside the Society In cooperation with the SIP Vavra, Sprague and others published monographs on Biology and Taxonomy of microsporidia (1976, 1977). A recent continuatiomn is the monograph edited by Wittner and Weiss in 1999. The actual progress in research of microsporidia by members of SIP concentrates on sequencing of series of microsporidia from vector insects (Becnel, Fukuda) together with the laboratory of Andreadis and Vossbrink. Further work with microsporidia is in progress in the laboratory of Ann Cali (Rutgers) and L. Solter (Champain), former laboratory of Brooks and Maddox . In Europe long lasting studies of ultrastructures of microsporidia are presented by Ronny Larsson (Sweden), E. U. Canning (U.K.) and Jiri Vavra, J. Lom and J. Weiser (Czech Republic). Other studies of microsporidia were presented by Huger (Germany), Loubes and Maurand (Montpellier, France). In Russia the work with microsporidia is concentrated in Irma Issi's and Voronin's laboratory at St. Petersburg. In Africa the laboratory of Toguebaye in Dakar is in good contact with Marchand and Bouix at Montpellier. Expected progress in research of microsporidia is in continuation of the sequencing concentrated on the realistic presentation of the sequenced tree of microsporidia in comparison with the morphological tree. There are several aspects of interactions of microsporidia with their hosts where a detailed research of physiology and pathophysiology is expected (sex- related development of Amblyospora, Octosporea efeminans in Crustacea, xenomas in insects and Glugea cysts in fish). A further deep research is expected in microsporidia of mammals including man. Experiments with introductions and colonization of microsporidia for introduced quarantine organisms may offer some limiting organisms for pests in areas of national parks or natural reserve areas without intensive management. It is difficult to predict further development in the field, it depends very much of the financial support and needs for solution of new important economic situations. Efforts in protection of useful insects and support of bio-control of important pests will be continued.

11:20 FROM METCHNIKOFF TO MONSANTO AND BEYOND: THE PATH OF MICROBIAL CONTROL

Jeffrey Lord, *USDA-ARS, USA*

Abstract: In 125 years since Metchnikoff proposed the use of Metarhizium anisopliae to control the wheat cockchafer, microbial control has progressed from the application of naturalists' observations to biotechnology and precision delivery. There is a dichotomy in the current paths. While Bt transgenic crops are now planted on millions of hectares, the successes of more narrowly defined microbial control are mainly in small niches, with forestry being a notable exception. Commercial enthusiasm for traditional microbial control agents has been unsteady in recent years, and there has been a great deal of industry start-up, shut-down, and consolidation. The prospects of fungal and viral insecticide use on vast areas of maize, cotton and soybeans are now viewed more realistically. A successful future will depend on creative approaches. There is likely to be increased emphasis on monitoring and conservation of natural microbial controls. Microbial agents will be integrated with chemical pesticides, exploiting synergies where possible. Governmental regulation will encourage imaginative approaches to niche microbial control agents. Where regulatory cost and restrictions are prohibitive, avoidance tactics such as site-of-origin production may be used. We will likely see more examples like the successful conservation program for cotton aphid control with Neozygites fresenii in the US. The involvement of governments and commodity cooperatives will continue to play an important role, as has been the case for mycoinsecticide use on sugar cane in Brazil and forest Lepidoptera in China. The less developed countries are favorable venues for microbial control because of many factors including low labor costs, mild regulatory climates, modest chemical inputs, and small scale farming. Future progress will be retarded by regulatory costs and constraints, resistance from activist pressure groups, new benign and efficacious chemical alternatives, and limited research funding. Progress will be advanced by growing organic agriculture, regulation harmonization, and clever scientists who devise creative strategies for conservation and integration.

11:40 INSECTICIDAL BACTERIA IN HISTORICAL PERSPECTIVE: AN OVERWHELMING SUCCESS FOR INVERTEBRATE PATHOLOGY

Brian A. Federici, *Department of Entomology, University of California, UNITED STATES*

Abstract: A little over a century ago, S. Ishiwata described the sotto-kin bacillus as a cause of silkworm disease in Japan. Not long after, a similar bacterium, named Bacillus thuringiensis (Bt), was described by E. Berliner in Germany as the cause of disease in larvae of the flour moth, Ephestia kuhniella. For many years, these bacteria remained interesting curiosities, but in France just prior to WWII, variants of the latter were used for insect control. WWII interrupted these studies, but after the war, research on Bt by E. Steinhaus, and B. popilliae (Bp) by S. Dutky led to commercialization of both bacteria for control of important insect pests, Bt for lepidopterous pests, and Bp for scarabs. Aside from the commercial utility of these bacteria, their insecticidal properties stimulated basic research on the mechanisms by which these caused disease in insects and tactics to prevent disease, as in the case of foulbrood of bees. During the 1950's, the Bt parasporal crystal was shown to be an endotoxin protein and the principal component responsible for insect death though destruction of the midgut epithelium. In contrast, Bp was shown to act by causing an infectious disease in which the bacterium reproduced primarily in the hemolymph. Basic and applied research continued with a focus on Bt throughout the 1950's and 1960's and during the latter decade, the first commercially successful products based on H. Dulmage's HD1 isolate of B. t. subsp. kurstaki were developed for control of lepidopterous pests. For many years, it was thought that all Bt's were only active against lepidopterous insects, but discovery of B. t. subsp. israelensis by L. Goldberg and Y. Margalith in Israel in 1976, a subspecies highly toxic to mosquito and blackfly larvae, and later the discovery by A. Huger and his colleagues of the tenebrionis strain of B. t. subsp. morrisoni active against coleopterous insects, showed that a wide variety of Bt strains had evolved that could be put to commercial use. These discoveries stimulated a rapid increase in our basic knowledge of Bt that eventually led to the cloning of the first Bt endotoxin gene by H. Whiteley's group, and subsequently the first structure of a Bt Cry protein by D. Ellar's laboratory. The cloning of Bt genes led quickly to the development of insecticidal transgenic crops in the mid-1980's, an industry that has grown to a market value of greater than $5 billion per year. Aside

from this progress, other advances include discovery and development of B. sphaericus for control of Culex mosquitoes, and Serratia entomophila for grass grub control in New Zealand. These contributions to basic science and their subsequent commercial development changed the landscape of agriculture and medicine with respect control of insect pests and vectors of disease, and will continue to do so well into the future.

12:00 **FROM BERGOLD TO BURAND: A JOURNEY WITH INSECT VIRUSES**

Basil Arif, *Great Lakes Forestry Centre, CANADA*

Abstract: Research in insect viruses started in the 19th century when it was discovered that Jaundice in the silkworm was caused by refractive bodies that, today, we define as viral occlusion bodies. Earlier investigators such as Steinhaus, Aizawa and Bergold had set the stage for modern baculovirology. The pioneering work of Bergold provided the first insight into the nature and structure of baculoviruses. Interest in these viruses was initially driven by their potential to replace chemicals in the control of economically important agricultural and forest insect pests. Indeed, successes with viruses of sawflies had further consolidated this idea. However, it was soon realized that the success of viruses was not a generalized phenomena and, in fact, their slow acting nature was a major drawback. The advent of permissive tissue culture systems was the coming of age for insect viruses. Studies on the molecular biology and replication were manifested in a plethora of excellent published works. Concomitantly, it became clear that baculoviruses were particularly amenable to genetic modification and gave further impetus to their use in pest management strategies as carriers of genes deleterious to insect pests. It also resulted in their prolific use as systems for the expression of foreign proteins. The latter property of baculoviruses has impacted positively on various sectors of science. Today, genomics of insect viruses are giving insight into their co-evolution with the larval host and may lead to understanding of the factors that determine host range and specificity.

12:20 **THE FUNGAL PAST, PRESENT AND FUTURE: GERMINATION, RAMIFICATION AND REPRODUCTION**

John D. Vandenberg, *USDA-ARS, U.S. Plant, Soil & Nutrition Laboratory, U.S.A.*

Abstract: The history of observation and research on fungal pathogens of invertebrates dates back thousands of years. In the era before microscopes, fungi were visible to the naked eye as organisms and observation of them helped give birth to invertebrate pathology as a modern field of study. The twentieth century brought phenomenal advances in our knowledge of fungal biology, cultivation and use. The present is filled with a worldwide community of researchers working on many fronts to grasp the dynamics of fungal populations, to reveal their organismal and cellular mechanisms, and to decipher their genetic code. We are striving to deploy fungi to help manage pests and to exploit fungal genes and their products for new uses. We are gaining a much deeper understanding of the interactions of fungi with other agents of pest management and the trophic cascades in which they are involved. New technologies allow us to track, with increasing accuracy, the fate of fungi released into the environment. This encouraging present points to a future that is daunting but bright. The current assemblage of invertebrate fungal pathologists is relatively small and the struggle for adequate research funding is never-ending. However, in coming years, an ever-wider array of techniques will be available to biologists, and I am certain our creativity will allow us to take full advantage of them. In this presentation, I will draw on the contributions of many other SIP members for essential background and data. I will use case studies to illustrate the rich past, exciting present and promising future of research on fungi. This history can be told chronologically, but I hope to tell it in other ways as well. Our future success will depend on appreciating the history of international efforts by many laboratories and institutions. It will depend on acknowledging past initiatives to manage devastating insect pests. It will depend on recognizing our failures and our breakthroughs. Finally, our success depends on cultivating our international cooperation, our sanguinity, our indefatigable diligence, and our vision.

12:40 **INSECT PARASITIC NEMATODES: FROM LAB CURIOSITIES TO MODEL ORGANISMS**

S. Patricia Stock, *Department of Plant Sciences. University of Arizona. Tucson AZ 85721-0036, USA, USA*

Abstract: Interest in insect parasitic nematodes was originally focused on their potential as biological control agents of insects and other arthropod pests. Now, after 30 years of intense basic and applied research, realization of the practical use of insect parasitic nematodes, particularly of entomopathogenic nematodes and their symbiotic bacteria, has spurred developments across a far broader scientific front. We are now entering a new era of discovery in which tools of molecular genetics are being increasingly used to address a range of biological questions. The knowledge gained from these efforts will directly benefit the practical application of insect parasitic nematodes as more effective biopesticides. Moreover, these studies will advance these nematodes as unique and intrinsically interesting biological model systems not only for basic research but also in applied fields such as plant health, human medicine, pharmaceutical bioprospecting and genetic engineering. In this presentation, the current state of insect parasitic nematode research will be reviewed and future research priorities and goals will be identified and discussed.

Sunday, August 1^{st}, 2004
Time: 13:30 - 15:30, Corridor levels 2 and 3

Setting up posters

Sunday, August 1^{st}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Workshop (Cross-Divisional)

The graduate student's guide to the galaxy

Chair: Todd Udine

Sunday, August 1^{st}, 2004
Time: 19:00 - 22:00, Marina Congress Center

Welcoming reception

Monday, August 2^{nd}, 2004
Time: 08:00 - 09:30, Lecture Room 1

Plenary (Cross-Divisional)
Invertebrate pathogens as pests

Presenter: Heikki Hokkanen

08:00 **EPIDEMIOLOGY IN HONEY BEES**

Ingemar Fries, *Department of Entomology, Swedish University of Agricultural Sciences, SWEDEN*

Abstract: From an insect pathology perspective, honey bee reproduction is of fundamental interest for understanding the host-parasite relationships in this host. Within colony, honey bees reproduce through sexual reproduction, as well as by parthenogenesis. At colony level, however, honey bees reproduce by colony fission as colonies divide during swarming. This mode of reproduction offers vertical transmission opportunities for any parasite/pathogen that can be carried with adult bees. The degree to which a disease evolves to be virulent depends, in part, on whether the pathogen is transmitted horizontally or vertically. Horizontal transmission often selects for more virulent pathogens, whereas vertical transmission, where the reproductive interest of the host and the pathogens are aligned, often develop more benign host-parasite relationships. Within colony, only horizontal pathogen transmission is known, but at colony level vertical transmission is likely the most important route of pathogen infection of new colonies in natural systems. Inter-colony transmission of pathogens also occurs horizontally (by drifting or robbing), but drifting of worker bees is probably mainly an apicultural phenomenon and significant robbing between colonies, when not clumped in apiaries, is likely to occur only in weak colonies unable of defense. In theory, the reproductive system at colony level in honey bees should generally select for benign host-parasite relationships. The implications of horizontal and vertical pathogen transmission for virulence of honey bee diseases is discussed in the light of current ideas in evolutionary epidemiology. The implications from the reproductive system of honey bees and modes of parasite transmission in this system has important epidemiological consequences. To understand the host-parasite adaptions in this system it is necessary to study, and to quantify, parasite transmission rates (horizontal as well as vertical) at colony level.

08:30 **CRAYFISH PLAQUE (APHANOMYCES ASTACI) IN FINLAND: PAST, PRESENT AND FUTURE**

Satu Viljamaa-Dirks, *National Veterinary and Food Research Institute, Kuopio Department, FINLAND*

Abstract: Crayfish plaque is the most serious disease threatening the populations of European freshwater crayfish species. The causative agent, an oomycete fungus Aphanomyces astaci, originating from North-America, was apparently introduced in the end of the 19th century into Europe, where it had a devastating effect on native crayfish populations. The disease appeared in Finland 1983 and has ever since caused more economic losses in fishing industry than any other disease of aquatic animals. Noble crayfish Astacus astacus was a common inhabitant of the Finnish lakes and rivers before the crayfish plaque destroyed many of the main populations. Because the attempts to re-introduce noble crayfish in main water courses were unsuccessful, a North-American species, signal crayfish Pasifastacus leniusculus, was used for stockings in the southern part of Finland. North-American crayfish species are relatively resistant to the disease, with mortality only in stress situations. These species can carry the fungus in their cuticle for extended periods as a latent infection. Most of the signal crayfish populations in Finland are now recognised as carriers of the fungus Aphanomyces astaci, representing a permanent threat for the remaining populations of noble crayfish. Following the introduction of the signal crayfish in the 1960s, new genotypes of the fungus were documented as the cause of disease outbreaks in several countries including Finland. Geographical distribution of the two genotypes found in Finland corresponds with the stocking area of signal crayfish. There is some epidemiological evidence suggesting differences in virulence of these two genotypes. The present situation concerning crayfish plaque in Finland and the strategies for prevention will be discussed.

09:00 **IMPORTANCE OF BLOOD CELLS AND HEMATOPOIESIS IN HOST DEFENCE IN CRUSTACEANS**

Irene Söderhäll, *Department of Comparative Physiology, Evolutionary Biology Centre, Uppsala University, SWEDEN*

Abstract: Crustaceans as members of the invertebrate group of animals, lacking the memory of vertebrate immunity, have to rely solely on innate immune mechanisms in their host defense. Cellular defense mechanisms are of great importance both in vertebrate and invertebrate innate immunity as well as in wound repair. In crustaceans host defence the hemocytes play a key role in immobilising or destroying invasive microorganisms. Three classes of hemocytes, which all play a key role in immobilising or destroying invasive microorganisms, are observed within the hemolymph, the hyaline cells the semigranular cells and the granular cells. In the freshwater crayfish, Pacifastacus leniusculus, hemocytes have been extensively characterized and their importance for the innate immune system is recognized mainly due to that several immune factors have been determined both structurally and functionally. These include factors involved in clotting and coagulation reactions, phagocytosis and cellular encapsulation, antimicrobial peptides, lectins, pattern recognition proteins, and components of the prophenoloxidase-activating cascade (proPO-system). Injection of microbial polysaccharides into the hemolymph of insects as well as of crustaceans is known to cause a rapid decrease in hemocyte number shortly followed by a recovery. In P. leniusculus this recovery is the result of increased synthesis and release of hemocytes from the hematopoietic tissue. How this septic injury response leads to increase in circulating hemocytes has remained unclear, and regulation of hematopoiesis is largely unknown in invertebrate animals. In vertebrates, cytokines act as important coordinators of innate and adaptive immune response as well as of growth and differentiation of new blood cells. The search for invertebrate cytokines during the past decades has been intense and to further study the regulation of hematopoiesis we have created an in vitro system for culture of hematopoietic stem cells and hemocyte progenitors from crayfish. This stem cell culture was used to identify an endogenous cytokine-like factor that is required for differentiation and growth of these cells. Interestingly this factor contains a protein domain hitherto only found in vertebrates, and is the first invertebrate cytokine involved in hematopoiesis sharing a protein domain with a protein in the vertebrate phyla. In order to investigate regulation of the hematopoietic process we used gene silencing by RNA interference technique. DsRNA was designed from cDNA for the cell adhesion protein peroxinectin and as a result differentiation of the stem cells was affected indicating some role for peroxinectin during the differentiation process.

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 1

Symposium (Division of Bacteria)
Second generation transgenic crops

Chair: Sarjeet Gill

10:00 **QUANTIFICATION OF LEPIDOPTERAN ACTIVITY IN COTTON EXPRESSING TWO BT CRY PROTEINS.**

Ty Vaughn, James Baum, Sakuntala Sivasupramaniam, John Greenplate, *Monsanto, USA*

Abstract: Abstract not available at this time

10:25 **USING BT'S TO ACHIEVE ECONOMIC LEVELS OF HOST-PLANT NON-PREFERENCE: HERCULEX * I VS. BLACK CUTWORM**

Steve Lefko, Laura Higgins, Bill McCutchen, *DuPont Agriculture & Nutrition, USA*

Abstract: HerculexTM I is a new insect protection trait developed through a research collaboration between Dow AgroSciences / Mycogen Seeds and Pioneer Hi-Bred International Inc. Herculex uses the Cry1F protein from Bacillus thuringiensis (Bt) var. aizawai. This trait protects corn from more major Lepidopteran insect pests than any other commercial Bt product; one example of its advantage is protection from the seedling corn pest black cutworm. Laboratory and greenhouse studies were conducted to investigate the relative importance of the antibiotic and non-preference categories of host plant resistance in Herculex protection from black cutworm. Preliminary results suggest that, although relatively high concentrations of Cry1F may have an antibiotic effect on black cutworm, the predominant resistance mechanism in seedling plants likely is non-preference. These preliminary results and a brief discussion of the relative importance of Cry1F antibiosis and non-preference in other target pests will be presented.

10:50 **BACILLUS THURINGIENSIS BINARY INSECTICIDAL PROTEINS FOR CORN ROOTWORM CONTROL: MODE OF ACTION STUDIES**

Meibao Zhuang, Tarlochan S. Dhadialla, *Dow AgroSciences LLC, UNITED STATES*

Abstract: Cry34Ab1 and Cry35Ab1 are insecticidal crystal proteins (ICPs) isolated from Bacillus thuringiensis (Bt) strain PS149B1 that are active against economically important corn rootworm species including the western corn rootworm (WCRW), Diabrotica virgifera virgifera. Maximum insecticidal activity is observed when both ICPs, Cry34Ab1 (14 kDa) and a Cry35Ab1 (44 kDa), are administered to susceptible larvae although Cry34Ab1 alone possesses some activity against southern corn rootworm, Diabrotica undecimpunctata howardi, in artificial diet bioassays. These binary ICP are not active on other insect and non-insect species tested, indicating a selective mode of action. In an attempt to understand the mode of action and the selective toxicity of these insecticidal proteins, we have conducted experiments using brush border membrane vesicles (BBMVs) isolated from whole WCRW larvae and from the dissected mid-guts of corn ear worm larvae. Ligand overlay blots have been conducted to identify BBMV proteins that specifically bind individual or combined components of the binary ICP or Cry1Ac. BBMVs were also used to determine pore-formation capability of individual and combined proteins of the binary ICP. Pore formation capability of recombinant Cry34Ab1, Cry35Ab1 or mixture in WCRW larval BBMVs was determined using an assay in which a positively charged fluorescent dye, 3,3'-dipropylthiocarbocyanine, is used to measure changes in membrane potential. Results from these experiments that elucidate the mechanism of action of the binary Cry34Ab1 and Cry35Ab1 proteins will be presented

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Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 12

Symposium (Division of Nematodes)

Significance of the entomopathogenic nematode infected-host in the soil ecosystem, and potential impact on microbial control

Chair: David Shapiro-Ilan

10:00 **INFECTED HOST'S ROLE IN INFECTION DYNAMICS OF ENTOMOPATHOGENIC NEMATODES**

Parwinder S. Grewal, *Department of Entomology, Ohio State University, U.S.A.*

Abstract: Infected hosts can play an important role in the infection dynamics of entomopathogenic nematodes. We found that odor-mediated resource assessment occrs in entomopathogenic nematodes which plays an important role in reducing inter- and intra specific competition. Steinernema carpocapsae infective juveniles were repelled from hosts infected by most heterospecific nematodes except S. anomali, whereas S. glaseri were repelled only from S. riobrave-infected hosts. Steinernema feltiae did not differentiate any heterospecific or heterogeneric infections. Steinernema glaseri were attracted to two of the five heterospecific infections. Both S. anomali and S. glaseri were more attracted to hosts infected with the out-group Heterorhabditis bacteriophora than those infected by conspecific nematodes. Infective juvenile S. carpocapsae, S. anomali, and S. glaseri were more attractive to hosts colonized by conspecific nematodes than to ininfected insects. Infective juvenile S. carpocapsae were repelled from the 24-hr-old conspecific infections, whereas S. glaseri were less attracted to 24- than 4-hr. old conspecific infections. Experiments with insects injected with bacteria from the nematodes suggested the bacteria as a source of active volatiles. Recruitment of conspecfic nematodes during the initial phases may ensure mate-finding and host-death though mass-attack.

10:20 **INFECTED HOST INTERACTION WITH ANTAGONISTS**

Harry Kaya, *University of California-Davis, USA*; Heidi Goodrich-Blair, *University of Wisconsin -Madison, USA*

Abstract: Entomopathogenic nematodes (EPNs), like all other organisms, have their own guild of natural enemies (Kaya, 2002). The natural enemies of the free-living, infective stage of EPNs include nematophagous fungi, protozoan and bacterial pathogens and predatory nematodes, mites tardigrades, collembolans, etc. Until recently, the fate of EPNs in nematode-killed insects was not examined in detail. Baur et al. (1998) showed that ants could serve as scavengers of nematode-killed insects, but that, in some cases, the cadavers with nematodes were only partially consumed or were not consumed at all. On the other hand, Bacillus thuringiensis-killed or frozen-killed insects were entirely consumed by these ants. The associated bacteria of EPNs in nematode-killed insects were producing an ant deterrent factor(s) (ADF) that turned off the ants from consuming these cadavers. In further studies, Zhou et al. (2002) showed that Xenorhabdus nematophila and Photorhabdus luminescens, the symbiotic bacteria of the nematodes Steinernema carpocapsae and Heterorhabditis bacteriophora, respectively, produced an ant deterrent factor(s) (ADF) was tested in vitro and in vivo. When nutrient broth was used as a culture medium, X. nematophila HgB007 and P. luminescens HgB008 required 108 and 132 h, respectively, to produce maximum levels of ADF. The different bacterial isolates varied in their ability to produce ADF in vivo and in vitro, with X. nematophila HgB007 and P. luminescens HgB008 showing the highest level of activity. ADF was heat stable (at 121aC for 20 min) and retained its activity after passage through a 0.45 Ým Millipore filter. Thus, ADF was extracellular and a non-proteinaceous compound. Host size affected ant behavior; that is, nematode-killed hosts that were small (27-28 mg/larva) were carried into the ant nests under laboratory conditions no matter whether they were killed by S. carpocapsae or H. bacteriophora. However, H. bacteriophora-killed hosts were discarded from the nest within 2 to 24 h, whereas S. carpocapsae-killed hosts were retained within the nest and consumed. These results demonstrated that the symbiotic bacteria of entomopathogenic nematodes produce compounds that deter scavengers such as ants and thus protect the nematodes within the hosts from being eaten.

Baur, M. E., H. K. Kaya, and D. R. Strong. 1998. Foraging ants as scavengers on entomopathogenic-killed hosts. Biol. Control 12: 231-236. Kaya, H. K. 2002. Natural enemies and other antagonists. In: Entomopathogenic Nematology, R. Gaugler, ed. CAB International, Wallingford, UK, pp. 189-203. Zhou, X., H. K. Kaya, K. Heungens, and H. Goodrich-Blair. 2002. Response of ants to a deterrent factor(s) produced by the symbiotic bacteria of entomopathogenic nematodes. Appl. Environ. Microbiol. 68: 6202-6209.

10:40 **EMERGENCE DYNAMICS FROM THE INFECTED HOST AND QUALITY OF EMERGED NEMATODES**

Christine T. Griffin, Martin J. Downes, Alec N. Rolston, *Department of Biology, National University of Ireland, Maynooth, Co. Kildare, IRELAND*; Jon J. Ryder, *School of Biological Sciences, Queen Mary, University of London, London E1 4NS, ENGLAND*

Abstract: A single insect cadaver can produce up to half a million infective juveniles (IJs) of the entomopathogenic nematodes Heterorhabditis and Steinernema. These IJs emerge over days or weeks. It is to be expected that IJs emerging even from a single cadaver will be physiologically and behaviourally diverse. The amount of genetic variation in the emerging cohort will depend on variation within the source population and on the number of individuals from that population that established the infection. Nematodes can cycle through up to three generations in a cadaver, and this is expected to create heterogenous developmental environments for the IJs. The most obvious difference between IJs developing at different times is the progressive decrease in size of later emerging IJs, which is presumed to be due to the progressive decline in quality of cadaver resources. Other phenotypic differences in the behaviour and tolerances of IJs emerging at different times have also been reported. Not only do IJs emerging early and late differ, but so does the environment in to which they emerge. Earlier waves of IJs may occupy nearby hosts or attract nematode pathogens or predators. Some of the factors influencing the timing of emergence from host cadavers and the phenotype of emerging IJs will be discussed.

11:00 **RESPONSE OF SOIL FAUNA TO INUNDATIVELY AND CADAVER-APPLIED ENTOMOPATHOGENIC NEMATODES**

Mary Barbercheck, *The Pennsylvania State University, USA*; C. Marie Greenwood, *North Carolina State University, USA*

Abstract: Soil organisms provide the foundation for such critical processes as soil structure development, nutrient cycling, decomposition, and biological control. Complex assemblages of organisms with both broad and narrow host ranges lead to complex trophic webs. Entomopathogenic nematodes are widely distributed and function as a naturally-occurring biological control agent of arthropods that live or spend part of their lives in the soil. Existing studies suggest that predation on nematodes could affect the efficacy of entomopathogenic nematodes against soil dwelling insect pests. The understanding of biological interactions in the soil is complicated by the high prevalence of omnivory, which has previously been assumed rare in food webs. The impact of predation on entomopathogenic nematodes in the soil is also confounded by both physical and biotic complexities of the soil environment. More heterogeneous systems, with less distinct trophic levels, have the capacity to buffer the effects of predation on lower trophic levels. In this presentation we will review examples of the effects of various agricultural practices and soil characteristics on soil biota, including the interaction between soil microarthropods and entomopathogenic nematodes applied inundatively and as infected cadavers. In our research, we have found that the response of soil fauna to an application of nematodes is specific, and is not detectable at coarse levels of taxonomic identification, arthropod abundance or by calculated diversity indices.

11:20 **POTENTIAL FOR APPLICATION OF INFECTED HOSTS IN MICROBIAL CONTROL**

David I. Shapiro-Ilan, *USDA-ARS, SAA, U.S.A.*; Edwin E. Lewis, *Virginia Tech, U.S.A.*

Abstract: Entomopathogenic nematodes are generally applied for insect control in aqueous suspension using various sprayers or irrigation systems. Novel methods of application can reduce costs and improve efficacy of pest suppression. One potential alternative is to apply nematodes in their infected hosts. Applications of nematodes in infected hosts can result in significant pest suppression under field conditions. Our research has addressed the following questions: 1) Are there advantages to applying nematodes in infected hosts Vs aqueous application? 2) Is it feasible to apply infected hosts from a technical standpoint? Our laboratory experiments indicate advantages of greater dispersal, infectivity, and survival when applying nematodes in infected hosts compared with aqueous application (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999; Perez et al., 2003). In greenhouse studies, superior efficacy was observed in suppression of Diaprepes abbreviatus and Otiorhynchus sulcatus when nematodes were applied in infected hosts (Shapiro-Ilan et al., 2003). Further, our studies indicate it is feasible to store and package nematode infected hosts for commercial application. Fragile cadavers such as Galleria mellonella can be coated with formulations to facilitate storage and application (Shapiro-Ilan et al., 2001). Alternatively, hard-bodied insects (such as Tenebrio molitor) can be ideal hosts because they are naturally resistant to rupturing or sticking together. Acknowledgement: This research was partially funded by a grant from USDA-SBIR (PI = Louis Tedders, H&T Alternative Controls, LLC). Shapiro, D.I., and I. Glazer. 1996. Environ. Entomol. 25:1455-1461. Shapiro, D.I., and E.E. Lewis. 1999. Environ. Entomol. 28: 907-911 Perez, E.E., E.E. Lewis, and D.I. Shapiro-Ilan. 2003. J. Invertebr. Pathol. 82:111-118. Shapiro-Ilan, D.I., E.E. Lewis, R.W. Behle, and M. R. McGuire. 2001. J. Invertebr. Pathol. 78:17-23. Shapiro-Ilan, D.I., E.E. Lewis, W.L. Tedders, and Y. Son. 2003. J. Invertebr. Pathol 83:270-272.

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 6

Symposium (Division of Viruses)

Virus ecology

Chair: Linda King

10:00 **ECOLOGY AND EPIDEMIOLOGY OF WHITE SPOT SYNDROME VIRUS OF SHRIMP**

Just M. Vlak, *Wageningen University, NETHERLANDS*; Bui Thi Minh Dieu, *Can Tho University, VIETNAM*; Hendrik Marks, Angela Vermeesch, *Wageningen University, NETHERLANDS*; Tran Phuoc Duong, *Can Tho University, VIETNAM*; D. Zuidema, *Wageningen University, NETHERLANDS*

Abstract: White spot syndrome virus (WSSV, Nimaviridae), Taura syndrome virus (TSV, Dicistroviridae) and Yellow head virus (YHV, Roniviridae) are the most important viral pathogens affecting cultured shrimp. These viruses emerged in the last decade and quickly spread around the world. WSSV is a notorious scourge as it not only affects shrimp, but also other crustaceans such as crabs and crayfish. Intervention strategies are being sought to control virus diseases in shrimp, in particular against WSSV, and vaccination - although in its infancy - shows some promise (Witteveldt et al., 2004). The virus emerged in the early nineties near China and spread quickly through tide and trade in SoutEast Asia and beyond. WSSV is a sole member of the virus family Nimaviridae and contains a large, circular, double-stranded DNA molecule with 180 computational open reading frames (ORF). The function of about 25 ORFs is known, most of these either encoding structural virion proteins or enzymes involved in nucleotide metabolism and DNA replication. The genome is further characterized by the presence of multiple homologous repeat regions (hr's). WSSV isolates originating from Taiwan (WSSV-TW), China (WSSV-CN) and Thailand (WSSV-TH) show genotypic differences allowing the identification of each isolate (Marks et al., 2004). The variable loci were mapped by alignment of the genome sequence of these three WSSV isolates. These loci can be divided into deletions, differences in number of repeat units and SNPs. The variation within these loci suggests a recent geographical spread from a common ancestor. We hypothesize that a genetic gradient exists through the natural spread of WSSV over time from Taiwan to Thailand and onwards via China, Vietnam and Cambodia. Analysis of WSSV isolates obtained along the coast of Vietnam (WSSV-TH) showed that all contained the same but unique deletion in the variable region ORF23/24 and that this region can be used as a diagnostic marker for WSSV isolates. The existence of a genetic gradient was further investigated by analysis of the non-hr unidirectional tandem repeats dispersed along the viral genome. These repeats may be suitable as markers to study the spread of WSSV at the regional or local level. The potential of molecular genetics to understand WSSV epidemiology and ecology is discussed.

This research is supported by a Nuffic Training Grant (MHO-7) to BMD and, in part, by Intervet International, Boxmeer. E-mail: just.vlak@wur.nl

10:25 **THE ECOLOGY OF INVERTEBRATE IRIDESCENT VIRUSES (IRIDOVIRIDAE): RECENT ADVANCES**

Trevor Williams, *Univ. Publica Navarra, SPAIN*; Carlos F. Marina, *CIP, MEXICO*; Anaximandro Gomez, Alvaro Hernandez, *ECOSUR, MEXICO*; Peter Christian, *Nat. Inst Standards & Biol Contr., UK*

Abstract: Invertebrate iridescent viruses (IIVs) are little studied DNA viruses that infect invertebrates, especially insects, in damp and aquatic habitats. We review recent advances in four aspects of the ecology of these viruses. 1. Persistence - the persistence of Invertebrate iridescent virus 6 (IIV-6) has recently been studied in water and soil. The half life in soil was dependent on soil humidity and microbial activity. The persistence in water was reduced by exposure to sunlight, whereas the presence of sediment caused daily fluctuations in the virus titre. Binding to clay minerals was highly dependent on the type of clay tested, with extremely high affinity for bentonite and low affinity for kaolin. 2. Transmission experiments with Aedes aegypti larvae revealed that the overall prevalence of infection was positively influenced by host density and increased with exposure time. The transmission coefficient was 3 times greater at a high density than at a low density probably due to an increase in the frequency of aggression at high densities. Experiments with mosquito larvae exposed to IIV-6 in mixtures with an abrasive and optical brightener indicated that the insect midgut does not appear to be the principal site of infection. In contrast, laboratory and field experiments revealed that cannibalism was a highly efficient mechanism of transmission in Spodoptera frugiperda larvae. Introduction of the disease into experimental microcosms significantly reduced survival to pupation and emergence of adult moths. Parasitoid vectoring of IIV from infected to healthy hosts was demonstrated in an endoparasitoid of S. frugiperda but was not observed in an ectoparasitoid. The IIV was capable of infecting and killing developing parasitoid larvae. 3. Genetic heterogeneity in IIV populations is very evident although the factors that favour the persistence of such heterogeneity are presently unclear. 4. Sublethal effects are commonly observed in mosquitoes and Lepidoptera with covert (inapparent) IIV infections. We review the impact such infections on correlates of insect fitness (fecundity, longevity, body size, development rate, etc.) and the consequences of IIV infections in host populations.

10:50 **FUNCTIONAL IMPORTANCE OF DELETION MUTANT GENOTYPES IN A NUCELOPOLYHEDROVIRUS POPULATION**

Oihane Simón, Trevor Williams, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Miguel López-Ferber, *Laboratoire de Patologie Comparée, UMR 5087, INRA-CNRS-Université de Montpellier II, FRANCE*; Primitivo Caballero, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

Abstract: A nucleopolyhedrovirus (SfMNPV) that attacks the fall armyworm, Spodoptera frugiperda, survives as a complex mixture of genotypes. In vitro cloning and endonuclease analysis revealed that all variants presented genomic deletions, except variant B (complete genotype), whereas variants C and D were not infectious per os. All pure genotypes were less pathogenic than the wild-type isolate. Previous studies demonstrated a significant positive interaction between genotypes B and C, when coccluded in viral occlusion bodies, that restored pathogenicity to the level of the wild-type population. We compared the pathogenicity, speed of kill and time-mortality distribution of single genotypes (A, B, C, D, F) and coccluded genotype mixtures (B+A, B+D, B+F, A+C, F+C in a 3:1 ratio). Pure genotypes were all less effective than the wild SfNIC isolate, but differed markedly in their virulence (speed of kill) ranging from a maximum of 130 h for variant A, which did not differ from the SfNIC isolate (129 h), to a minimum of 89 h for variant F. The mixtures B+A, B+D, B+F showed increased pathogenicity or virulence, although only B+D restored the activity of the mixture to that of the natural population. Mixtures of two deletion variants (A+C, F+C) did not show interactions in pathogenicity or virulence. Clearly, the genes present in the deleted regions (currently being investigated) are likely to define the mechanisms underlying the observed changes in phenotype. A bimodal time to death distribution of the virus population suggests two conflicting outcomes of mixed infection. It appears that the minority genotypes, A and F, have an important influence on the overall virulence of the population. These results clearly demonstrate the importance of retaining genotypic diversity in virus biopesticide products.

11:15 **PERSISTENT INFECTIONS OF BACULOVIRUSES AND CYPOVIRUSES**

Rosie Hails, John Burden, *NERC Centre for Ecology and Hydrology, UK*; Clare Nixon, *School of Biological and Molecular Sciences, Oxford Brookes University, UK*; Rob Graham, *NERC Centre for Ecology and Hydrology, UK*; Steve Sait, *Centre for Biodiversity and Conservation, University of Leeds, UK*; Mike Bonsall, *Imperial College, London, UK*; Linda King, *School of Biological and Molecular Sciences, Oxford Brookes University, UK*; Robert Possee, *NERC Centre for Ecology and Hydrology, UK*

Abstract: The prevalence of pathogens in wild populations has often been estimated by the appearance of overt symptoms in the host, and this is typically used as the sole gauge of the impact of the pathogen on host dynamics. However, the development of molecular methods has increased the sensitivity with which we can detect asymptomatic infections. Here, we present evidence for asymptomatic, covert infections of both baculoviruses and cypoviruses in natural populations of three Lepidopteran species. These infections may persist for many generations, in some cases with undetectable impacts on host fitness. In a model laboratory system known to be free from persistent infections, survivors of a sublethal challenge were found to transmit baculovirus to subsequent generations. The ecological costs of carrying the sublethal infection were ameliorated after one generation, yet presence and expression of baculovirus genes persisted for five generations. We suggest that this is the route by which individuals become persistently infected in the field. Population models of host-pathogen interactions including persistent infections predict that under many conditions, persistent infections would become endemic, excluding susceptible clean hosts. This may explain the frequency with which we find persistent infections in the field. These results have broad ranging implications for our understanding of host pathogen interactions in the field.

Monday, August 2^{nd}, 2004
Time: 10:00 - 12:00, Lecture Room 10

Symposium (Cross-Divisional)

Honeybee pathology

Chair: Ingemar Fries

10:00 **MOLECULAR CHARACTERISATION OF THE EUROPEAN BUMBLE BEE MICROSPORIDIAN PARASITE NOSEMA BOMBI BASED ON RIBOSOMAL RNA AND BETA-TUBULIN GENES**

W. T. Tay, *School of Biology and Biochemistry, Queens University Belfast, UNITED KINGDOM*

Abstract: Microsporidian parasites have recently emerged as important pathogens in modern medical (e.g., in immuno-compromised individuals infected with the HIV virus) and agricultural settings (e.g., widespread in agriculturally important insects). In Europe, bumblebees are important pollinators in both natural ecosystems and for greenhouse crops (e.g., tomatoes and cucumbers). Bumblebees infected with microsporidian parasites may be asymptomatic or show a wide range of symptoms ranging from decline of worker pollination performance and queen mating ability to death of colonies. Because of their efficiency as greenhouse pollinators, commercial interests in trading bumblebee hives within the Europe Union is high, leading to great mobility of hives and therefore potential cross-infection of microsporidian parasites (e.g., from imported commercial bumblebee hosts to local bumblebee populations). Although widespread in various bumblebee hosts, only the microsporidian parasite Nosema bombi from Bombus terrestris has nevertheless been described so far, based on morphological characters such as size, cell wall structures and number of

polar filament coils. In this study, and in line with common practice, the ribosomal RNA (consisting of the small subunit (SSU-rRNA), the internal transcribed spacer (ITS) region, and the large subunit (LSU-rRNA)) DNA sequences of microsporidian parasites from different European Bombus species were sequenced to determine the levels of genomic differences. In addition, partial beta-Tubulin (-Tubulin) DNA sequences of Nosema parasites from respective hosts were also sequenced and characterised. The results from both rRNA and -Tubulin DNA sequences were analysed and the implications of characterising N. bombi based on a both rRNA and -Tubulin genes will be presented.

10:20 **FUNGAL DISEASES IN BEES: A STORY OF ASCOSPHAERA**

Rosalind James, Craig Huntzinger, Ellen Klinger, *USDA, ARS, Bee Biology & Systematics Laboratory, USA*; Jeff Skinner, *Oregon State University, USA*

Abstract: Chalkbrood is a disease of bee larvae caused by fungi in the genus Ascosphaera. Twenty-one species of Ascosphaera have been described to date, and all are associated with bees in one way or another. Ascosphaera apis it the most common species causing chalkbrood in honeybees. Although this disease can become quite prevalent in a honeybee colony, it is not usually serious because it can be fairly easily controlled using management techniques that increase colony strength in general. Chalkbrood can, however be a serious disease in the alfalfa leafcutting bee (Megachile rotundata, Megachilidae), causing mycosis in 20-50% of the larvae in managed populations. The alfalfa leafcutting bee is used extensively in the U.S. and Canada to pollinate alfalfa seed crops. An effective method for managing chalkbrood in this bee has not yet been found. Sanitation of nesting boards is of minimal effectiveness because emerging adult bees are heavily contaminated with spores already, and thus do not need to acquire them from the boards in order to transfer the pathogen to the larvae. For this reason, a method is needed to reduce the load of live spores on adults. We have screened some fungicides for activity against A. aggregata and for non-target effects on the leafcutting bees. In addition to A. aggregata, we found that other species are fairly common in the alfalfa leafcutting bee, even though A. aggregata has been attributed to the majority of chalkbrood cases in the literature. We have developed PCR markers that are genus- and species-specific for Ascosphaera, using the nine species that have been found on Megachilidae and A. apis. We hope to use these DNA markers to determine the occurrence and prevalence of different Ascosphaera species in alfalfa leafcutting bee populations.

10:40 **MOLECULAR AND BIOCHEMICAL DIFFERENTIATION BETWEEN PAENIBACILLUS LARVAE SUBSP. LARVAE AND PAENIBACILLUS LARVAE SUBSP. PULVIFACIENS.**

Elke Genersch, Ainura Ashiralieva, *Institute for Bee Research, GERMANY*; Jochen Kilwinski, *SVUA Arnsberg, GERMANY*

Abstract: Paenibacillus larvae subsp. larvae (P. l. larvae) is the etiological agent of American foulbrood (AFB), the most virulent bacterial disease of honey bee brood. In many countries, AFB is a notifiable disease since it is highly contagious, in most cases incurable, and able to kill affected colonies. For the correct and early laboratory diagnosis of AFB it is absolutely necessary to be able to unambiguously identify P. l. larvae and to discriminate between P. l. larvae and close relatives like Paenibacillus larvae subsp. pulvifaciens (P. l. pulvifaciens). The development of suitable methods for the differentiation between these two subspecies is hampered by the fact that they seem to be indistinguishable by cultural characteristics as well as by PCR protocols. Production of an orange pigment and a weak, delayed catalase-positive reaction was attributed to some strains of P. l. pulvifaciens only. Thus, colonies showing these characteristics were often ruled out as P. l. larvae. In order to find a reliable method to differentiate between these two subspecies we performed an extensive analysis of several P. larvae reference strains (DSM 7030 for P. l. larvae; DSM 3615, DSM 8442, DSM 8443 for P. l. pulvifaciens) and numerous field isolates of P. l. larvae originating from clinically diseased, AFB-positive hives. We employed conventional culture techniques as well as several molecular methods, like diagnostic PCR, rep-PCR, biochemical fingerprinting, and sequencing of 16S rDNA. We present evidence that P. l. pulvifaciens reference strain DSM 3615 is clonally related to P. l. larvae reference strain DSM 7030. Hence, this strain should be reclassified as P. l. larvae. Given that the correct classification of DSM 3615 is P. l. larvae our results indicate (a) that a negative catalase-test is not sufficient to identify P. l. larvae since some strains are also weak, delayed catalase-positive, (b) that an orange-pigmented colony morphology is not necessarily indicative for P. l. pulvifaciens since orange-pigmented variants are also possible with P. l. larvae, (c) that biochemical fingerprinting using the BIOLOG-system allows identification of P. l. larvae, and (d) that PCR-based methods (16S rDNA, 35-kD-metalloprotease gene) are a reliable means to rule out P. l. pulvifaciens and unequivocally identify P. l. larvae.

11:00 **SAMPLING OF ADULT BEES FOR DETECTION OF AMERICAN FOULBROOD (PAENIBACILLUS LARVAE SUBSP. LARVAE) SPORES IN HONEY BEE (APIS MELLIFERA) COLONIES**

Anders Lindström, Ingemar Fries, *Swedish University of Agricultural Sciences, Department of Entomology., SWEDEN*

Abstract: Fifty nine apiaries with a total of 489 honey bee colonies in a beekeeping operation with a previous history of American foulbrood (AFB) were inspected outside Uppsala, in central Sweden. In each colony a visual inspection of the clinical disease status was made, and the number of brood cells clinically diseased by AFB were estimated. During the visual inspections in 94 of these colonies, we collected individual samples of >100 live honey bees from the brood room and the supers respectively. Furthermore, two composite samples consisting of >100 adult bees from each individual colony in each inspected apiary were also taken, from 1) all brood boxes and 2) all supers respectively. We took samples on two levels, the individual colony and the apiary. The samples were analyzed in the lab for presence of Paenibacillus larvae subsp. larvae using microbiological techniques. Spores were found in all bee samples from clinically diseased colonies and often in colonies without clinical symptoms, in particular in apiaries with one or more clinically diseased colonies. There was only a slight difference in spore load between bees in brood boxes and in supers, which indicates that from a practical point of view, the quicker sampling of adult honey bees in supers can be used when screening apiaries for AFB. No clinically diseased colonies were negative when adult bees were investigated for the bacteria. No apiary, where at least one clinically diseased colony was present, was negative in composite bee samples.

11:20 **INVESTIGATING INTERACTIONS BETWEEN VARROA DESTRUCTOR, VIRUSES AND HONEY BEES**

Brenda Ball, Judith Wilson, Norman Carreck, *Rothamsted Research, UK*

Abstract: Honey bees are hosts to a large number of serologically distinct small ssRNA viruses most, if not all of which persist in populations as latent or inapparent infections. There are a number of different factors that naturally limit the transmission of individual viruses within colonies and damaging overt infections causing disease outbreaks are uncommon. However, the world-wide distribution of the honey bee parasitic mite Varroa destructor has had a significant impact on the type and prevalence of viruses causing mortality in infested colonies as many of these control mechanisms have been overcome. The adult female mite feeds on the haemolymph of both adult bees and brood and can act as an efficient vector of a number of unrelated honey bee viruses. This host-parasite-pathogen association provides a unique opportunity within an arthropod system to investigate further the nature of these interactions. Field studies in the UK on the causes of mortality in honey bee colonies in areas where the mite had recently become established determined that the death of both adult bees and brood was primarily due slow paralysis virus (SPV) infection. This virus was known only as an inapparent infection and had never previously been found to be responsible for mortality in nature. Activation of SPV multiplication has been demonstrated in the laboratory by the injection of foreign protein. Analysis of individual live adult bees, brood and mites by ELISA from these naturally infested colonies provided further insight into virus dynamics. Laboratory experiments designed to test the ability of V.

destructor to transmit SPV and Kashmir bee virus (KBV) to both adult bees and brood showed that honey bee pupae were very susceptible to infection and mites were efficient vectors. However, although both viruses are rapidly fatal by injection and few particles are required to cause infection, surviving experimental adult bees and pupae surprisingly contained significant amounts of virus. These elevated levels of virus in surviving individuals in natural circumstances could provide a reservoir of infection in the honey bee population which mites could activate and then transmit.

11:40 **VARROA MITES (VARROA DESTRUCTOR) AND HONEY BEES (APIS MELLIFERA) A DYNAMIC RELATIONSHIP**

Ingemar Fries, *Department of Entomology, Swedish University of Agricultural Sciences, SWEDEN*

Abstract: In an isolated site on Gotland, in the Baltic sea, we have studied the development of Varroa mite (Varroa destructor) population development and honey bee colony mortality in non-managed colonies (N=150) without any mite control for over 4 years. Swarming of bee colonies reduce the mite burden in swarming colonies only at medium to low infestation levels (< 0.35 mites per bee). When heavily infested colonies (>0.35 mites per bee) manage to swarm the effect from swarming on mite population is not pronounced, probably because the swarming per se creates better breeding conditions for the mites compared to heavily infested colonies that do not manage to swarm because of mite damages. Furthermore, swarms from infested colonies do not have better survival chances than swarming colonies, although most mites remain in the brood as the bee population divides during swarming. Some colonies (N=8) still remain alive 5 breeding seasons post mite introduction. Data suggest that one explanation for prolonged survival may be a dynamic relationship between the mite and the bee population. Data demonstrate that heavily infested colonies have a significantly reduced chance of surviving the winter. If heavily infested colonies do survive the winter, the bee cluster is likely to be small sometimes only 1000-1500 bees. Analysis of infestation level in such colonies demonstrate that most mites die with their hosts under such circumstances. Such colonies are then able to outgrow the mite population and produce colonies which are strong for the next winter, with moderate mite infestations. The next season, when more bees manage to survive the winter in such colonies, they will again be heavily infested in the fall, with increased risk of colony collapse over winter. Or possibly surviving with only a small number of bees. Further work has been initiated to investigate if mites may be less virulent or bees may be more mite tolerant in the remaining bee population, compared to the bee and mite material they originate from.

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 6

Contributed Papers (Division of Viruses)

virus / contributed paper session 1

Chair: H. J. R. Popham; K. Hoover

13:30 **THE PERITROPHIC MATRIX AS A BARRIER TO FATAL BACULOVIRUS INFECTION IN COTTON-FED HELIOTHIS VIRESCENS**

Ruth Plymale, Diana Cox-Foster, Dan Jones, Kelli Hoover, *Penn State University, USA*

Abstract: It has been well documented that Heliothis virescens larvae fed cotton foliage shortly before oral inoculation with occlusions of Autographic californica nucleopolyhedrovirus (AcNPV) experience decreased viral mortality compared with larvae fed on lettuce or artificial diet. We hypothesize that one of the mechanisms responsible for this difference involves diminished permeability of the peritrophic matrix (PM) to occlusion derived virus, reducing the establishment of primary midgut infections in cotton-fed insects. To test this hypothesis, we fed enhancin, a metalloprotease packaged in granules of Trichoplusia ni GV known to disrupt the PM in several lepidopteran species, to fourth instar H. virescens prior to inoculation with AcNPV. Enhancin increased mortality by AcNPV in cotton-fed larvae to the same level as mortality in diet-fed larvae. Although enhancin also increased mortality in diet-fed insects, it was not statistically significant, suggesting that the PM is not a substantial barrier to AcNPV infection in diet-fed H. virescens. Western blots from PM surrounding frass collected from these larvae showed that enhancin removed mucin from the PM, indicating that the PM had in fact been disrupted in these insects. Detailed pathogenesis studies designed to document the impact of enhancin on the timing and number of midgut and tracheal infections are underway. To date, our results support the hypothesis that inhibition of baculoviral disease in cotton-fed insects involves reduced permeability of the PM to occlusion-derived virus.

13:45 **SELENIUM IMPACTS THE INFECTIVITY OF ACMNPV IN TRICHOPLUSIA NI**

Holly Popham, *USDA ARS Biological Control of Insects Research Laboratory, UNITED STATES*; Kent Shelby, *USDA ARS Biologcial Control of Insects Research Laboratory, UNITED STATES*

Abstract: Herbivorous insects encounter a range of dietary nutrients, antioxidants, co-factors and plant secondary metabolites which may modulate their resistance to microbial infections. A colony of the lepidopteran pest insect Trichoplusia ni has been maintained at BCIRL for generations on an artificial diet with no added Se. These depleted or low Se, insects grow and reproduce normally. Supplementation of the diet of these Se-depleted larvae with 10 ppm or less Sodium Selenite resulted in no deleterious effects on larval growth. Larvae were reared on three different regimes of increasing levels of Se: 1) Se throughout their larval development; 2) Se depletion until the onset of the fourth instar then repletion of Se; and 3) Se up to the fourth instar followed by Se depletion. Selenium levels of pupae from Se fed larvae showed increasing levels depending on the amount of Se added to the diet while larvae fed Se until the fourth instar displayed the same amount of Se in all groups tested. Larvae reared on the three regimes were infected per os with increasing doses of the baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV). LC50s were not significantly different between control larvae and larvae fed 5 and 10 ppm Se, except larvae fed Se until the fourth instar and then moved to control diet. These larvae had 10 fold higher LC50 when fed 10 ppm Se. This study indicates that dietary selenium levels do impact the infectivity of AcMNPV in Selenium-depleted T. ni.

14:00 **INACTIVATION OF PHTHORIMAEA OPERCULELLA GRANULOVIRUS (POGV) DUE TO NATURAL RADIATION AND THE POTENTIAL OF UV-ADJUVANTS FOR VIRAL PROTECTION**

Marc Sporleder, Jürgen Kroschel, *International Potato Center (CIP), PERU*; Jürg Huber, *Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, GERMANY*; Octavio Zegarra, *International Potato Center (CIP), PERU*; Aziz Lagnaoui, *Environmentally and Socially Sustainable Development, The World Bank, USA*

Abstract: The potato tuber moth, Phthorimaea operculella Zeller (Lepidoptera: Gelechiidae) can be controlled by a granulovirus (PoGV). While virus applications in stored potatoes have had notable success, PoGV use in the field is limited by a rapid inactivation due to solar (UV-) radiation. The objective of our study was to assess the inactivation of PoGV at dif-ferent intensities of natural solar irradiation in Lima, Peru, and in the Peruvian Andes at various altitudes. Dry deposits of PoGV were exposed to the sun for different time inter-vals and bioassayed using an egg-dip method. During exposure a pyranometer and a UV-B sensor were used to measure intensity and energy of visible light (400 to 1100 nm) and erythermal light (265-315 nm), respectively. Inactivation (half-life) was determined in function of exposure time and accumulated exposure energy. Inactivation occurred as an initial steep decline followed by a slower decay. This was best described by a two-component (bisegmented) model incorporating two separate exponential curves. Initial inactivation curtailed when approximately 98% of the virus was inactivated. Thereafter, inactivation was 4.4 times

slower. The two-component model, modified by a coefficient for altitude, could be successfully fitted to the total data set. With each 100m rise in alti-tude, the speed of inactivation increased by 2.8%, while the proportion of UV-B in sunlight increased by only 1.4%. The method described above was also used to evaluate the protective capacity of UV-adjuvants. Several dyes, antioxidants, the optical brightener Tinopal LPW, and two types of formulations (a wettable powder [WP] and a soluble concentrate [SC], each with and without UV screen) were examined. The dye Congo Red was detrimental to viral activity. Tinopal LPW enhanced the viral activity at concentrations above 0.01% by factors rang-ing from 8.6 to 134. Higher mortalities obtained from irradiated PoGV with Tinopal in comparison to unprotected PoGV can be attributed to enhancement of remaining viral ac-tivity rather than to increased viral persistence. Compared to unprotected virus, the anti-oxidants propyl gallate and phenylthiocarbamide increased the viral activity (LC50) by factors of 18 and 6, and increased viral stability (t) 2- and 3-fold, respectively. PoGV with host derived material (macerated virus-infected larvae) displayed 6-fold increased stability compared to purified PoGV. Virus formulated as WP had 1.4 to 2.5 times greater persistence than unprotected PoGV. SC formulations, on the other hand, did not significantly alter viral stability.

14:15 **HORIZONTAL AND VERTICAL TRANSMISSION OF WILD-TYPE AND RECOMBINANT HASNPV**

Xiulian Sun, Mingzhe Zhou, *Wuhan Institute of Virology, Chinese Academy of Sciences,Wuhan, 430071, Hubei, CHINA*; Wopke Van der Werf, *Crop and Weed Ecology Group, Wageningen University, THE NETHERLANDS*; Just M. Vlak, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Zhihong Hu, *Wuhan Institute of Virology, Chinese Academy of Sciences,Wuhan, 430071, Hubei, CHINA*

Abstract: Transmission of baculoviruses plays an important role in their ecology and the population dynamics of their hosts. We studied the horizontal and vertical transmission of wild-type Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus (HaSNPV-wt) and a genetically modified variant (HaSNPV-AaIT) with enhanced speed of action through the expression of an insect-selective scorpion toxin (AaIT). While using inoculated 1st to 3rd instar larvae as infectors, the horizontal transmission rates of both HaSNPV variants were the highest with 3rd instar larvae and the lowest with 1st instar larvae. Transmission was greater at higher density of the infectors. HaSNPV-AaIT had a significantly lower rate of horizontal transmission than HaSNPV-wt. In the laboratory, the vertical transmission rate of HaSNPV-AaIT from infected females to offspring was 16.7 2.1%, which was significantly lower than that of HaSNPV-wt (30.9 2.9%). No vertical transmission from males was observed. Likewise, in the field, vertical transmission of HaSNPV-AaIT (8.4 1.1%) was significantly lower than of HaSNPV-wt (12.6 2.0%). Data obtained in this study provide a basis for building an epizootic model of wild-type and recombinant HaSNPVs in cotton and aids in the risk assessment of using genetically modified baculoviruses as bioinsecticides.

This research is supported by 863 grants (2001AA214031 and 2001AA212301), CAS grants (KSCX2-1-02 and Kscx2-SW-301-09), an NSFC grant (30025003) and a grant from the KNAW (01CDP023). E-mail: xiulian.sun@wur.nl.

14:30 **THE POLYHEDRIN GENE OF THE AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDRO-VIRUS IS A MOSAIC OF GROUP I AND GROUP II NPV POLYHEDRIN GENES**

Johannes A. Jehle, *Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate, GERMANY*

Abstract: The polyhedrin (polh) gene of nucleopolyhedroviruses (NPVs) encodes for the matrix protein of the virus occlusion body and is one of the most conserved baculovirus genes. Previous analyses of different NPV genes and polh genes provided conflicting results indicating that the Autographa californica nucleopolyhedrovirus (AcMNPV) is generally a member of the so-called group I NPVs and is most closely related to Rachiplusia ou (Ro) NPV, whereas the AcMNPV polh is more similar to the polh of the group II NPVs. A comparative analysis of the AcMNPV polh and its closest neighbours within group I and group II NPV, the RoMNPV and the Thysanoplusia orichalcea (Thor) NPV, was performed using Hidden Markov Models for detecting recombination. The analyses provided strong evidence that the AcMNPV polh is a chimerical gene which consists of a mosaic of group I and group II NPV specific sequences. The sites of recombination were located.

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 1

Symposium (Division of Nematodes)

Nematodes and cold adaptations

Chair: Patricia Stock

13:30 **COLD TOLERANCE STRATEGIES OF ENTOMOPATHOGENIC NEMATODES**

Ian M. Brown, *Biology, Georgia Southwestern State University, U.S.A.*; Randy Gaugler, *Entomology, Rutgers University, U.S.A.*

Abstract: Under optimal conditions, infective juveniles of entomopathogenic nematode(Rabditida: Steinernematidae, Heterorhabditidae) find, penetrate and quickly kill their hosts. Environmental extremes such as low and freezing temperatures force this free-living stage to postpone infection activities and adopt various cold tolerant survival strategies. Infective juveniles can survive temperatures above freezing (0aC) within either the cadaver of a dead host or in a live host. At 5aC, Steinernema carpocapsae survived in the cadaver of the wax moth Galleria mellonella for at least 12 days beyond the emergence time at 25aC (9 days post-infection). On transfer to 25aC, 100% of cadavers showed infective juvenile emergence. When exposed to suboptimal temperature regimes infective juveniles may also penetrate hosts and remain in a latent state until more favorable conditions occur. Latent infections have been documented in S. carpocapsae, S. riobrave and in G. mellonella and Heterorhabditis bacteriophora in G. mellonella and the grubs of Japanese beetle, Popillia japonica and the oriental beetle, Exomala orientalis. Infective juveniles are also capable of freezing survival. Lower lethal temperatures and prolonged freezing times at -4aC have been recorded for six steinernematid and heterorhabditid species. Infective juveniles of S. riobravis, S. carpocapsae S. feltiae, H. bacteriophora, and S. glaseriand S. anomali survived 19,15, 6, 5, 3, and 2 days at -4aC for periods respectively. S. riobravis, S. carpocapsae were still pathogenic after 6 days freezing, S feltiae, S.glaseri and S. anomali were pathogenic for 4, 3 and 2 days respecitively. Lower lethal temperatures for the S. feltiae, H. bacteriophora, and S. anomali were, -22, -19, and -14 respectively. Our data demonstrate that entomopathogenic infective juveniles exhibit various low temperature and freezing survival strategies under laboratory conditions. Therefore the potential for infective juveniles to overwinter in extreme environments is plausible.

13:50 **PHYSIOLOGY OF NEMATODE COLD TOLERANCE**

Parwinder S. Grewal, Ganpati B. Jagdale, *Department of Entomology, Ohio State University, U.S.A.*

Abstract: Entomopathogenic nematodes have been isolated from a wide range of habitats, where they face a challenge of daily and seasonal temperature fluctuations. We explored biochemical changes and consequent environmental tolerance of cold-adapted Steinernema feltiae, an intermediate S. carpocapsae, and warm-adapted S. riobrave during recycling or acclimation to different temperatures. Fatty acid composition of total lipids and phospholipids changed adaptively with recycling temperatures. The unsaturation indices of lipids increased as temperature decreased. Recycling temperatures also influenced the activities of glucose-6-phosphate dehydrogenase and hexokinase in an adaptive fashion. Isozyme patterns of malate dehydrogenase (MDH), mannose-6-phosphate isomerase (MPI) and phosphoglumutase (PGM) were also affected. S. feltiae synthesized additional isozymes of MPI, MDH and PGM in response to cold temperatures while S. carpocapsae synthesized isozymes of MDH in response to warm temper-

atures. All three species accumulated trehalose when acclimated at either 5 or 35oC, but the amount of trehalose accumulation differed by species and temperature. S. riobrave and S. carpocapsae accumulated high levels of trehalose when acclimated at 35oC, and S. feltiae at 5oC. Heat tolerance increased in acclimated S. carpocapsae and S. feltiae, but not in S. riobrave. Freezing tolerance increased in acclimated S. carpocapsae and S. riobrave, but not in S. feltiae. Desiccation tolerance of S. feltiae in 25% glycerol at both 5 and 35oC was enhanced by both cold and warm acclimation and the enhanced desiccation tolerance was positively correlated with the acclimation induced trehalose accumulation. At 5oC, desiccation tolerance of S. carpocapsae was enhanced by either cold or warm acclimation, but at 35oC it was increased by only cold acclimation. Similarly, at 5oC, desiccation of S. riobrave was enhanced either by cold or warm acclimation, but at 35oC, it was increased only by warm acclimation.

14:10 **STU** **SEASONAL DYNAMICS OF ENTOMOPATHOGENIC NEMATODES OF THE GENERA STEINERNEMA AND HETERORHABDITIS AND THEIR INSECT HOSTS, WITH COMMENTS ON THE WINTER PERIOD**

Vladimir Puza, Zdenek Mracek, *Institute of Entomology, Czech Academy of Sciences, CZECH REPUBLIC*

Abstract: Even though a number of field surveys have been carried out, the population biology of entomopathogenic nematodes (EPNs) under natural conditions is still poorly understood. In present study, seasonal dynamics of entomopathogenic nematodes of the genera Steinernema and Heterorhabditis in the interaction with their insect hosts abundance and soil temperature and moisture was studied during one season (2002) in meadow and oak wood habitat in the vicinity of eské Budìjovice. Additionally the abundance of entomopathogenic nematodes and insect hosts was observed in the part of the winter period in February and March 2004 in the oak wood habitat. EPN abundance was assessed by Galleria baiting method while insects were quantified using Tullgren's apparatus. Four entomopathogenic nematode species were found during the investigation. Steinernema affine dominated in both habitats. Moreover, oak wood was inhabited by S. kraussei and S. weiseri while meadow by Heterorhabditis bacteriophora. The mean abundance of total EPN community was 28 000 ind.m-2 in oak wood and 11 000 ind.m-2 in meadow. Winter nematode abundances in the oak wood habitat were about 4 000 ind.m-2. The host range of entomopathogenic nematodes in both habitats was formed predominantly by larvae of dipteran and coleopteran families, particularly Asilidae or Empididae (Diptera) and Carabidae or Curculionidae (Coleoptera). Seasonal dynamics of entomopathogenic nematodes in both habitats was characteristic by high nematode densities in the beginning of the season, followed by rapid decrease and stabilization. Nematode abundance did not show any apparent correlation with soil temperature and moisture during the season, but it was significantly negatively correlated with abundance of suitable insect hosts. These insects were infrequent in spring and most abundant in autumn. Winter nematode and insect abundances did not fluctuate apparently. Competition and parasitisation probably played major role in nematode and suitable insect seasonal dynamics: high nematode and low insect densities at the beginning of the season probably led to severe competition and nematode density decreased. Then insect numbers arose and the balance between nematode and insect numbers in the following part of the season was established. Low nematode abundance in winter period and discrepancy between the high spring and low autumn nematode abundances (and an inverse state in insect numbers) may be explained partly by overwintering of nematodes in insect bodies. However, further investigation is needed.

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Lecture Room 12

Contributed Papers (Division of Microbial Control)

microbial control / contributed paper session 1

Chair: Lawrence Lacey; Shawn McLaughlin

13:30 **EVALUATION OF COMMERCIAL FORMULATIONS OF THE CODLING MOTH GRANULOVIRUS AGAINST NATURAL CODLING MOTH INFESTATIONS IN PACIFIC NORTHWEST APPLE AND PEAR ORCHARDS**

Steven Arthurs, Lawrence Lacey, *USDA-ARS, USA*

Abstract: Inundative applications of the codling moth (CM), Cydia pomonella L., granulovirus (CpGV), which target neonate larvae before or during initial entry into fruit, offer potential for selective control of this key pest. In field tests on apple we compared the persistence and efficacy of single applications of three CpGV products approved for organic orchards in North America. In addition the success of repeated (2-14) applications of one product (Cyd-X) as a principal control measure for CM in apple orchards was monitored following operational use by cooperating growers at four separate locations. In the first study, an early season application of all products at label rates remained highly effective for the first 24 hours (averaging 94% larval mortality relative to controls) and moderately effective after 72 hours (averaging 71% mortality) during dry sunny conditions. Significant activity remained up to 14 days, suggesting prolonged survival of the virus in UV-protected locations, such as the calyx of fruit. A second application later in the season was slightly less effective. Data obtained from commercial sites provides circumstantial evidence for the effectiveness of well-timed CpGV applications against CM outbreaks. In all cases where 1st generation larvae were targeted beginning at egg hatch (Ö 250 degree days) and treated areas monitored (0.3 - 1.6 ha plots), fruit damage during 2nd generation was reduced or eliminated. Based on the number of live larvae recovered throughout the season, mortality rates remained high (80.3 C 100% across sites). The cumulative number of moths caught in pheromone-baited traps was reduced (66-94%) in the second flight. Data from tree bands placed to catch diapause-destined larvae indicated overwintering generations in treated sites remained low (Ü 0.18 larvae/band). Experiments are currently underway in 2004 to compare the impact of CpGV and spinosad on nontarget organisms in apple and pear.

13:45 **CONTROL OF THE BROWNTAIL MOTH, EUPROCTIS CHRYSORRHOEA, IN THE UNITED STATES WITH A BACULOVIRUS**

James Slavicek, *USDA Forest Service, USA*; Joseph Elkinton, *University of Massachusetts, USA*; John D. Podgwaite, *USDA Forest Service, USA*

Abstract: The browntail moth, Euproctis chrysorrhoea, was introduced into the United States in 1867. We performed studies in the spring and fall of 2003 to determine if the Euproctis chrysorrhoea nucleopolyhedrovirus (EcNPV) could be used as an effective browntail moth control agent. EcNPV was added to a lignosulfonate-based formulation and applied to test trees at a rate of 5 x 1012 polyhedra/ha. Larvae were collected from ten test apple trees and two control apple trees prior to virus application. Five trees were sprayed on May 7 and five additional trees on May 20. The same formulation was used in an application on September 8 to branch tips on oak, cherry, and hawthorn trees with winter webs. Larvae were collected 1, 2, 3, and 4 weeks after the May 7 and May 20 applications and reared until death or pupation. Larvae were collected 1 and 2 weeks after the fall virus application and reared until death or the reformation of the winter web. Webs were collected 7 weeks after the fall application, opened, the number live and dead larvae counted, and the dead larvae were inspected for the presence of EcNPV. No virus mortality was observed in the pretreatment larvae. EcNPV mortalities ranged from 75-85 % in larvae collected from trees treated on May 7, and from 8288 % in larvae collected 1-3 weeks and 50% in larvae collected 4 weeks after the May 20th virus treatment. Mortality levels of an average of 62% and 55% were found in larvae collected 1 and 2 weeks, respectively after the September 8th virus application. Mortality on larvae from oak and cherry trees was similar ranging from 70% to 82% and 60% to 80%, respectively. In contrast, mortality in larvae from hawthorn was less, ranging from 30% to 35%. An average of 94% of the larvae were alive in the control webs collected 7 weeks after the September 8th virus application, and no virus was found in the dead larvae. In virus treated nests an average of 60% of the larvae were alive, and 77% of the dead larvae contained EcNPV. Overall, these results suggest that the EcNPV could be an effective browntail moth control agent. Spring

application of virus gave very high levels of browntail moth control. Fall application of EcNPV gave good levels of control; however, once the final results are obtained in 2004 the fall application may prove to be the most effective time for treatment.

14:00 **DEVELOPMENT OF SPODOPTERA EXEMPTA NUCLEOPOLYHEDROVIRUS (SPEXMNPV) FOR THE CONTROL OF AFRICAN ARMYWORM IN EAST AFRICA**

David Grzywacz, Mark Parnell, *Natural Resources Institute, UK*; Wilfred Mushobozi, *Pest Control Services, TANZANIA*; Ken Wilson, *Lancaster University, UK*

Abstract: The African armyworm Spodoptera exempta is a major migratory crop pest over much of Eastern and Southern Africa. Tanzania is a focal point for primary outbreaks of armyworm that attack both pasture and grain crops and are a serious threat to the food security of farmers and subsistence growers. Control to date has largely depended upon the use of chemical insecticides but this paper reports on progress in utilising the homologous baculovirus of this species as an alternative biological pesticide for strategic control. A research project has been initiated to explore alternative non chemical controls for armyworm, including the indigenous armyworm NPV.
The SpexMNPV virus occurs widely during major outbreaks of armyworm but normally it appears too late in the pest cycle to prevent serious damage to crops and rangeland. A stock of a SpexMNPV strain originally isolated from East Africa was produced at NRI and has been used to conduct a series of field trials in Tanzania to evaluate its potential in controlling armyworm outbreaks. Small scale field trials in 2001 and 2002 showed that SpexMNPV can be as effective as chemical insecticide in destroying armyworm outbreaks when applied early to outbreaks of larvae. Application of 1x1012 occlusion bodies of SpexMNPV per hectare using standard ground application equipment to armyworm outbreaks on pasture initiate major outbreaks of NPV disease and population collapses within 4-5 days. Trials in 2004 repeated the successful ground trials and also completed successful aerial application trials on pasture. The data from these trials indicate that NPV can be used to control armyworm outbreaks and could be a viable replacement for the use of chemical insecticides in Tanzania and other parts of Eastern and Southern Africa.
The project is also exploring the potential field production of SpexMNPV as a low cost method for local mass production of this agent. Related studies of the ecological role of SpexMNPV in the population ecology of African armyworm are also underway as part of this initiative and should illuminate our understanding of the role that NPV may play in the population dynamics of this pest.

14:15 **IMPACT OF DISEASES ON SOFTSHELL CLAM (MYA ARENARIA) POPULATIONS**

Shawn M. McLaughlin, *NOAA National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research/Cooperative Oxford Laboratory, U.S.A.*

Abstract: Large softshell clam abundances located subtidally in the Chesapeake Bay became an important commercial fishery in the 1950's upon the development of the escalator dredge. A steady decline of landings over the last few decades has been associated with natural and anthropogenic factors including mortalities caused by disease. The most well studied diseases of the softshell clam are proliferative in nature; namely disseminated sarcomas and gonadal neoplasms. Disseminated sarcomas have been linked with severe epizootics of juvenile and adult softshell clam populations in the Chesapeake Bay since the early 1980's. Mortalities of softshell clams have only recently been associated with Perkinsus sp., a parasite rarely observed in softshell clams before 1990. Mya arenaria is also susceptible to infection by other potential pathogens that have been little studied. For example, rickettsia-like organisms (RLOs) have been reported in digestive diverticula and gills of softshell clams with no apparent host effects. Studies of softshell clams collected from several sites in the upper Chesapeake Bay revealed the presence of two morphologically distinct RLOs in digestive diverticula. Prevalence and infection intensity of the RLOs appeared to increase in the fall. Differences in host responses to infection were also observed. Large numbers of Ancistrocoma sp. ciliates adjacent to gills have been reported to cause harm in clams in the presence of stressors. Interestingly, a ciliate-like organism was found in the hemolymph of some softshell clams. Historical and current impacts of potential pathogens on softshell clam populations of the Chesapeake Bay will be presented.

14:30 **MICROBIAL CONTROL OF VARROA: FIELD ADVENTURES**

Rosalind James, Craig Huntzinger, Ellen Klinger, *USDA-ARS Bee Biology and Systematics Laboratory, U.S.A.*

Abstract: Varroa destructor is a mite that is parasitic to honey bees and is a serious pest in beekeeping operations wherever Apis mellifera is used. Chemical control methods can be successful, but have been met with the development of pesticide resistance in the mite. We have been investigating the use of the fungi Metarhizium anisopliae and Hirsutella thompsonii as microbial control agents for this pest. Some strains of H. thompsonii are very pathogenic in the laboratory, but have proved ineffective in the field due to difficulties in production and formulation of the spores, most likely because of their mucous coating. M. anisopliae gives lower infection rates in the laboratory, but is more effective in the field. We report on our field trials and application strategies with these fungi, including two strains of M. anisopliae that are being considered for commercial production.

Monday, August 2^{nd}, 2004
Time: 13:30 - 14:45, Corridor, II and III levels

Poster Session 1: Posters for fungi and bacteria

B-1 **ACTIVITY OF BACILLUS THURINGIENSIS TOXINS AGAINST COCOA POD BORER LARVAE**

Tetty Chaidamsari, *Plant Research International, NETHERLANDS*; Djoko Santoso, *Biotechnology Research Institute for Estate Crops, INDONESIA*; Soekadar Wiryadiputra, *Indonesian Coffee and Cacao Research Institute, INDONESIA*; Ruud De Maagd, *Plant Research International, NETHERLANDS*

Abstract: Cocoa pod borer (CPB) (Conopomorpha cramerella), is a serious insect pest for the cocoa tree throughout Southeast Asia. Larvae of this insect bore into the pod after hatching and cause the pods of the cocoa tree to ripen prematurely, with serious effects on bean production. This pest is difficult to control with conventional measures. In our collaborative project we aim at expressing insecticidal (Cry) proteins from Bacillus thuringiensis (Bt) in the pod walls of transgenic cocoa trees to make these resistant to pod borer larval attack. Crystal (Cry) proteins from Bt are formed during sporulation of this bacterium and form a large family of homologous proteins with different insecticidal activities. Bt has a long history of safe use as biological control agent in agriculture. More recently we have seen the commercialization of crop plants made resistant to insect pests by expression of Cry proteins through genetic modification. To test whether this strategy might work for CPB, we first tested activity of Cry proteins to the larvae of this insect. Twelve Cry proteins from Bt were tested in bioassays on cacao plantations in Indonesia for activity against the larvae of cocoa pod borer. Preliminary assays identified five toxins that were more active than others. In two subsequent bioassays the activity of some toxins was determined more accurately. Three Cry1 proteins with relatively little homology were all found to be toxic, opening perspectives for controlling cocoa pod borer by expression of Cry proteins in transgenic plants.

Part of this work was supported by the BIORIN Programme (Scientific Programme Indonesia-Netherlands) of the Royal Netherlands Academy of Arts and sciences (KNAW), and an Indonesian International Joint Research Grant (RUTI) of the Ministry Research and Technology of the Republic of Indonesia

B-2 **INTERACTION BETWEEN P20 AND CYT1AA IN VIVO USING THE TWO-HYBRID SYSTEM OF SACCHAROMYCES CEREVISIAE**

Olga Burgazliev, Robert Manasherob, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

Abstract: The insecticidal crystal proteins of Bacillus thuringiensis subsp. israelensis (Bti) include four major polypeptides, Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa. The latter is the major -endotoxin protein (50% of total crystal). It is not homologous to and less specific than the Cry toxins, but is haemolytic and cytotoxic in vitro. Expressing cyt1Aa in Escherichia coli arrests biomass growth and reduces viability by 4 orders of magnitude. This lethal affect of Cyt1Aa on E. coli is abolished by co-expression with p20. P20 is a Bti-encoded helper polypeptide that stabilizes Cyt1Aa. Testing the hypothesis that the two proteins physically interact in vivo, the yeast two-hybrid interaction trap was used. As corollaries, the effect of Cyt1A on eukaryotic cells and the potential of Saccharomyces cerevisiae as a bio-pesticide will be studied.

Student Poster

B-3 **AN ATTEMPT TO IMPROVE MOSQUITO LARVICIDAL ACTIVITY OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS**

Nadine Sela-Baranes, Robert Manasherob, Eitan Ben-Dov, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

Abstract: Attempts to isolate a strain of Bacillus thuringiensis (Bt) with higher mosquito larvicidal activity than that of subsp. israelensis (Bti) have not been successful to date. Among the major -endotoxin proteins of Bti, Cry11Aa is less active than two similar proteins, Cry11Ba (of Bt jegathesan) and Cry11Bb (of medellin). It is anticipated that replacing cry11Aa by a gene for one of the latter will raise the potential of Bti as a mosquito bio larvicide. Such a new composite may, in addition, contribute to regaining sensitivity among resistant populations. A collection of field-isolates includes at least 22 strains toxic against larvae of Aedes aegypti. A pair of universal primers were designed from conserved sequences, and used to identify strains containing cry11. A pair of cry11Bb-specific primers amplified an appropriate fragment from the DNA of one of the 11 cry11-positive strains. Cloning for expression of this new cry11Bb-like gene together with various combinations of the genes encoding the major crystal proteins of Bti, cry4Aa, cry4Ba, cry11Aa and cyt1Aa, should increase toxicity and delay appearance of resistance.

Student Poster

B-4 **LARVICIDAL ACTIVITY OF TRANSGENIC ESCHERICHIA COLI EXPRESSING TOXIN GENES FROM BACILLUS THURINGIENSIS TO SUSCEPTIBLE LEPIDOPTERA**

Maria Menin, *Ben-Gurion University of the Negev, ISRAEL*; Vadim Khasdan, *Dept. of Entomology, ARO, Gilat Research Center, ISRAEL*; Eitan Ben-Dov, Robert Manasherob, Sammy Boussiba, *Ben-Gurion University of the Negev, ISRAEL*; Rami Horowitz, *Dept. of Entomology, ARO, Gilat Research Center, ISRAEL*; Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

Abstract: The gene cry1Ac of Bacillus thuringiensis subsp. kurstaki was introduced into previously constructed Esherichia coli clones expressing cyt1Aa and p20, encoding, respectively, cytolytic and accessory proteins of B. thuringiensis subsp. israelensis. Seven clones with all possible combinations of the three genes were obtained and found to express the genes included. Cyt1Aa, produced with and without P20 in E. coli, lacks toxicity towards susceptible larvae of Cotton Bollworm (Helicoverpa armigera) and Pink Bollworm (Pectinophora gossypella) in bioassays performed with leaves and artificial diet bioassays, respectively. Only two clones, pMVER-A and pMVER-ARCyt, expressing cry1Ac without and with p20-cyt1Aa, respectively, displayed toxicity to Lepidopteran larvae. Toxicity of pMVER-A against susceptible larvae of H. armigera and P. gossypella was highest. Cyt1Aa thus seems to antagonize the toxic activity of Cry1Ac toward susceptible Lepidopteran larvae, but the question whether it synergizes the activity of Cry1Ac towards a resistant strain is yet to be resolved.

Student poster

B-5 **PHAGOCYTOSIS BY INSECT MACROPHAGES: A MORPHOLOGICAL AND BIOCHEMICAL STUDY**

Sonia Costa, Carlos Ribeiro, *Departamento de Biologia, Universidade dos Açores, PORTUGAL*; Robert Zumbihl, Fabienne Vigneux, Noel Boemare, Michel Brehélin, *EMIP Unité INRA UMII 1133, Université de Montpellier II, FRANCE*

Abstract: Insects present an apparently simple immune system which only proceeds from the innate immunity. However, phagocytosis constitutes an evolutionary conserved complex process used by all metazoan organisms to eliminate potentially pathogenic microbes. Although there are some evidence that innate immune responses in insects share a high degree of structural and functional homology with the vertebrate immune system, little is known on the onset and on the regulation of the phagocytic process. In the present work, morphological and biochemical studies of phagocytosis were achieved in the lepidoptera Spodoptera littoralis. Using light and electron microscopy techniques, we observed that the granulocytes are the cells displaying the highest phagocytic properties among hemocytes, both in vivo and in vitro. We also identified two main models of phagocytosis described in mammals: the sinking-type of engulfment and the macropinocytosis. The initial event in phagocytosis is the recognition of pathogens by receptors present on the plasmatic membrane. In order to characterize receptors involved in phagocytosis of bacteria by insect hemocytes, we analysed the effect of different soluble ligands (IgG, C3, LPS, lipoteichoic acid, laminarine and polyribonucleotides). The analysis of binding reveals that there is no diminution in the adhesion of bacteria onto hemocytes when incubated in presence of immunoglobulins or complete bovine serum. Our data also indicate that binding of Gram- and Gram+ bacteria on hemocytes, prior to phagocytosis, seems to be achieved by a scavenger-like receptor which is able to recognize LPS, lipoteichoic acids and laminarine. We show that the specific scavenger receptor inhibitor, the polyinosinic acid, reduces the number of bacteria adherent to granulocytes. The reorganization of actin cytoskeleton is essential for the accomplishment of phagocytosis and is regulated by proteins of the Rho GTPases family in mammals. Overexpression of Rac and Cdc42 in insect hemocytes led to the formation of membrane ruffling and filopodia, respectively, as in mammal macrophages. The results obtained denote a great conservation of the recognition and internalization of pathogens during the phagocytic process in the Animal Kingdom and suggest an implication of Rho GTPases.

B-6 **PARTIAL RESISTANCE OF PLUTELLA XYLOSTELLA TO COMMERCIAL FORMULATES OF BACILLUS THURINGIENSIS IN AGRICULTURAL FIELDS IN MXICO***

Artemisa Perea, Magdalena Iracheta-Cardenas, *Facultad de Ciencias Biologicas/UANL, MEXICO*; Rafael Bujanos-Muñiz, *INIFAP-Celaya, MEXICO*; Luis Galan-Wong, Benito Pereyra-Alferez, *Facultad de Ciencias Biologicas/UANL, MEXICO*

Abstract: Two colonies of Plutella xylostella collected from different broccoli fields, named as East (Guanajuato State) and North (Querétaro State), were bioassayed with JavelinT and XentariT formulates. Results of initial LC50 (mg.L) for East colony was of 0.279 and 1.913 for XentariT and JavelinT. The North colony had a LC50= 0.294 for XentariT and 0.216 for JavelinT. Resistance ratio (RR) (LC50 field colony / LC50 of laboratory colony) showed higher differences among formulates, specially for the East colony. The RR for JavelinT, was of 18 and 2 for East and North, respectively. While with XentariT, the RR was of 1.3 for East and 1.6 for North. Both colonies were subject to selective pressure, separately, increasing the LC50 formulate concentration. After five generations, both colonies showed no resistance to 10-fold (10X) of JavelinT. On the other hand, we obtained a resistant colony derived from the North one, which showed resistance to 50X, 150X and 200X of XentariT, after five, six and seven generations, respectively. Resistant colony (200X) had a LC50= 16.43 (FL95= 14.52 - 24.06), 56-fold in comparison with its initial susceptibility and 89-fold than laboratory one. In order to know if some Cry toxin was the responsible of partial resistance, we tested Cry1Aa, Cry1Ab and Cry1Ac solubilized protoxins. Significant differences in LC50 (micrograms.ml) did not occur for

Cry1Ab among original colonies (LC50= 0.003, 0.005 and 0.005 for laboratory, North and East, respectively); however the 200X colony had 1.8 and 5-fold resistance than laboratory for Cry1Aa and Cry1Ab, respectively. Midgut protease content was partially analyzed and results demonstrated differences not only in the protease activity on Cry1Ab and Cry1Ac, but also in its protease profile. Although is necessary to perform experiments of binding, our bioassay results suggest that the partial resistance of the diamondback moth to B. thuringiensis formulates in México could be due to both phenomena, binding and protoxin processing. *CONACYT 38040-N

B-7 **CLONING AND EXPRESSION OF NOVEL CRY GENES FROM A MOSQUITOCIDAL STRAIN OF BACILLUS THURINGIENSIS SEROVAR SOTTO**

Akira Ohgushi, *Graduate School of Agriculture, Kyushu University, JAPAN*; Hiroyuki Saitoh, Naoya Wasano, *Biotechnology & Food Research Institute, JAPAN*; Michio Ohba, *Graduate School of Agriculture, Kyushu University, JAPAN*

Abstract: Two new crystal protein genes, cry24A-like and sotorf2, were cloned from Bacillus thuringiensis serovar sotto strain 96-OK-85-24. The cry24A-like and sotorf2 genes encoded the 76- and 61-kDa protein, respectively. These two proteins each possessed the five (block 1-5) and three (block 6-8) conserved regions. The amino acid sequence of the SOTORF2 had a high homology to that of the ORF2 protein of B. thuringiensis serovar jegathesan. Southern hybridization experiments with a cry24A-like gene-specific probe revealed that these genes are located on two large plasmids of $>$ 50 kb. The cry24A-like and sotorf2 genes were expressed in an acrystalliferous B. thuringiensis host. The proteins were synthesized and accumulated as inclusions. Experiments are currently under way to determine larvicidal activity against three dipteran species: Aedes aegypti, Culex pipiens molestus and Anopheles stephensi.

B-8 **CLONING AND CHARACTERIZATION OF A NOVEL GENE, CRY9EC1, ENCODING A LEPIDOPTERA-SPECIFIC CRYSTAL PROTEIN FROM A BACILLUS THURINGIENSIS SEROVAR GALLERIAE STRAIN**

Naoya Wasano, Hiroyuki Saitoh, Eiichi Mizuki, *Fukuoka Industrial Technology Center, JAPAN*; Minoru Maeda, *Kyushu Medical Co. Ltd., JAPAN*; Akira Ohgushi, Michio Ohba, *Kyushu University, JAPAN*

Abstract: A novel -endotoxin gene of the Lepidoptera-specific Bacillus thuringiensis serovar galleriae strain 92-KU-149-8 was cloned in a lambda phage and full sequence of the cry gene was determined. The cloned 6.5-kb DNA fragment includes full sequence of the cry gene and three open reading frames located upstream of the cry gene. The gene, designated cry9Ec1, encodes a polypeptide of 1154 amino acid residues with a predicted molecular weight of 130,237. The deduced amino acid sequence of the Cry9Ec1 protein had highest homology (77.7%) with the Cry9Ea1 protein when compared with existing Cry proteins and the Cry9Ec1 contained five conserved regions of the Cry proteins. An orf1 gene encoding 19,187-Da protein was located 207-bp upstream from the cry9Ec1 gene, and a putative terminator sequence (14-bp inverted repeat sequence) was located downstream from the stop codon of cry9Ec1 gene. The expression, in an acrystalliferous B. thuringiensis strain, of the cry9Ea1 gene was on a high level when controlled by the cyt1A promoter, leading to the formation of large spherical inclusions. Interestingly, these inclusions lacked the membranous surface structure which was associated with wild-type inclusions of the strain 92-KU-149-8. Purified inclusions from the recombinant strain were toxic to Bombyx mori and Plutella xylostella, while nontoxic to Spodoptera litura, S. exigua, Plodia interpunctella, Helicoverpa zea, and Culex pipiens molestus.

B-9 **EXPRESSION OF A VEGETATIVE INSECTICIDAL PROTEIN GENE UNDER THE CONTROL OF PROMOTER PLUS SD SEQUENCES OF CRY GENES FROM BACILLUS THURINGIENSIS**

Jianwu Chen, Fan Sun, Mujing Tang, Yongxia Shi, Wei Xu, Jianxiu Yu, Yi Pang, *State Key Laboratory for Biocontrol & Institute of Entomology, Zhongshan University, CHINA*

Abstract: Vip3A is a novel 89-kDa protein produced by Bacillus thuringiensis during vegetative growth. Derived from the shuttle vector pHT3101, various recombinant plasmids were constructed to investigate the vip184P gene expression time course and Vip184 protein yield under the control of promoter and Shine-Dalgarno (SD) sequences of cry3A or cry1A genes. The combination of vip184P gene (in Cry-B[pHTP3AP]) with the promoter plus SD sequence of cry3A gene could be expressed during vegetative growth and yielded about 2.5 fold the maximum amount of Vip184 obtained with the native vip184 gene (in Cry-B[pHPT3]) at the onset of the stationary phase. vip184P gene (in Cry-B[pHTP1AP]) could be also expressed under the overlapping promoter and SD sequence of cry1A gene in Cry-B. Its expression time course was consistent with that of cry1A gene. The maximum yield obtained with a construct that contained the vip184P gene and promoter plus SD sequences of cry1A gene was increased to about 4.8 and 2.2 fold as compared with the amount obtained with the native vip184 gene early and later in sporulation phase respectively. The results showed that the cry3A or cry1A promoter / SD combination could enhance synthesis of Vip184 and change its expression time. However, among the recombinant strains 2ndE[pHPT3], 2ndE[pHTP3AP] and 2ndE[pHTP1AP], Vip184 protein only in the 2ndE[pHTP3AP] at 9 h could be detected and it showed 2 and 4 folds higher toxicity against Spodoptera exigua and Helicoverpa armigera than that of 2ndE[pHPT3] and 2ndE[pHTP1AP] respectively when pellets were from an equivalent number of spores.

B-10 **EXPRESSION OF VIP1/VIP2 GENES IN ESCHERICHIA COLI AND BACILLUS THURINGIENSIS**

Yongxia Shi, Wei Xu, Meijin Yuan, Mujing Tang, Jianwu Chen, Yi Pang, *State Key Laboratory for Biocontrol & Institute of Entomology, Zhongshan University, CHINA*

Abstract: A kind of new binary toxin genes encoded by a single operon from Bacillus thuringiensis, encoding the vegetative insecticidal protein Vip2A(c) and Vip1A(c), had been cloned and sequenced. The ADP-ribosyltransferase Vip2A(c) shared significant sequence similarity with the enzymatic components of some binary toxins, and Vip1A(c) protein also revealed significant conserved domain similarity with Clostridium difficile toxin B. The individual genes (vip2A(c) or vip1A(c)), 5-teminus truncated genes deleting the signal peptide (vip2AGS or vip2AGS) and the operon were all ligated into pET28b(+) vector containing 6eHis-tagged coding sequence and expressed in Escherichia coli BL21(DE3). Western blot analysis showed that Vip2A(c) protein could be detected in the induced DE3 containing vip2A(c) gene and found in both soluble protein and the inclusion bodies, while most of the expressed Vip1A(c) in the induced DE3 containing vip1A(c) gene formed the insoluble inclusion bodies. Protein purification and analysis revealed that only 49kDa Vip2A(c)GS and 86kDa Vip1A(c) GS could be obtained, which deleted the N-terminus signal peptides compared with the primary Vip2A(c) and Vip1A(c), while the expressed proteins in other samples were processed and their N-terminal signal peptides after 6eHis-tag were cleaved. In addition, the individual gene and the operon were also expressed in B. thuringiensis. Both proteins were mostly secreted into the cell supernatants. The expression level of Vip1A(c) was influenced because of the interruption of vip2A(c) gene on the operon. Neither separate protein (Vip2A(c) or Vip1A(c)) nor both showed any toxicity against some lepidopteran, and coleopteran insects. It was probably owing to no proper target insects or loss of the toxicity because of the variable Vip1A(c) protein.

B-11 **RECOVERY OF BACILLUS THURINGIENSIS FROM ACTIVATED SLUDGES OF A WASTE WATER TREATMENT PLANT IN A MISO FACTORY**

Tokio Ichimatsu, Kazuhiko Higuchi, *Fukuoka Industrial Technology Center, JAPAN*; Kumiko Kagoshima, *Kyushu University, JAPAN*; Eiichi Mizuki, *Fukuoka Industrial Technology Center, JAPAN*; Michio Ohba, *Kyushu University, JAPAN*

Abstract: Bacillus thuringiensis was isolated at a high frequency from activated-sludge system environments in a waste water treatment plant of a miso (fermented soybean paste) factory. The organism was recovered from eight (88.9%) out of nine materials tested, sampled at several treatment steps. The frequency of B. thuringiensis colonies was 20.7% among 633 colonies of the Bacillus cereus/B. thuringiensis group. The highest density of this bacterium was 2.5 x 104 cfu/ml in a sample from the second aeration basin. Serological tests revealed that the serotype H14/23 was the predominant. All of the isolates from the activated-sludge system produced spherical or irregular-shaped parasporal inclusions with no insecticidal activities. This is in contrast to the results that most of B. thuringiensis isolates, recovered from litters of raw-materials storehouse in the factory, formed bipyramidal inclusions toxic to dipteran and/or lepidopteran insect larvae. Cytocidal activity against liver cancer cells (HepG2) was associated with 11 isolates from activated-sludge system.

B-12 **CANCER CELL-KILLING ACTIVITY OF PARASPORAL INCLUSION PROTEINS FROM JAPANESE ISOLATES OF BACILLUS THURINGIENSIS**

Eiichi Mizuki, *Fukuoka Industrial Technology Center, JAPAN*; Yoshitaka Murata, Masako Nomaguchi, *Kyurin Corporation, JAPAN*; Hiroyuki Saitoh, Satoko Yamashita, *Fukuoka Industrial Technology Center, JAPAN*; Yasuyuki Sasaguri, *University of Occupational and Environmental Health, JAPAN*; Michio Ohba, *Kyushu University, JAPAN*

Abstract: Parasporal inclusion proteins from a total of 2780 Japanese isolates of Bacillus thuringiensis were examined for cytotoxicity against 29 human cancer cell lines from ten organs: esophagus (4 cell lines), stomach (3), colon (5), pancreas (2), liver (1), lung (6), uterus (4), ovary (1), testis (1) and bile duct (2). Eighty-nine non-haemolytic B. thuringiensis strains showed in vitro cytocidal activity with different cytotoxicity spectra and varied activity levels. The cancer cell-toxic strains were from soil, fresh water, phylloplane and activated sludge, and consisted of several H serovars including kurstaki, alesti, pakistani, dakota, tohokuensis, shandongiensis, coreanensis, seoulensis and other unidentified serogroups.

B-13 **LYOPHILIZATION OF LEPIDOPTERAN MIDGUTS: A PRESERVING METHOD FOR BACILLUS THURINGIENSIS TOXIN BINDING STUDIES**

Carmen Sara Hernández, Ana Rodrigo, Juan Ferré, *Universitat de València, SPAIN*

Abstract: Binding assays with brush border membrane vesicles (BBMV) from insect midguts are commonly used in the study of the interactions between Bacillus thuringiensis Cry toxins and their receptors. Collaboration between laboratories often require that frozen insect samples are sent in dry ice. Because of customs restrictions and delays, sample thawing is always a risk and often the biological material becomes ruined during shipping. We have tested lyophilization as an alternative method for preserving insect midguts for binding studies with B. thuringiensis Cry toxins. For this purpose, BBMV were prepared from both frozen and lyophilized midguts from three lepidopteran species: Spodoptera exigua, Manduca sexta, and Helicoverpa armigera. Higher membrane protein recovery was always obtained from lyophilized midguts compared to frozen midguts, and similar membrane marker enzyme activities were found in BBMV from either treatment. Comparable equilibrium dissociation constants and binding site concentrations, calculated from binding experiments with labeled 125ICry1Ab toxin, were found using BBMV from either method. In the light of these results, lyophilization is a good preserving method of lepidopteran midguts to study binding of B. thuringiensis Cry toxins.

B-14 **MOLECULAR STUDIES OF A BACILLUS THURINGIENSIS PUTATIVE VIRULENCE OPERON**

Jinhong Wang, David Ellar, *Biochemistry Department, University of Cambridge, UNITED KINGDOM*

Abstract: Pathogenic members of the Bacillus cereus group are important medically as agents of human and animal diseases (B. anthracis and B. cereus) and industrially as the principal biopesticide in agriculture and insect vector control (B. thuringiensis). Although increasing evidence of chromosomal similarity of the three organisms suggests that they belong to one species, their pathogenic targets are quite different. Investigation of their virulence mechanisms at the genetic and molecular level will contribute significantly to our fundamental understanding of pathogenesis. A previous signature-tagged mutagenesis (STM) study of virulence determinants in B. thuringiensis with transposon Tn917 using the Manduca sexta model identified and cloned 12 unique attenuated mutants. Attenuation of one of these mutants (6F8) in M. Sexta was confirmed by in vitro and in vivo competition assays. Primer walking yielded 7312bp DNA sequence from clones flanking the transposon insertion site and 16 potential ORFs were predicted by GeneMark. In 6F8 the transposon was inserted into the 3 end of ORF4. DNA sequencing revealed that ORF 2 and ORF3 were 42% and 61% identical to Clostridium tetani phage-related proteins, ORF11 was 70% homologous to a B. anthracis conserved hypothetical protein with unknown function, while no significant similarities were found between the other ORFs and any sequence in the databases. To confirm that the gene mutated in 6F8 is essential for pathogenesis, insertional inactivation of the wild type by homologous recombination was used to eliminate the putative virulence gene and determine the effect on pathogenesis. Three null mutants of ORF4, ORF5-6 and ORF2-7 were successfully constructed, in which the wild-type genes were disrupted by insertion of a kanamycin cassette. Surprisingly, the competition assays with these three mutants showed them to be as virulent as the wild type in M. sexta. One explanation is that the 6F8 mutant might be a polar mutant. This was verified by RT-PCR analysis of the wild type growing in LB broth. Expression of all ORFs was detected and the transcript size spanned a distance from ORF1 to ORF16, which suggested that all these ORFs might exist in one operon. Generation of null mutants in the remaining ORFs is in progress to identify the part of the operon responsible for virulence and the transcription patterns of the wildtype and 6F8 mutant during M. Sexta infection.

B-15 **A NOVEL TOXIN FROM A BRAZILIAN STRAIN OF BACILLUS THURINGIENSIS REPORTED TO KILL THE COTTON BOLL WEEVIL (ANTHONOMUS GRANDIS)**

Joseilde Silva-Werneck, David Ellar, *University of Cambridge, UK*

Abstract: Bacillus thuringiensis (Bt) is a gram-positive bacterium that synthesises cytoplasmic protein crystals during sporulation. These crystals are composed of one or more -endotoxins (Cry proteins or Cyt proteins), which are toxic to crop and forest insect pests, and insect disease vectors. Bt has been the most successful commercial bioinsecticide for decades against various insect pests, and is also a source of genes for transgenic expression to provide insect pest resistance in plants. However, because several important insect pests have poor susceptibility to the existing portfolio of Bt insecticidal proteins, intensive searches continue for novel genes. The Brazilian Bt strain S725 was selected for its toxic activity against the cotton boll weevil, Anthonomus grandis (Martins, et al., 2002. In: Program and Abstracts VIII International Colloquium on Invertebrate Pathology and Microbial Control, Foz do Iguassu, Brazil, Society for Invertebrate Pathology, p. 86). Purified crystals of Bt strain S725 contain 130 kDa proteins which convert to 65 kDa after proteolytic activation. Amino-terminal sequence of a trypsin activated fragment revealed a high level of similarity to Cry9 proteins. PCR analysis of total DNA revealed a cry9-like and a cry1I gene in this strain. The cry9-like gene was PCR amplified, cloned in Escherichia coli, and the ORF was sequenced. Blast searches for the deduced amino acid sequence revealed 73% identity to the Cry9B toxin, and

lower identity levels to other Cry9 proteins. The novel protein sequence was modeled and showed a three domain structure, similar to the known Cry proteins. The Cry9-like protein was expressed in an acrystalliferous strain of Bt subsp. israelensis, purified and analysed by scanning electron microscopy, in vitro processing assays, and immunoaffinity against Cry antibodies. The results of bioassays of the Cry9-like protein and the Bt strain S725 crystals against A. grandis and other larvae will be reported.

B-16 ROLE OF BACILLUS THURINGIENSIS TOXINS DOMAINS II AND III IN TOXICITY AND BINDING TO MIDGUT RECEPTORS OF SPODOPTERA EXIGUA (HÜBNER).

Joel González-Cabrera, *Universidad de Valencia, ESPAA*; Salvador Herrero, Petra L. Bakker, Ruud De Maagd, *Plant Research International B.V., THE NETHERLANDS*; Juan Ferré, *Universidad de Valencia, ESPAA*

Abstract: Most Cry proteins are arranged in a three domain structure. Domain I is involved in the insertion and pore formation in the midgut epithelial membrane of susceptible larvae and domains II and III in interaction and binding to specific receptors in the membrane. The role of domains II and III appears not to be the same for different susceptible pests. Here hybrids toxins were used to study the role of these domains in the mode of action of Cry1 proteins against Spodoptera exigua. Hybrid proteins H04 (domains I and II from Cry1Ab and domain III from Cry1Ca) and H205 (domains I and II from Cry1Ca and domain III from Cry1Ab) together with Cry1Ab and Cry1C toxins were compared for their toxicity, protease stability and binding properties in S. exigua. Analysis of their toxicity against first instar larvae showed that H04 toxin was the most toxic with around 3- 25- and 100-fold higher toxicity than Cry1Ca, Cry1Ab and H205, respectively. Binding competition experiments with 125I-labelled Cry1Ab and Cry1Ca revealed that hybrid toxins were able to compete for binding of these toxins when sharing the same domain I and II. Despite previous evidence that supports the involvement of domain III in the interaction and binding, its involvement is not obvious from our results. We found that binding specificity seems to be dominated by domains I and/or II.

B-17 CRY1C-TOLERANCE STUDIES USING SF9 CELLS AS A MODEL SYSTEM

Dror Avisar, Michal Segal, Baruch Sneh, Aviah Zilberstein, *Tel Aviv University, ISRAEL*

Abstract: The Spodoptera frugiperda Sf9 cell line is highly and specifically sensitive to the Bacillus thuringiensis ?-endotoxin Cry1C. Sf9 cells were used as a model system for unraveling Cry1C interaction with the cell membrane. Two types of Cry1C tolerant Sf9 cells have been defined. The first consists of Cry1C-resistant cell lines (denoted as rSf9) generated by a random silencing approach based on antisensing of a cDNA library. Sf9 cells arrested at the G2/M-phase by nocodazole constitute the second type of Cry1C-tolerant cells (denoted as mSf9). These cells revealed transient Cry1C insensitivity during mitosis and regained Cry1C sensitivity in the G1-phase that occurred after nocodazole removal. Time lapse photographing of normal Sf9 culture treated with Cry1C also showed that cells at M-phase were not sensitive to the toxin. Cry1C dose response experiments showed higher LC50 values for both rSf9 and mSf9 cells (3-fold and 5-fold, respectively) compared with the LC50 of normal Sf9 cells. Correlatively, rSf9 and mSf9 cells bound 3- to 10-fold less toxin, respectively, in whole cell binding assays. No lipid rafts could be isolated from mSf9 cells, while clearly defined lipid rafts were isolated from normally grown Sf9 cells. Caveolin-1 was identified as a lipid raft component in normal cells but in mSf9 cells caveolin-1 was shifted to the membrane soluble fraction. Hence M-phase linked changes in lipid rafts organization may account for reduced Cry1C binding and toxicity. Analysis of lipid raft proteins in Cry1C-resistant rSf9 cells showed a reduction in porin (a voltage dependent ion channel protein) content. However, no corresponding reduction in porin transcript level was observed, suggesting that the lower level of porin in rSf9 lipid rafts reflects a structural change that also reduces its capacity to interact with Cry1C. Taken together, these results indicate that membrane raft integrity is playing an important role in Cry1C interaction and toxicity.

B-18 POTENTIAL NON-TARGET IMPACTS OF BT-CANOLA

Peter G. Mason, Lorraine Braun, Suzanne I. Warwick, *Agriculture and Agri-Food Canada, CANADA*; C. Neal Stewart, *University of Tennessee, USA*

Abstract: Laboratory and field studies were carried out to assess impacts of Bt canola on two non-target secondary pest species that feed on canola, and on parasitoids associated with diamondback moth and the non-target species. Both Pieris rapae and Mamestra configurata were susceptible to canola containing Bt-cry1Ac and GFP. In a field cage study, number of Diadegma insulare (diamondback moth parasitoid) was significantly reduced on Bt canola plants. Successful parasitism of M. configurata by Microplitis mediator was significantly reduced when M. configurata fed on Bt canola in a lab study. The results suggest that Bt canola can have a significant impact on non-target species and its use in pest management should be carefully considered.

B-19 BIOCHEMICAL CHARACTERIZATION OF FIELD EVOLVED RESISTANCE TO BACILLUS THURINGIENSIS TOXIN CRY1AC IN DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA

Maria Sales Ibiza-Palacios, *Department of Genetics, Universitat de València, SPAIN*; Ali Sayyed, Ben Raymond, Denis Wright, *Department of Biological Sciences, Imperial College London, UK*; Baltasar Escriche, *Department of Genetics, Universitat de València, SPAIN*

Abstract: Crucifer crops have an important pest, Plutella xylostella L. (diamondback moth), which is widely distributed around world and causes significant economical losses. This lepidopteran insect is the only pest that has developed significant resistance to Cry toxins of Bacillus thuringiensis (Bt) in the open field. The development of resistance populations can threaten the benefits of Bt toxin use, both in formulated solutions and in transgenic crops, and the diamondback moth is thus an important model for the management of Bt resistance. In the present study, we have analysed a field resistant population (Karak) of diamondback moth from Malaysia, which has kept the resistance in the laboratory during 11 generations without re-selection with Cry toxins. The Karak population shows a high resistance to four pure activated toxins, Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa, and to two commercial products based on Cry1A toxins, when compared with a laboratory standard susceptible population (LAB-UK). Biochemical analysis of midgut brush border membrane vesicles prepared from Karak and LAB-UK populations using 125I-labeled pure activated toxins Cry1Ab and Cry1Ac, and Cry1Ca as a binding control, shows that the most important biochemical mechanism that underlines the resistance is the strong reduction of specific binding to Cry1Ab and Cry1Ac.

B-20 PURIFICATION AND CHARACTERIZATIONS OF A NEW BACILLUS THURINGIENSIS VIRULENCE FACTOR : THE INHA2 METALLOPROTEASE

Myriam Hajaij, *Unité Génétique Microbienne et Environnement, INRA, la Minière, FRANCE*; Michel Gohar, *Unité Génétique Microbienne et Environnement, INRA, Unité Microbiologie et Génétique Microbienne, INRA, FRANCE*; Sinda Fedhila, *Unité Génétique Microbienne et Environnement, INRA, la Minière, FRANCE*; Didier Lereclus, Christina Nielsen-LeRoux, *Unité Génétique Microbienne et Environnement, INRA, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*

Abstract: The main insecticidal activity of Bacillus thuringiensis (Bt) is due to the larval ingestion of the insect specific Cry toxins. However, strains of both crystal minus Bt and of B. cereus are known to produce other factors contributing to the overall virulence of these bacteria toward insects. The importance of the Bt pleiotropic PlcR regulator was demonstrated by reduced mortality in larvae of the greater wax moth Galleria mellonella infected with spores from a Bt 407 cry- plcR mutant (Salamitou et al., 2000). PlcR governs many putative virulence factors (phospholi-

pases, enterotoxins, hemolysins, proteases etc.), and recently the putative PlcR-controlled zinc protease InhA2 was discovered to be important for pathogenesis via the oral route (Fedhila et al. 2002, 2003). InhA2 may interfere with intestinal barriers (peritrophic membrane and/or intestinal midgut cells). InhA2 is found as a 72 kDa polypeptide in the secretomes of Bt 407 cry- cultures in early stationary phase. InhA2has 66% homology with InhA (inhibitor A) which degrade some insect antimicrobial peptides. Purification of InhA2 was performed in order to characterize its enzymatic activity and specificity and to correlate this with the possible mode of action of InhA2 during the larval infection process. Since InhA2 is lethal for E. coli, purification was obtained from supernatants of a recombinant Bt 407 cry- plcR mutant transformed with the plasmid [pHT315 Omega (papha3-inha2)] where inhA2 is placed downstream of the constitutive promoter of apha3 resulting in high level expression. Following precipitation by 85% ammonium sulphate, InhA2 was fully purified by anion-exchange chromatography in the presence of Ca2+ which is required for stability. Using azo-casein as a colorimetric substrat for measuring the enzymatic activity, InhA2 was found to be active in a large range of temperatures, from 25 to 55C, with an optimum at 45C, and in a rather acid and neutral pH spectrum, from pH 6 to 8. Enzymatic activity was inhibited by several protease inhibitors, both specific metallo and serine inhibitors as well as EDTA and EGTA chelators and by high concentrations of zinc. Besides its activity on casein, InhA2 was also found to degrade albumin, collagen, gelatin, and actin. No direct larvicidal activity was observed when pure InhA2 was ingested by Galleria mellonella. Further investigations related to cellular targets in the larvae are under process.

Salamitou S. et al., 2000 The regulon PlcR is involved in the opportunistic properties of Bacillus thuringiensis and Bacillus cereus in mice and insects. Microbiology 146 :2825-2832 Fedhila, S., Nel, P., Lereclus,D., 2002. The InhA2 metalloprotease of Bacillus thuringiensis strain 407 is required for pathogenicity in insects via the oral route. J. Bacteriol. 184: 3296-3304. Fedhila, S., Gohar, M., Slamti, L., Nel, P., Lereclus,D., 2003. The Bacillus thuringiensis PlcR-regulated gene inhA2 is necessary, but not sufficient, for virulence. J. Bacteriol., 185: 2820-2825.

B-21 HOST RANGE EXTENSION OF BACILLUS THURINGIENSIS CRY TOXINS TO THE SPINY BOLLWORM EARIAS INSULANA (BOIS.) (LEPIDOPTERA: NOCTUIDAE)

María A. Ibargutxi, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*; Anna Estela, Juan Ferré, *Departamento de Genética Universidad de Valencia, SPAIN*; Primitivo Caballero, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*

Abstract: Transgenic cotton plants expressing the insecticidal protein Cry1Ac from Bacillus thuringiensis (Bt) have been produced to make them insect-resistant. Cotton has also been successfully transformed to express Cry1Ac and Cry2Ab. Lepidopteran species targeted by Bt transgenic cotton are primarily Heliothis virescens, Helicoverpa zea, and H. armigera. The spiny bollworm, Earias insulana, constitutes an important component of the cotton lepidopteran pests complex in Southern Europe and Northern Africa. However, the susceptibility of E. insulana to the insecticidal proteins produced by B. thuringiensis has not been properly tested. We analyzed the toxicity and binding of 11 of the most common lepidopteran-specific Cry proteins (all of them belonging to the protein classes Cry1, Cry2 or Cry9) for larvae of E. insulana. Insect bioassays were performed by incorporating the protein into an artificial diet. First, the susceptibility of E. insulana larvae to each Cry toxin tested was determined at a high toxin concentration (100 g/ml). A second experiment involved determining the median lethal concentration (LC50) for the most active Cry proteins.. The most active proteins against E. insulana were: Cry9Ca1, Cry1Ac, Cry1Ba, Cry1Ab, Cry1D and Cry1Ia7. A number of Cry proteins (Cry1Aa, Cry1C, Cry1Ea, Cry1Fa, Cry1J, Cry2Aa, and Cry2Ab) were nontoxic for E. insulana. Competition binding experiments with 125I-labeled Cry1Ac and unlabeled Cry1Ab showed that Cry1Ab competed completely for Cry1Ac binding sites in E. insulana. In contrast, no competition for binding sites was found between Cry1Ac and any of the following proteins: Cry1Ba, Cry1Da, Cry1Ia7 or Cry9Ca1. These results contribute to our understanding of the potential of B. thuringiensis toxins to control the spiny bollworm.

B-22 ISOLATION OF A NEW BACILLUS THURINGIENSIS STRAIN CYZ-13 AND CRY-TYPE GENE ANALYSIS BY PCR-RFLP

Li Changyou, *1. Dept. of Plant Protection, Laiyang Agricultural College, P.R. CHINA*; Lu Xiujun, *2. Dept. of Entomology Agricultural University of Hebei, P.R. CHINA*; Zheng Guiling, *1. Dept. of Plant Protection, Laiyang Agricultural College, P.R. CHINA*; Cheng Linyou, *2. Dept. of Entomology Agricultural University of Hebei, P.R. CHINA*; Li Guoxun, *1. Dept. of Plant Protection, Laiyang Agricultural College, 2. Dept. of Entomology Agricultural University of Hebei, P.R. CHINA*

Abstract: Spodoptera exigua is very important pest with that damage to many plants is getting more and more serious in China. Bacillus thuringiensis (Bt) cyz-13 strain, one of 37 Bt isolates in Hebei, China, appeared more toxin against S. exigua than that from Bt HD-1 with mortality of 97.94.47% and 68.83.35% at concentration of 5.0106 cells/g respectively. The Cry-type genes were analyzed by PCR-RFLP technique. The results showed three cry type genes of cry1Ac, cry1Bc, and cry2Ab in cyz-13 strain. The restriction enzyme analysis showed that the fragments from the strain were different from all of published genes.

B-23 HIGH LEVEL OF CYT1A SYNTHESIS IN BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS IS DUE TO THREE PROMOTERS AND A STRONG 3' MRNA STEM-LOOP STRUCTURE

Yuko Sakano, *Department of Entomology, University of California, U.S.A.*; Baoxue Ge, *Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, U.S.A.*; Hyun-Woo Park, *Department of Entomology, University of California, U.S.A.*; Brian A. Federici, *Department of Entomology, Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, U.S.A.*

Abstract: The insecticidal bacterium, Bacillus thuringiensis subsp. israelensis, produces a spherical parasporal body during sporulation that is highly toxic to larvae of insects such as mosquitoes, blackflies, and midges. This parasporal body consists primarily of the Cyt1A protein that makes up approximately 55% of the parasporal body's mass. The other proteins are Cry11A, which accounts for about 35%, and Cry4A and Cry4B that together account for the remaining 10% of the parasporal body. The genetic basis of the comparatively large amounts of Cyt1A produced by B. thuringiensis subsp. israelensis is not known. In the present study, sequence analysis of the 5' untranslated region of cyt1A identified a third promoter for this gene in addition to the well known sporulation-dependent BtI and BtII promoters. Use of constructs containing BtI and BtII or only the third promoter, BtIII, demonstrated that latter promoter was functional and capable of directing the synthesis of Cyt1A. In addition, we show that a strong 3' mRNA stem-loop structure in the untranslated region of cyt1A results in more Cyt1A synthesis than a similar structure in the cry11A gene. These results indicate the third promoter and 3' stem-loop structure contribute substantially to Cyt1A synthesis in B. thuringiensis subsp. israelensis and are likely responsible for larger amounts of this protein in the parasporal body in comparison to the Cry proteins.

B-24 THE 20-KDA PROTEIN OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS ENHANCES

Hyun-Woo Park, Dennis K. Bideshi, *Department of Entomology, University of California, U.S.A.*; Brian A. Federici, *Department of Entomology, Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, U.S.A.*

Abstract: The mosquitocidal bacterium Bacillus thuringiensis subsp. israelensis (Bti) produces four major mosquitocidal proteins of Cry4A (128 kDa), Cry4B (135 kDa), Cry11A (72 kDa) and Cyt1A (27 kDa). The

gene encoding Cry11A occurs as the second gene in an operon that is co-transcribed with genes not involved in toxicity. A 20-kDa protein encoded as the third ORF of the Cry11A operon apparently acts like a chaperone, assisting Cry11A synthesis. This protein was originally shown to be required for efficient Cyt1A production in Escherichia coli. It has been reported to enhance net synthesis of Cry4A and Cry11A in E. coli and B. thuringiensis, as well as Cyt1A production and crystal formation in this species. In addition, it has been demonstrated that this protein can enhance the production of a truncated Cry1C. Binary (Bin) toxin of B. sphaericus (Bs) 2362, the active ingredient of VectoLex, a commercial bacterial larvicide used for mosquito control, is composed of two proteins, a 42-kDa toxic domain (BinA) and a 51-kDa binding domain (BinB) assembled in small parasporal inclusions highly toxic to Culex mosquitoes as well as certain Aedes and Anopheline species. The quantity of Bin produced per cell is of commercial interest because the higher the yield, the higher the toxicity per unit weight. To determine whether the 20-kDa protein of Bti can improve the yield of Bin, the bin 2362 operon was expressed with and without the 20-kDa protein gene using three different expression systems, (1) bin toxin promoter; (2) cyt1A promoter, and (3) cyt1A promoter combined with STAB-SD sequence, in the 4Q7 acrystalliferous strain of Bti. Using the latter construct, a 1.3-fold increase in the yield of Bin was obtained in comparison to that obtained with the wild type operon.

B-25 BACILLUS CEREUS SENSU LATO POPULATION FROM SOW BUG: VIRULENCE GENE PROFILES VERSUS CHROMOSOMAL DNA RELATIONSHIP REVEALED BY PFGE

Izabela Swiecicka, *Department of Microbiology, University of Bialystok, Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, POLAND*; Jacques Mahillon, *Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, BELGIUM*

Abstract: Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus anthracis, as well as the psychrotolerant Bacillus weihenstephanensis are genetically closely related and are often considered as varieties or subspecies of the same species, B. cereus sensu lato. Although these bacteria share strong taxonomic relationships, they differ significantly by their ecological features and/or symbiotic associations with other organisms. B. cereus can cause food poisoning due to the combinatory action of various toxins such as the three component enterotoxin HBL or the single components hemolysin 2, cerulolysin O, and cytolysin K. Yet, the entomopathogen B. thuringiensis appears to be indistinguishable from B. cereus, except for the accumulation of crystalline inclusions specific against target insect larvae. Also, little is known about the possible virulence of the rhizoid B. mycoides and B. pseudomycoides, and more generally, information related to toxin synthesis by natural B. cereus s.l. isolates remains rather limited. Although these bacteria are generally regarded as soil microorganisms, some authors have suggested that B. cereus s.l. are residents of invertebrate intestinal track, displaying symbiotic relationships with their hosts. Thus, given their taxonomic similarity, their possible common habitats, and the presence of some of these organisms in the human food chain, the study on of the potential virulence of natural existing B. cereus s.l. is of major importance. In present study, the occurrence of B. cereus s.l. in the intestine of sow bugs was investigated. The genetic relationship among these invertebrate bacteria was assessed on the basis of their chromosomal DNA profiling by pulsed-field gel electrophoresis (PFGE). The diversity of their virulence genes was also investigated by PCR amplification. In total 25 strains of B. cereus s.l. were isolated from 30 animals: 16 isolates were identified as B. cereus, 3 as B. thuringiensis, and 6 as B. mycoides. Whereas the gene coding for cereolysine O was found in all isolates, the frequencies of hemolysin HBL, hemolysin 2, and cytolysine K differed for each particular species. Following digestion with NotI, PFGE analysis revealed a high level of genomic diversity among all these B. cereus s.l. from sow bugs. Interestingly, no correlation could be observed between the DNA pulsotypes of the isolates and their content of virulence genes. The commensal behaviour of these arthropod bacteria is currently under investigation using genetically tagged derivatives reintroduced in sow bugs.

B-26 MOLECULAR CLONING OF A NEW GENE ENCODING A CRY PROTEIN EFFECTIVE TOWARDS COTTON BOLL WEEVIL, ANTHONOMUS GRANDIS

Maria F. Grossi de Sa, Mariana T. Q. De Magalhães, João A. N. Batista, Shirley M. B. Da Silva, Rodrigo R. Fragoso, Osmundo B. Oliveira-Neto, Rose Monnerat, Edson L. Z. Figueira, *Embrapa Recursos Genéticos e Biotecnologia. Parque Estação Biológica, BRAZIL*

Abstract: The Bacillus thuringiensis (Bt) represents an efficient alternative to control many insect pests. Its crystalline inclusions formed during sporulation, composed by delta-endotoxin or crystal proteins (Cry), are toxic to larvae of several insect orders and harmless to mammals. Aiming to identify proteins that are toxic to the cotton boll weevil larvae, we have characterized a Bt strain from a microorganism germoplasm bank of EMBRAPA - Genetic Resources and Biotechnology that is highly toxic to this insect. The cotton boll weevil, Anthonomus grandis, is an economically important pest of cotton in tropical and subtropical areas of several countries in the Americas, causing severe losses due its damage in cotton floral buds. Biochemical and electronic microscopy characterization showed the presence of spherical and bipyramidal crystals composed with proteins of molecular masses around 100 kDa, 68 kDa and 30 kDa. By using cry8 specific gene primers and TAIL-PCR technique, we have isolated a novel cry gene, called cry8Ea, containing 2688 bp, which encodes a protein with 896 amino acid residues. The codified protein presents 58% identity with other Cry8 protein class. While the N-terminal and C-terminal extensions of this protein are highly conserved when compared with other Cry8 endotoxins, the three domains (Domain I, II and III) involved on the receptor specificity show lower identities, suggesting be a novel toxin with different insecticide specificity. The cry8Ea gene was expressed in an acrystalliferous B. thuringiensis strain and the recombinant-protein showed similar activity against the cotton boll weevil as was found with the native Bt strain. This new gene isolated represents a great potential to be used in genetic improvement program of cotton crop to A. grandis control. Supported by EMBRAPA, FACUAL, FIALGO, CNPq.

B-27 DIVERSITY OF BACILLUS SPP. POPULATIONS IN THE DIGESTIVE TRACT OF LUCILIA CAESAR AND LUCILIA SERICATA BLOWFLIES (DIPTERA: CALLIPHORIDAE)

Sophie Buyle, Alan Fauconnier, *Nivelles Laboratories, BELGIUM*; Izabela Swiecicka, Jacques Mahillon, *UCL, BELGIUM*

Abstract: The so-called greenbottle flies (blowflies, Diptera: Calliphoridae) actually cover a wide range of different species that belong to distinct genus. These insect species share a similar ecological niche, feeding on meat, animal carcasses, decaying vegetation or organic garbage. In this study, we have addressed the microbial flora of the digestive tract of the greenbottle flies Lucilia caesar and Lucilia sericata, hypothesising that they could be the reservoir of food intoxication and potentially threatening bacteria. More generally speaking, to our knowledge, no study has been achieved on the bacterial flora of these dipteran insects. In a first step, we decided to focus on sporulating bacteria and, more specifically, on species belonging to the Bacillus genus. To this end, seven campaigns of insect catching were conducted in the south part of Belgium, between June and September 2003. One hundred and sixty L. caesar and L. sericata flies were captured, alcohol treated for insect surface decontamination and submitted to dissection. Care was taken to keep both head and thorax intact for further species determination. The fly digestive tract was removed, ground in sterile PBS buffer and treated for 10 minutes at 80C degrees. The selected heat-resistant bacteria were spread on LB medium and incubated at 30C. In total, 431 isolates were classified according to their colony morphology, including 226 Bacillus-like strains. Among these, further microbiological tests allowed the identification of 38 Bacillus cereus sensu lato, 3 Bacillus circulans and 1 Bacillus megaterium. A particular attention was then brought to discriminate among members of the B. cereus s.l. group: microscope observation of endotoxin crystal inclusions in sporangia, haemolytic activity on sheep blood agar and growth on MYP agar (Mannitol Egg Yolk Polymyxin Agar, Oxoid). Interestingly, most of these strains (37) turned

out to be B. cereus sensu stricto, with only one isolate of Bacillus mycoides but no strain of Bacillus thuringiensis. Detailed microbiological and molecular characterisations of these B. cereus s.l. strains are in progress, including their antibiotic resistances, plasmid profiles, genomic relationships and enterotoxin production.

B-28 THE DEVELOPMENT OF AN ASPOROGENIC STRAIN OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS BY DISRUPTING THE SIGK GENE AFFECTS CRYSTAL PROTEIN EXPRESSION AND TOXICITY

Adriana González, Gemma Armengol, *Biotechnology and Biological Control Unit, Corporation for Biological Research, COLOMBIA*; Sergio Orduz, *Biotechnology and Biological Control Unit, Corporation for Biological Research, Universidad de Pamplona, COLOMBIA*; Neil Crickmore, *School of Biological Sciences, University of Sussex, UNITED KINGDOM*

Abstract: Commercial preparations of bioinsecticides based on Bacillus thuringiensis contain viable spores and recently environmental concern has appeared from the spread of living spores to the environment, and it is thought that spores could be a source of environmental contamination that could probably cause changes in natural microbial populations in water and soils. Therefore, we developed a sporulation deficient strain of B. thuringiensis subsp. israelensis (Bti spo-) disrupting the sigK gene by inserting a kanamycin resistance gene. The Bti spo- did not formed spores after 72 hr of culture, and formed a crystal inclusion which remained encapsulated within the cell. Analysis by Western blot of 72 hr cultures of the Bti spo- strain indicates that the only crystal protein expressed is the Cyt1A, while the Cry4A, Cry4B, and Cry11A proteins were not expressed; interestingly, the polyclonal antibody used detected a protein of 80 kDa, whose role in toxicity is unknown The encapsulated crystals were not toxic towards Aedes aegypti third instar larvae; however, toxicity of the Bti spostrain become detectable when 72 hr cultures were lysed by sonication in order to release the crystals, reaching an LC50 value of 423 ng/ml when assayed against third instar Ae. aegypti larvae. The low toxicity showed by the Bti spo- strain is correlated to the lack of expression of the Cry4A, Cry4B and Cry11A proteins.

B-29 GENOMIC SEQUENCE OF A CADHERIN-LIKE GENE FROM THE EUROPEAN CORN BORER (OSTRINIA NUBILALIS, HÜBNER)

Yolanda Bel, Baltasar Escriche, *University of Valencia, SPAIN*

Abstract: Transgenic corn expressing the insecticidal toxin Cry1Ab from the bacterium Bacillus thuringiensis (Bt-corn) has been grown through the corn belt of U.S.A. This Bt-corn is successfully protected from damages produced by the lepidopteran major pest, the European Corn Borer (Ostrinia nubilalis, ECB). However, the mechanisms underlying the process of toxicity in ECB are not totally clarified. Cadherin genes have shown tight genetic linkage to Bt-toxins resistance in Heliotis virescens and Pectinophora gosypiella populations. ECB cadherin-like cDNA sequence has been reported in a patent (WO01/36639) as a Cry1Ab insect midgut receptor. Our analysis of that sequence showed that it shares a homology between 56%-64% with the rest of lepidopteran Bt-related cadherins described, the same percentage that share these cadherins among them. In addition, protein structure prediction was very similar to the described ones. We have determined the genomic sequence of this gene. Genomic DNA was isolated from the thorax of adult insects. Gene fragments were amplified using primers based on the cDNA reported sequence. Amplified fragments were sequenced and sequences overlapped as contigs. The final sequence has some blocks almost identical to the described cDNA and others with no homology. In the connecting areas, intron splicing signals were found. Then, sequence blocks without homology were considered introns and the others as exons. The genomic sequence is about 4 times longer than the cDNA one (5498 bp). We have found 33 introns, ranging from 69 to 1620 bp. This is the first genomic sequence of a lepidopteran cadherin-like gene reported. Reported position of a intron in cadherin-like gene from P. gossypiella suggests that both genes are ortologous. The genomic structure of the gene is similar to the one found in mammals (human and mouse) and dipteran (fruit fly and mosquito). However, sizes of the genes and introns are directly related with its evolutionary scale and number of introns follow the same scale. Further studies are needed to determine the implication of the studied ECB gene in Bt resistance and to determine if its sequence can be used in strategies of resistance management.

B-30 MOLECULAR EPIDEMIOLOGY OF PAENIBACILLUS LARVAE SUBSP. LARVAE

Jaana Pentikäinen, Eija Kalliainen, Sirpa Heinikainen, Sinikka Pelkonen, *National Veterinary and Food Research Institute, Kuopio Department, FINLAND*

Abstract: American foulbrood is the most virulent brood disease of the honey bee and the most significant disease in Finnish apiculture. It is caused by gram-positive bacterium Paenibacillus larvae subsp. larvae. Last year we examined 1096 honey samples and 93 brood samples for the presence of Paenibacillus larvae subsp. larvae spores and 34% and 39% were positive, respectively. The aim of this research was to study molecular epidemiology of P. l. larvae regionally in Finland and temporally in apiaries of the same beekeeper. Macrorestriction profiles (MRP) of the isolates were characterized by pulsed-field gel electrophoresis (PFGE). Strains were also characterised by biotyping (nitrate reduction, mannitol and salicin fermentation). To this study 145 P. l. larvae isolates were selected. They were isolates from honey and brood samples in the years 1997, 1999 and 2001. Strains were divided to 53 different MRP. Profiles had many conserved fragments and they had 83% degree of similarity measured by Dice factor. Forty-one of the isolates (28%) shared the most common MRP PF1. The second and third most common MRPs were PF4 (7%) and PF35 (6%). Other profiles had few or only one representative. Forty-one (28%) of the strains were found only from apiaries of one beekeeper. Apiaries from the same area showed often quite similar restriction profiles but some genotypes were spread throughout the country. When isolates of the same beekeeper were compared during a four-year period, some strains remained identical and some had encountered minor changes. A single beekeeper could also have two or more quite different isolates at a same year or during different years in his apiaries. In biotyping most isolates (61%) produced acid from salicin but not from mannitol and reduced nitrate to nitrite. There was no clear correlation between biotypes and genotypes. In conclusion macrorestriction analysis by PFGE proved to be a powerful tool for epidemiological studies of American foulbrood. There is no dominant virulent strain but several similar strains causing the disease. The disease easily spreads when bees rob other hives nearby or when the owner sells infected colonies to other beekeepers. Both infection routes can bee seen in our results.

B-31 GLOBAL ASSESSMENT OF BACILLUS THURINGIENSIS CRY1 GENE CONTENTS USING DNA MICROARRAYS.

Jaroslaw Letowski, *NRC-Biotechnology Research Inst.,, CANADA*; Alejandra Bravo, *Instituto de Biotecnologia UNAM, MEXICO*; Roland Brousseau, Luke Masson, *NRC-Biotechnology Research Inst.,, CANADA*

Abstract: The cry genes, found in Bacillus thuringiensis strains, are responsible for the synthesis of crystalline proteins, also known as Bt toxins for their insecticidal properties. The diversity of cry genes is huge. At the present time approximately 280 different cry genes, grouped in 43 families based on the similarity of their sequences, are known. With over 130 genes, cry1 is the most represented family (Fig.1). The vast majority of DNA microarrays studies are concerned with differential gene expressions, however, new microarrays applications are emerging in the detection of microbial species and genes of medical or environmental importance. Here, we present an application of DNA microarrays (cryArray) for the identification of B. thuringiensis cry1 family genes which consists of 50-mer oligonucleotide probes targeting cry1 family genes, and a few other cry genes at primary rank. To insure more reliable identification at the secondary and tertiary rank level of cry gene classification, when possible, we have used a redundancy approach, where multiple hybridization positives are necessary before the presence of a gene can be ascertained. By using this strategy false positives are minimized. The majority of our probes have unique targets (at least 10 distributed mismatches to the next closest

matching gene). In a few cases the specificity of the probe was slightly lower (fewer mismatches to the next matching gene) and cross hybridizations are to be expected. In order to validate the cryArray, we hybridized it with BioPrimeTM Cy5-labeled genomic DNA from strains with known cry gene contents. Several other B. thuringiensis strains, uncharacterized for their cry genes content are being tested and validated by cry gene-specific PCR. Our cryArray is able to rapidly and efficiently detect and identify the known cry1 genes. It is an excellent tool for fast screening of new B. thuringiensis isolates presenting interesting pesticidal activity. Although this is a model study, the next cryArray version will be expanded to include a more thorough representation of other important cry gene subclasses.

B-32 A VIP NOMENCLATURE?

Neil Crickmore, *University of Sussex, UK*; Dan Ziegler, *Bacillus Genetic Stock Center, USA*; Alejandra Bravo, *National University, MEXICO*; Ernest Schnepf, *Independent, USA*; Didier Lereclus, *Institiut Pasteur, FRANCE*; Jim Baum, *Monsanto, USA*; Jeroen Van Rie, *Bayer Crop Science, BELGIUM*; Donald Dean, *Ohio State University, USA*

Abstract: In 1993 a committee was set up to devise and maintain a nomenclature system for the ever increasing number of Cry toxins isolated from Bacillus thuringiensis and other entomopathogenic bacteria. The current committee, comprising the authors of this paper, continue to assign names to newly characterized toxins and publish these via a publicly available website. Only proteins that are located within a crystalline inclusion, or are related to such proteins, are included in the nomenclature. These criteria thus resulted in the exclusion of the Vip (vegetative insecticidal protein) toxins since these are unrelated to the Cry toxins and are secreted rather than found within the crystal. In recent years the number of characterized Vip toxins has increased, and as with the Cry toxins a decade ago newly discovered toxins are being allocated names that do not necessarily provide information as to their relatedness to other Vip proteins. We have analysed the Vip proteins employing the same methods used for the Cry proteins and on the basis of this propose a nomenclature for the Vip proteins. The presented poster will present this data and it is hoped that the SIP community can consider the proposal and decide on whether a Vip nomenclature should be adopted.

B-33 INHIBITORY EFFECT OF THE ENTOMOPATHOGENIC BACTERIUM PHOTORHABDUS LUMINESCENS ON MANDUCA SEXTA PHENOLOXIDASE

Ioannis G. Eleftherianos, Nicholas Waterfield, Richard ffrench-Constant, Stuart E. Reynolds, *Department of Biology & Biochemistry, University of Bath, UNITED KINGDOM*

Abstract: Photorhabdus luminescens is a motile Gram-negative bacterium that lives in symbiosis with nematodes of the family Heterorhabditidae. The bacteria produce a variety of toxins (e.g. mcf1 gene), immune inhibitory compounds and antimicrobial secondary metabolites. One aspect of the bacterium's pathogenicity is its interaction with phenoloxidase (PO), an important enzyme in the insect defensive melanisation response (prophenoloxidase cascade). We investigated PO activity contained in the haemolymph of the tobacco hornworm (Manduca sexta) in the presence of different P.luminescens strains. Using a simple spetrophotometric assay, it was shown that the culture supernatant from strains TTO1 and K122 significantly suppressed the activity of the enzyme. Screening individual P. luminescens TTO1 cosmids in E. coli led to the isolation of cosmid clone F12/2B, which inhibited the active form of PO in vitro. Inhibition was dose-dependant and heat stable. The inhibitor was effective regardless of the method of prophenoloxidase (PPO) activation. PPO activators used were the detergent CPC, E. coli lipopolysaccharides (LPS) and 1,3 beta-glucan polysaccharides (laminarin). To identify the gene(s) associated with PO inhibition, F12/2B was subjected to insertional mutagenesis and transposon mutants were then re-screened for PO inhibitory activity. A single transposon within a 968 bp open reading frame (ORF) abolished inhibition of the enzyme. Although a number of insect pathogens and parasites are known to interfere with PPO activation, as far as we know this is the first instance of a pathogen inhibiting the active form of the enzyme.

F-1 IMPROVEMENT OF MYCOINSECTICIDE BY SIMULTANEOUSLY OVEREXPRESSING A SUBTILISIN-LIKE GENE AND AN ENDOCHITINASE GENE IN BEAUVERIA BASSIANA

Wei-Guo Fang, Jin-Cheng Ma, Kai Jin, Yong-Jun Zhang, Yan Pei, *Biotechnology Research Center Southwest Agricultural University, P.R.CHINA*

Abstract: Insect cuticle is mainly made up of chitin fibril embedded in a protein matrix. Penetration of the cuticle is pivotal for the infection of entomopathogenic fungi. The complex refractory nature of insect cuticle suggests that penetration would require the synergistic action of several different enzymes including chitinase and protease. Based on the deduction, we tried to enhance the virulence of B. bassiana by constitutively expressing protease gene CDEP-1 and endochitinase gene Bbchit1 simultaneously. CDEP-1 and Bbchit1 were both placed under the promoter Pgpd and terminator TtrpC. These two expression cassettes were then ligated into pBANF-bar to form a recombinant plasmid, pBANF-bar-pAN52-gCDEP-1-pAN52-Bbchit1. By using Agrobacterium tumefaciens-mediated transformation system, CDEP-1 and Bbchit1 were integrated into the genome of a B. bassiana strain, Bb0062-15. 30 transformants were randomly selected from 256 herbicide resistance colonies and subjected to CDEP-1 and Bbchit1 activity assay in basal salt medium supplemented with 0.5% (v/v) glucose. Two transformants, named CG5 and CG7, showed high expression level of CDEP-1 and Bbchit1. Using third instar larva of Pieris rapae as target insect, we compared the virulence of CG5 and CG7, and CC2, a transformat highly expressed only CDEP-1, with that of the wild type. Bioassay results showed that, the LT50 of CG5, CG7, CC2 and wild type were 58.5h, 75.4h, 85.8h and 110.4h, respectively. Against Bb0062-15, the LT50 of CG5, CG7 and CC2 was reduced by 47.1%, 31.7% and 22.3%, respectively. In comparison with wild type strain, Bb0062-15, the average weight of food consumed by one larva infected with CG5, CG7 and CC2 was reduced by 54.5%, 36.3% and 18.2%, respectively. These results indicate that by overexpressing simultaneously both of CDEP-1 and CDEP-1 can enhance the killing speed of B. bassiana.

F-2 MYIOPHAGUS UCRAINICUS, A CHYTRIDIOMYCETE FUNGAL PATHOGEN OF SPODOPTERA FRUGIPERDA IN NON-IRRIGATED RICE IN COLOMBIA

Richard A. Humber, *USDA-ARS Plant Protection Research, USA*; Ruber J. Delgado C., *Apartado Aero No. 03, Chigorodo Antioquia, COLOMBIA*

Abstract: The fungus Myiophagus ucrainicus (Chytridiomycetes; probably Blastocladiales) was repeatedly found from 1981 through 1997 as a pathogen of fall webworm, Spodoptera frugiperda (Lepidoptera: Noctuidae), on non-irrigated rice in Antioquia, northwestern Colombia. Epizootic outbreaks of this fungus repeatedly caused significant mortality of the host; these are the only known occurrences of Myiophagus ucrainicus from any lepidopteran host and constitute a first report of this genus from South America. Field observations allowed many insights about the life history of Myiophagus and about how a watermold can operate successfully against terrestrial insects. The production of posteriorly uniflagellate zoospores was observed from the fungus in cadavers drowned in the rainwater pools standing on the soil surface below the plants. Infected larvae on the surfaces of the rice leaves were usually found to be filled with golden-brown to orange-yellow masses of the resistant sporangia having thick, walls and a prominently raised (mostly hexagonal) reticulation of the spore surface. The placement of Myiophagus in the order Blastocladiales within the Chytridiomycetes is discussed.

F-3 A NOVEL TECHNIQUE TO INOCULATE CONIDIA OF ENTOMOPATHOGENIC FUNGI AND ITS APPLICATION FOR INVESTIGATION OF SUSCEPTIBILITY OF THE JAPANESE PINE SAWYER TO BEAUVERIA BASSIANA

Mitsuaki Shimazu, *Forestry and Forest Products Research Institute, JAPAN*

Abstract: The Japanese pine sawyer, Monochamus alternatus (Coleoptera: Carembycidae) is the most important pest insect of pine forests in Japan, because it vectors the pinewood nematode, Bursaphelenchus xylophylus, the causative agent of pine wilt disease. We have demonstrated that Beauveria bassiana is an effective control agent for this insect. Recent experiments revealed that this fungus kills adults of M. alternatus in around 14 days when the adults walked on nonwoven fabric strip formulation of B. bassiana. Virulence of a fungus using for this system cannot be analyzed in a conventional manner, such as dipping of insects into conidial suspensions, because the insects in the case infected with by walking on dry conidia. A novel technique to measure the virulence of B. bassiana by exposing dry conidia on tarsus of adults was developed to evaluate effectiveness of nonwoven fabric strip formulation of this fungus for controlling adults of M. alternatus. To regulate inoculum density without suspending conidia in water, dead conidia were made by heating at 100C for 1 h, and a step dilution series of conidia were prepared by mixing dead conidia with live conidia in different ratios. The conidial mixtures were attached onto tarsus of CO2-anesthetized adults using a fine hairbrush. The 50% lethal doses determined by this method at 14 d were 5.5 x 106 conidia/individual for adults over 10 days after emergence (aged adults) and 1.9 x 106 conidia/individual for those within 4 days (young adults), and those at 30 d were 2.8 x 105 conidia/individual for aged adults and 2.4 x 104 conidia/individual for young adults. The number of conidia produced on a nonwoven fabric strip was 3.5 x 108 conidia/cm2, and adult beetles which walked on the strip got 8.5 x 105 conidia/individual. Based on the results, the validity of the biological control method for M. alternatus to prevent vectoring the pine wilt disease was discussed.

F-4 MOLECULAR CHARACTERISATION OF BEAUVERIA BASSIANA ISOLATES OBTAINED FROM OVERWINTERING SITES OF SUNN PESTS IN WEST ASIA AND THE MIDDLE EAST

Marilena Aquino de Muro, Sarah Elliott, *CABI Bioscience, UK*; David Moore, *CABI Biosciene, UK*; Bruce Parker, Margaret Skinner, William Reid, *University of Vermont, USA*; Mustapha El Bouhssini, *ICARDA, SYRIA*

Abstract: Wheat and barley, very important food crops in the Near East, Middle East, and South-Western Asian countries, are attacked by complexes of bugs called Sunn Pests (Hemiptera). These can cause extensive crop loss over the 15 million hectares affected annually. Eurygaster integriceps (Scutelleridae) is the most important economic pest species, but the complexes include Eurygaster maura, and Aelia, Carpocoris and Dolycoris (Pentatomidae) spp. Natural biological control plays an extremely important role in the regulation of Sunn Pest populations. One possibility for biological control is to develop mycoinsecticide products based on B. bassiana to control summer or winter Sunn Pest populations. One hundred and twelve isolates of Beauveria spp (106 Beauveria bassiana, 5 Beauveria spp and 1 Beauveria brongniartii) were obtained from Sunn Pests (Eurygaster and Aelia spp), litter and other insect samples at overwintering sites in seven countries in the Middle East and West Asia. DNA was extracted from these isolates and four techniques were used to characterize and investigate the genetic diversity of these at the molecular level: ISSR-PCR (inter-simple-sequence-repeat-anchored polymerase chain reaction), AFLP (amplified fragment length polymorphism), ITS-RFLP (internal transcribed spacer restriction fragment length polymorphism) and ITS sequencing. The ITS-RFLP and ITS sequences did not detect genetic variation among the isolates. However, our results from both ISSR-PCR and AFLP analyses gave clear indications of genetic diversity among the isolates and revealed some intraspecific groupings related to geographical origin, but no association with host. There was no grouping of B. bassiana isolates from Eurygaster integriceps, perhaps suggesting the overwintering populations were infected by generalist isolates, rather than host-specific ones most suitable for biocontrol purposes.

F-5 BEAUVERIA CALEDONICA AS A NATURALLY OCCURRING PATHOGEN OF HYLASTES ATER AND HYLURGUS LIGNIPERDA IN NEW ZEALAND

Travis Glare, *Agresearch, NEW ZEALAND*; Stephen Reay, *SBFR, NEW ZEALAND*; Tracey Nelson, *AgResearch, NEW ZEALAND*

Abstract: The fungal genus Beauveria is well known as containing species which are pathogenic to a range of insect species. However Beauveria caledonica is an exception. This fungus was originally isolated from moorland soil in Scotland and has not been reported as a pathogen. Recently, we began searching for pathogens of two exotic bark beetles, Hylastes ater and Hylurgus ligniperda (Curculionidae: Scolytinae), which are pests of plantation pines in New Zealand. In particular, H. ater is a damaging pest of seedlings. During routine surveys, Beauveria-infected beetles of both species were commonly found. Some of these strains were consistent with B. bassiana, commonly found in New Zealand on many insect species. However, other strains had longer conidia and were often pink in colouration on standard media. Sequence analysis using rDNA showed these unusual strains to be identical to B. caledonica. Bioassays of several B. caledonica strains from two geographically distinct areas in New Zealand demonstrated pathogenicity to both the larvae and adults of both bark beetles. This discovery means that now all commonly recognised species of Beauveria are confirmed insect pathogens.

F-6 EFFECTS OF SELECTED PESTICIDES ON THE GROWTH AND GERMINATION OF CONIDIA OF THE APHID PATHOGENIC FUNGUS ERYNIA NEOAPHIDIS REMAUDIERE ET HENNEBERT

Cezary Tkaczuk, *Department of Plant Protection, University of Podlasie, POLAND*

Abstract: Erynia neoaphidis (Zygomycetes, Entomophthorales) is one of the most widely distributed fungal pathogen of aphids, and it is an important natural factor for reducing pest aphid number in many crops. The use of insect pathogenic fungi as microbial control agents often needs to be integrated with the use of different agrochemicals and particularly pesticides. Successful integration requires detailed knowledge of the compatibility of the pesticides and pathogens. The aim of this study was to determine the influence of selected pesticides on conidia germination of fungus Erynia neoaphidis. 16 pesticides were used in the experiment. Chlorothalonil, copper, difenconazole, mancozeb, procymidone, sulphur and triadimefon were chosen from fungicides, alfa-cypermethrin, deltamethrin, fenitrotion, fozalon and pirimicarb from insecticides and chizalofop-P-ethyl, glifosat, MCPA and pendimetalin from herbicides. Three concentration of insecticides and herbicide were used : 0,1; 1 and 10 times recommended field rate. For fungicides instead of 10 times higher rate 100 times lower than recommended one was used. Pesticides were added to the sterilized medium. Plates were kept at 200C and the colony diameter was measured 5, 10, 15, 20 days after inoculation. To determine the effects of pesticides on germination of conidia microscope slides were covered with thin layer of water agar containing the concentrations of pesticides described previously. Slides with agar containing pesticides were exposed to the shower of primary conidia ejected from sporulating mummies of A. pisum infected by E. neoaphidis. Germination of primary conidia was assessed microscopically 6 and 12 hours after collection of the conidia. Most of tested pesticides strongly inhibited growth and germination of conidia of E. neoaphidis in vitro. Among all the fungicides used in the experiment, mancozeb, copper and sulphur showed the strongest inhibiting effect on the growth of fungus. Procymidone and triadimefon seem to be the most selective to E. neoaphidis among investigated fungicides. Fungus was unable to grow on media containing herbicides at 1 and 10 times recommended field rate but MCPA prevented growth even at 0.1 field dose. The insecticides showed relative the least inhibiting effect on the growth of fungus. The most toxic was alfa-cypermethrin and the least were deltamethrin and pirimicarb. Among all the pesticides used in the experiment, fungicides showed the strongest inhibiting effect on germination of conidia.. Chlorothalonil and mancozeb were particularly toxic and prevented germination of spores in all concentrations. Procymidone and sulphur seem to be the most selective to E. neoaphidis. Insecticides fozalon and fenitrotion prevented germination of fungus conidia in all concentrations but deltamethrin and pirimicarb were less toxic. Among all the pesticides tested, herbicides exhibited a weak inhibitory effect on germination of conidia.

F-7 **HORIZONTAL AND VERTICAL TRANSMISSION OF ENTOMOPATHOGENIC FUNGI AND ENDOSYMBIONT BACTERIA IN APHID POPULATIONS**

Annette Bruun Jensen, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*; Lise Petersen, *Bioinformatics Centre, University of Copenhagen, DENMARK*; Lars Monrad Hansen, *Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, Department of Crop Protection, DENMARK*; Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*

Abstract: We will present the aims and scopes of a project, which was initiated spring 2004. The interactions between aphids and microorganisms will be the focus and these interactions will be elucidated using novel molecular techniques and bioinformatics tools. The eco-system subjected to study is cereals and the model organisms are the cereals aphids: Sitobion avenae and Rhopalosiphum padi (hosts), entomopathogenic fungi from Entomophthorales (horizontal transmission) and the endosymbiont bacteria Buchnera aphidicola (vertical transmission).
The specific aims:
1. Describe the genetic structure of aphid populations in Danish cereal with focus on their clonal distribution 2. Describe the variation of obligate insect pathogenic fungi on cereal aphids on three levels. A) between aphid species, B) between aphid populations, C) between aphid clones 3. Describe the variation of the endosymbiont Buchnera in cereal aphids on three levels. A) between aphid species, B) between aphid populations, C) between aphid clones 4. Elucidate basal evolutionary processes of the three groups of organisms by including transmission mechanisms, selection pressures and genetic variation. 5. Evaluate the significance of horizontal and vertical transmitted microorganisms on aphid populations with respect to different biological control strategies in the eco-system studied

F-8 **EFFECTS OF DETRIVORES ON A PLANT HERBIVORE ENTOMOPATHOGEN SYSTEM.**

Karsten Dromph, Jakob Magid, Jørgen Eilenberg, Peter Esbjerg, *The Royal Veterinary and Agricultural University, DENMARK*

Abstract: The aim of a recently initiated project is to study how changes at one trophic level, the detrivore fauna in the soil, affect the interactions at higher trophic levels between plants and herbivores and between herbivores and their pathogens. It has previously been shown that the detrivore community, including collembolans, have an important impact on the mobilisation of plant nutrients. This does not only affect the growth of the plant, but also its quality as host plant for herbivores, which has a marked effect on the interaction between the herbivores and their natural enemies. However, the importance of these trophic connections still has to be tested using a more holistic approach. The objectives of the present project is elucidate such links between trophic levels by utilising a model system consisting of winter wheat grown in microcosms where the populations of soil dwelling detrivores, collembolans, herbivores, aphids, and their natural enemies, entomopathogenic fungi, are manipulated. This allows us to use isotope tracers of nitrogen and carbon to study the impact of the soil fauna on decomposition of isotope labelled organic matter and the resulting mobilisation and flow of nutrients through the food web. The effect of the altered nutrient flow on the performance of the aphids and their susceptibility to infection with entomopathogenic fungi will be measured in parallel experiments utilising a similar system, but without the isotope labels. The applied methology and preliminary results for nutrient flow and aphid development and susceptibility will be presented and discussed.

F-9 **BIOLOGICAL CONTROL OF VARROA DESTRUCTOR DISSEMINATION AND IMPACT OF SPORE INOCULUM**

Caroline Birchall, *Rothamsted Research, UK*; Gillian Davidson, *Warwick HRI, UK*; Brenda Ball, Judith K. Pell, *Rothamsted Research, UK*; David Chandler, *Warwick HRI, UK*

Abstract: Varroa destructor is a damaging ectoparasitic mite of the European honey bee, Apis mellifera. It originates in Asia, but has extended its range and now causes severe damage to A. mellifera populations worldwide. Adult female mites feed on the haemolymph of adult bees and brood, and in doing so can activate and transmit honey bee viruses. This causes a reduction in the size and health of the colony and subsequent decline in pollination efficiency and honey production. Currently, beekeepers attempt to control mite populations with a range of acaricides, but chemical resistance has developed rapidly and alternative, sustainable methods of control are urgently needed. We are investigating entomopathogenic fungi as potential microbial control agents of V. destructor. Initial laboratory bioassays identified isolates of fungi that were pathogenic to V. destructor under the abiotic conditions of the honey bee colony, but that had low impact on bees and other beneficials. Two isolates of Beauveria bassiana, four of Metarhizium anisopliae and two of Lecanicillium lecanii are being assessed further for impact on bee and mite populations. We are examining the effects on V. destructor and bees of fungi applied as conidial powders to bee populations. The transfer of inoculum between foragers and nurse bees, and the dissemination of fungus from bees to mites is being investigated over a range of spatial scales caged laboratory populations, observation hives and nucleus colonies. Honey bees dusted with conidia powder are likely to acquire inoculum orally as a result of grooming behaviour or by the ingestion of contaminated pollen loads. To evaluate the susceptibility of bees to fungal infection by this route we will feed honey bee adults and larvae conidia as powder mixed with pollen or in suspension. The results of these experiments will be presented.

F-10 **THE COST ACTION 842: STATUS OF RESEARCH ON ENTOMOPHTHORALES IN EUROPE**

Siegfried Keller, *Federal Research Station for Agroecology and Agriculture, SWITZERLAND*

Abstract: The COST action 842 Biological control of pest insects and mites with special reference to Entomophthorales entered into force on 16th December 1999 and will end with a half year extension on 15th June 2005. The action is signed by 17 countries which demonstrates the huge interest in biological pest control. The aim of the action is the promotion of the entomopathogenic fungi of the order of the Entomophthorales (working groups 1-3) and the development of biological methods to control pest insects and mites feeding on stored products (working group 4 lead by L. Stengaard Hansen). Working group (WG) 1 (leader: J. Eilenberg) covers biodiversity and population biology, WG 2 (leader: R. Meadow) covers selection, production, formulation and application, and WG 3 (leader C. Santiago Alvarez) covers performance, risk assessment and registration. The fungus order of the Entomophthorales contains more than 240 species that attack specifically insects and mites. About 60 species are known to infect and kill pest arthropods and vectors of human diseases. Economically important arthropods are among their hosts like spider mites, thrips, aphids, flies, plant- and leafhoppers, locusts and grasshoppers, lepidopteran and tenthredinid larvae. They posses an enormous potential for microbial control (both by direct release and by conservation and environmental management) which has not been explored sufficiently. The members of the working groups 1-3 focus on some specific issues to overcome these difficulties. The main emphasis is on species and population ecology (for example life-cycles) and on strain selection and cultivation. The main activities are regular workshops with the aim to discuss specific subjects, for examplemethods for preparation of specimens s, methods for species identification,, methods to assess host and pathogen densities in the field

F-11 **INFLUENCE OF ZN ON GROWTH AND PRODUCTION OF ORGANIC ACIDS BY PAECILOMYCES FUMOSOROSEUS IN SOLID AND SUBMERGED CULTURE**

Ali Asaff, *UAM-Iztapala, MEXICO*; Octavio Gómez, Carlos Cerda, Mayra De la Torre, *CINVESTAV, MEXICO*; Gustavo Viniegra, *UAM-Iztapalapa, MEXICO*

Abstract: Paecilomyces fumosoroseus was cultivated in submerged (SmF) and solid statefermentation (SS) using a chemically defined medium. The main metabolites produced in both cultures were 2,6pyridindicarboxylic acid (DPA) and oxalic acid (OXA). Several entomopathogenic fungi secrete those acids that seem to play a role in pathogenesis and are toxic against certain order of insects. Therefore, the effect of the culture conditions on the kinetics production and yield of these acids were studied. In SmF experiments 500-ml Erlenmeyer flasks containing 150 ml of the media were inoculated with conidial suspension to a final concentration of 1.3 e 106 conidia mL-1. The flasks were shaken at 180 rpm. For SSF 250mL

Erlenmeyer flasks containing 20 mL of the same inoculated culture media adsorbed in 1 g of polyurethane foam (PUF) were employed. Incubation was carried out at 27 XC; samples were taking out and analyzed periodically. The effect of 100 gM and 2gM Zn2+ on the kinetics of fungal growth had the following results: .maximal specific growth rate, ŶM = 0.025 - 0.114 h-1, maximal biomass, XM = 13.28 V 22.47 g L-1, DPA/biomass yield YDPA/X = 0.006 0.0296 g g-1 and OXA/biomass yield YOXA/X = 0.1 0.4 g g-1 estimated using Logistic and LuedekingPiret equations. In SmF, Zn2+ enhanced fungal growth but decreased organic acid yields and under Zn2+ limitation, organic acid yields were enhanced but growth was decreased. Thus in SmF, low Zn2+ levels seem to be necessary to achieve high organic acids yields. In contrast in SSF Zn2+ had not effect on fungus growth neither on organic acid production. Citrate was excreted by the fungus in SSF. Apparently, Zn2+ limitation produces a bottle neck in the Tricarboxilic Acid Cycle.

F-12 **CONIDIAL COLOR IS IMPORTANT FOR SOLAR RADIATION TOLERANCE IN THE ENTOMOPATHOGENIC FUNGUS METARHIZIUM ANISOPLIAE VAR. ANISOPLIAE**

Gilberto Braga, *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, 14040-903,, BRAZIL*; Drauzio Rangel, *Department of Biology, Utah State University, USA*; Stephan Flint, *Dept. of Forest, Range and Wildlife Sciences, Utah State University, USA*; Anne Anderson, Donald Roberts, *Department of Biology, Utah State University, USA*

Abstract: Solar radiation tolerance in fungi is a multifactorial characteristic determined by morphological, physiological and biochemical factors. Information about the relative importance of each factor involved in solar-radiation tolerance would be of great importance for the production of strains more tolerant to solar-radiation by genetic and/or physiological manipulation of the fungus. Among the factors responsible for solar-radiation tolerance in fungi are pigments such as melanins and carotenoids present in mycelium and conidia. Pigments protect cells against the harmful effects of radiation by blocking penetration of radiation inside cells and by inactivating solar-radiation-induced toxic substances, especially free radicals induced by UV-A radiation. To determine the importance of pigmentation of M. anisopliae var. anisopliae conidia for solar radiation tolerance, we examined the effects of simulated solar radiation on the germination of four mutants with violet conidia (DWR 67, DWR 145, DWR 147 and DWR 149), five mutants with yellow conidia (DWR 69, DWR 142, DWR 144, DWR 146 and DWR 148) and two mutants with white conidia (DWR 180 and DWR 181) obtained from the wild-type strain ARSEF 23, which produces dark green conidia. The conidia of all strains were exposed to irradiance of 900 mW m-2 (weighted UV irradiance) for 2 h. The relative percent gemination was assessed after 12, 24 and 36 h of incubation. In general, tolerance was least in the white mutants, greater in the violet mutants, and then the yellow mutants compared to the green wild-type strain. ARSEF 23. However, significant differences in radiation tolerance were observed among mutants within each color group. Some yellow mutants, such as DWR 69 and DWR 142, had tolerances close to the wild-type strain. Part of this variation may be explained by varied tonality of pigmentation present within each color group, particularly among the yellow mutants. Green revertants obtained from the violet (DWR 149) and yellow (DWR 148) mutants displayed tolerance similar to that of the wild-type strain ARSEF 23, indicating that conidial pigmentation is one of the factors responsible for solar UV radiation tolerance in M. anisopliae.

F-13 **THE EFFECT OF AMMONIA ON CONIDIAL LONGEVITY OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE**

Tove Steenberg, Ole Kilpinen, *Danish Pest Infestation Laboratory, DENMARK*; Dave Moore, *CABI Bioscience, UNITED KINGDOM*

Abstract: Entomopathogenic fungi are currently being developed for control of chicken mites (Dermanyssus gallinae) and other arthropod pests in poultry production. Temperature and humidity levels in poultry houses are generally conducive for conidial germination, while the persistence of fungus inoculum might prove problematic, especially when conidial powders are applied in traps designed to remain active for several months. Gaseous ammonia is known to be fungistatic or even fungitoxic to some fungal species at 10-25 ppm, and thus may be potentially detrimental also to entomopathogenic fungi in poultry houses, where ammonia levels may reach up to 40 ppm. We studied the effect of ammonia on D. gallinae inoculated with high doses of dry conidia of B. bassiana and M. anisopliae. When mites were dusted with conidia and subsequently exposed to high levels of ammonia (250 ppm or higher) for 7 days, there were no significant adverse effect of ammonia on mite mortality. This indicates that ammonia will not influence the efficacy of entomopathogenic fungi negatively. Experiments are currently underway to study the effect of ammonia on the viability of dry conidial powders.

F-14 **INVESTIGATION OF THE SURVIVAL OF CONIDIA OF ENTOMOPATHOGENIC FUNGI WITH POTENTIAL FOR CONTROL OF VARROA DESTRUCTOR IN HONEY BEE COLONIES**

Gillian Davidson, *Warwick HRI, UNITED KINGDOM*; Caroline Birchall, Judith K. Pell, Brenda Ball, *Rothamsted Research, UNITED KINGDOM*; David Chandler, *Warwick HRI, UNITED KINGDOM*

Abstract: We are investigating entomopathogenic fungi as potential microbial control agents of the mite, Varroa destructor, which is a damaging ectoparasite of the European honey bee, Apis mellifera. In previous work, we have demonstrated that varroa is highly susceptible to entomopathogenic fungi. However, if a fungal biopesticide is to be used successfully, it is important to understand the persistence and fate of fungal inoculum. We have investigated the survival of fungal conidia at temperatures and humidities likely to be encountered in bee colonies. Conidia were placed onto polycarbonate membranes and maintained at 25 or 32.5aC and 40% or 70% RH for various time intervals up to 21 d. The upper temperature represents that of the drone brood rearing area in a colony in summer while the lower temperature represents that in broodless areas of the colony in summer. The relative humidities used in the experiment represent the average in brood areas in summer (40 % RH) and the maximum in brood areas (70% RH) during bouts of evaporative cooling. Membranes were then transferred to SDA plates at 25aC / 95% RH and germination measured after 24h. Isolates of Metarhizium anisopliae, Lecanicillium spp. and Hirsutella thompsonii behaved similarly and their survival could be described by the same equation. However isolates of Beauveria bassiana exhibited a different response. The survival of conidia was reduced at low humidity (40 vs 70 % RH) and high temperature (32.5 vs 25aC). For example, the incubation period under non-permissive conditions causing 50% germination of M. anisopliae conidia when transferred to SDA decreased from 95 h to 40 h as temperature was increased from 25aC to 32.5aC at 70% RH, and decreased from 95 h to 18 h as humidity was decreased from 70 % to 40 % RH at 25aC. Increasing the temperature from 25 to 32.5aC caused a larger shift in conidia survival at 70 % RH than at 40 % RH Even under the most favourable temperature / humidity combination (25aC / 70 % RH), conidia did not survive longer than an estimated 380 h of storage.

F-15 **PATHOGENS ASSOCIATED WITH THE ANT, MYRMICA RUBRA, IN ITS INTRODUCED AND NATIVE RANGE**

Eleanor Groden, Shicai Yan, Frank Drummond, *Department of Biological Sciences, University of Maine, U.S.A.*

Abstract: Myrmica rubra L. is a common species of ant in damp pastures, riverbanks and woodland edges in Europe and Central Asia. Although locally abundant in these habitats, M. rubra is not commonly considered pestiferous in Europe. This polygynous red ant has become a severe pest along the coast of Maine where it has become locally dense and aggressively stings humans, pets, and livestock. Research was initiated in 2002 to assess the presence of pathogens in introduced M. rubra populations on Mt. Desert Island in Maine, and in 2003 pathogens were surveyed in native M. rubra populations in the United Kingdom and Russia. On Mt. Desert Island, ME, cadavers were collected from middens associated with M. rubra at six sites in 2002 and three sites in 2003. Cadavers were surface sterilized and held at 100% RH to encourage sporulation of fungi or release of nematodes. In 2003, a subsample of cadavers were also surface sterilized, squashed on a microscope slide and observed under phase contrast microcopy for signs of other pathogen types. In the United Kingdom and Russia, middens were only found associated with M. rubra nests at one out of fourteen populations sampled. Therefore, colonies were collected from each site and held in the laboratory for 3 to 4 weeks. During this time, ants that died were surface sterilized and either incubated or squashed and observed microscopically for pathogens. Only Beauveria bassiana and Metarhizium anisopliae were recovered from cadavers collected from the introduced populations in Maine, whereas a greater diversity of pathogens were observed associated with the ants in their native range. In addition to B. bassiana and M. anisopliae, two other fungal pathogens, entomopathogenic nematodes, and a microsporidia were recovered from European sites.

F-16 **PRELIMINARY SURVEY OF ENTOMOPATHOGENIC FUNGI ASSOCIATED WITH THE AFRICAN ROOT AND TUBER SCALE STICTOCOCCUS VAYSSIEREI RICHARD (HEMIPTERA: STICTOCOCCIDAE)**

Leonoor Wijnans, Maurice Tindo, *International Institute of Tropical Agriculture, Humid Forest Center, CAMEROON*; Rachid Hanna, *International Institute of Tropical Agriculture, Biological Control Center for Africa, REPUBLIC OF BENIN*

Abstract: Stictococcus vayssierei Richard is a subterranean scale insect indigenous to the Congo Basin, where it is considered a major constraint to cassava production. A preliminary survey was undertaken in Cameroon to identify entomopathogenic fungi infecting S. vayssierei. Over 1100 adult scales were collected from 45 locations across Cameroon. Collections were made during a 3-months period at the end of the dry season and the beginning of the wet season (February 2004 - April 2004). Metarhizium anisopliae (Metschnikoff) Sorokin and Metarhizium flavoviridae Gams & Rozsypal were isolated from various locations, yet infection levels were lower than 1%. Additionally, a more detailed investigation was undertaken in one location in the center of Cameroon. Here large numbers of adult scales were collected from one field on two occasions. Before the start of the wet season, infection levels by Metarhizium anisopliae was 1.6 %, but increased to 23.5% after the start of the rains. More studies are clearly necessary to understand the importance of these fungi in the natural control of the scale and the role that environmental factors play in their persistence.

F-17 **CICADAPEPTINS, NEW AIB-CONTAINING PEPTIDES,**

Stuart B. Krasnoff, *Department of Plant Pathology, Cornell University, U.S.A.*; Donna M. Gibson, *USDA, ARS, Plant Protection Research Unit, U.S.A.*; Melissa Wagenaar, Ricardo Reátegui, James B. Gloer, *Department of Chemistry, University of Iowa, U.S.A.*

Abstract: Fermentation extracts of an undescribed Tolypocladium fungus derived from a Cordyceps teleomorph isolated from an Australian cicada yield a complex microheterogeneous family of novel non-ribosomal peptides containing 2 residues of alpha-aminoisobutyric acid (Aib). Complete structural elucidation of two major components of the peptide mixture, cicadapeptins I and II, was accomplished by amino acid analysis as well as mass and NMR spectral studies. Amino acid sequences of minor cicadapeptin analogs were deduced from mass spectra. Cicadapeptins display insecticidal activity as well as antibacterial activity against both gram positive and gram negative bacteria.

F-18 **STUDY OF THE SPORULATION OF PAECILOMYCES FUMOSOROSEUS VARYING CARBON AND NITROGEN SOURCE**

Ana Gabriela Osuna Paez, *Consejo Estatal de Ciencia y Tecnología, MEXICO*; Héctor Manuel Cárdenas Cota, *Centro de Ciencias de Sinaloa, MEXICO*; Rene Castro Montoya, *Facultad de Fisicomatemáticas, Ciudad Universitaria, MEXICO*

Abstract: The spores of entomopatogenic fungi Paecilomyces fumosoroseus have been used successfully in the control of diverse insects pests. The rice mixed with husk, maize, wheat and soybeen among others is used like solid medium to obtain spores in the microbial insecticide elaboration. These raw materials vary as far as the physical and nutritional conditions at each lot and as a consequence the sporulation levels are not constant and dificult to detect the problems in the production. For this reason the optimal sporulation of the Pfrd isolate P. fumosoroseus was determined in a the solid medium varying carbon and nitrogen sources. The composition of the solid medium (Fargues Medium) in g/L was: KH4PO4 0,39, NaHPO4.12 H2 1.06, MgSO4.7H2 0.60, KCl 1.0, NH4NO3 0.70 and bacteriology agar 20. The concentrations in g/L of glucose and yeast extract were of 30, 60, 90 and 3, 6, 9 respectively. Before that Fargues Medium solidified added 3 mL in sterile boxes of 49 mm of diameter. The suspension of spore of inoculate was obtained from 15 days culture in inclined tube in the medium with Saboraud-dextrose- agar (65 g/L), enriched with malt (10 g/L) and yeast (10 g/L) extract (Medium SDYM). The concentration and the volume of inoculate was of 5 x 106 spores/mL and 0.06 mL. Three inoculated boxes and three without inoculating were placed in acrylic boxes of 13.5 x 9.5 x 9.5 cm. The incubation of the boxes was 27 1 C with cycles 12 hours light/ 12 hours dark; varying the time of harvest (th) of 12, 15 and 18 days. The spores of the boxes were harvested by flood adding 120 mL of tween 80 to the 0.05%. The counts of spores were made by triplicate under a camera of Neubauer. The averages of total spores of each condition were obtained by means of a design of central composition with three variables of design with the methodology of response surface adjust a model of second order and the optimal one was obtained, using software STATISTICA 6.0. The optimal conditions of sporulation were th of 28 days and with a concentration in g/L of glucose and yeast extract of 42.01 and 9.80, respectively. The optimal production of spores was of 18.3 x 109 meaning 24 times superior to value obtained in SDYM Medium with the same isolate and diameter of box. The sporulation was significantly different when increasing th and the concentration in g/L of glucose and yeast extract (á = 0.05). With the equation of the model and the optimal values of glucose and yeast extract the different sporulation to th was determined. The sporulation to optimal th (28 days) was 1.2 times superior to traditional th (15 days); this optimal th for production aims would be not longer operative to adopt because it is duplicated and by consequence the costs.

F-19 **SUSCEPTIBILITY OF THE CEREAL APHID METOPOLOPHIUM DIRHODUM TO THE ENTOMOPATHOGENIC FUNGUS PANDORA NEOAPHIDIS ON GNA WHEAT**

Paresh A. Shah, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*; A.M.R. Gatehouse, *School of Biology, King George VI Building, University of Newcastle,, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

Abstract: Studies were carried out to determine if susceptibility of the cereal aphid Metopolophium dirhodum to the fungus Pandora neoaphidis was affected by wheat expressing snowdrop lectin (GNA). Aphid infection did not differ significantly between transgenic GNA and non-transgenic lines (91% and 82%, respectively). Fecundity was similar between P. neoaphidis-treated or untreated aphids (ca. 18 nymphs aptera-1. Time to infection was ca. 5 days for M. dirhodum with both varieties in two of three assays. Our results suggest that wheat expressing GNA would not compromise the efficacy of P. neoaphidis as a biocontrol agent.

F-20 **EFFECTIVENESS OF LOCAL FUNGAL ISOLATES FOR COLORADO POTATO BEETLE IN UZBEKISTAN**

Kerim K. Ergashev, Erkin N. Abdullaev, *Samarkand State University, UZBEKISTAN*

Abstract: On the territory of Zeraphshan valley and other district of the Uzbekistan was conducted the isolation of fungi from Colorado potato beetle cadavers and from soil samples. The cadavers were sterilized from surface and than the pathological material were homogenized in sterile water and the suspension was used for inoculation to the nutrient media. The samples from different depth of soil (5, 10, 15 cm) were using for preparation of the soil suspension with 5 double dissolutions. Each suspension was placed on three petry dishes with potato-dextrose medium contained antibacterial antibiotics penicillin (400 units) and streptomycin (300 units). As a result, we isolated 3 species of the entomopathogenic fungi, including Beauveria bassiana (13 isolates), Metarhizium anisopliae (6 isolates), and Paecilomyces fumosa-roseus (14 isolates). Frequency of isolations was following: B. bassiana 67%, M. anisopliae 8%, P. fumosa-roseus 25%. The spore productivity of isolates was from 6.5x105 to 1.0x106 per 1 cm2 of media surface. The most productivity isolates were tested on the second instar of Colorado potato beetles in the laboratory conditions. The fungi were grown on the potato-dextrose medium with agar at temperature 22-240C during 14 days. After fungal fruiting, the water suspension of spores was prepared helping 0.01% Twin. The water suspension of conidia with concentration around 5.0x108 per ml was tested. The experiments were conducted in 5 repetitions. The pest mortality was following: B. bassiana isolates from 15 to 100%; M. anisopliae from 85 to 95%; P. fumosa-roseus from 60 to 70%. The most effectiveness was showed some isolates of the fungi B. bassiana and M. anisopliae. These isolates will be recommended for the mass-production and for application in the Uzbekistan conditions.

F-21 **DIFFERENTIAL SUSCEPTIBILITY BETWEEN DIAPAUSING AND NON-DIAPAUSING COLORADO POTATO BEETLES (LEPTINOTARSA DECEMLINEATA) TREATED WITH BEAUVERIA BASSIANA**

C. Noronha, *Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, CANADA*; Mark Goettel, *Lethbridge Research Centre, Agriculture and Agri-Food Canada, CANADA*

Abstract: Mortality caused by Beauveria bassiana in diapausing beetles is generally considered to be very low when compared to non-diapausing adults. However there have been no studies to evaluate this difference; a better understanding of this difference in susceptibility is needed to exploit this pathogen to the fullest. A laboratory-based study was conducted to examine the difference in mortality in non-diapausing and diapausing CPB

adults following an inoculation with Beauveria bassiana. Newly emerged beetles from a laboratory colony were induced into diapause in an incubator. Twenty diapausing or non-diapausing beetles were placed in a petri dish (100x15mm) and were inoculated with either buffer (control), 104 or 106 conidia per cm2, using an airbrush. Treated adults were allowed to dry for 30 min before being transferred to 29.5ml plastic cups containing moist soil. Diapausing beetles were placed at the bottom of the cup and soil was placed over them; non-diapausing beetles were placed on the soil surface along with a leaf as food. The cups were placed in an incubator at 17C and 16:8 L:D and were checked every two days for a period of 30 days. Dead beetles were placed in a moist chamber to determine infection. Results show a higher percentage of mortality among the non-diapausing as compared to the diapausing beetles. At the end of 30 days, we observed 89% mortality among the non-diapausing beetles and 21% among the diapausing beetles at the 106 conidia /cm2 inoculation level. Beetles inoculated with 104 conidia/cm2 showed 20% mortality among the non-diapausing beetles and zero mortality among the diapausing beetles. No mortality was observed in the controls. An increase in mortality was observed beginning on day 12 in the non-diapausing and day 16 in the diapausing beetles. A similar study conducted with field-collected diapause-ready beetles gave similar results, with higher mortality being observed in the non- diapausing population. Further studies are under way with both field and lab-reared populations to confirm these results and determine if the physiology of diapause beetles plays a role in inhibiting disease development.

F-22 **METHODS FOR RISK ASSESSMENT OF BIOLOGICAL CONTROL PROGRAMS IN THE SAHELIAN REGION**

Eva Nølke Fisker, Niels Elmegaard, *The Danish National Environmental Research Institute, DENMARK*; Jørgen Eilenberg, *The Royal Veterinary and Agricultural University, DENMARK*; Christiaan Koyman, Jürgen Langewald, *The International Institute of Tropical Agriculture, BENIN*; Zakaria Ouambama, Abdoulaye Tonkoano, *AGRHYMET Regional Centre, NIGER*

Abstract: This Ph.D. project, which was initiated in 2003, is a part of 'A programme for environmentally sound grasshopper control in the Sahel' (PReLISS). The objective of the PReLISS project is to develop different integrated grasshopper management strategies. One component of these strategies is the application of Green MuscleTM, a fungal product developed by the LUBILOSA programme. A second component consists of classical biological control using a parasitoid wasp Scelio sp. This Ph.D. project will aim to evaluate the use of Green Muscle with respect to effects on non-target grasshoppers, transmission, and survival of the fungus in the field as well as to study the possibilities of using Scelio sp. as a biological control agent. Based on these practical case studies and a theoretical study on methods for risk assessment of biological control programmes, it is the ambition to identify limitations of current practices and to identify ways to improve the present methods. The study shall describe the strengths and weaknesses of methods to assess and compare risks of pest management strategies and give a general outline of what is needed for risk assessment of biological control agents. It shall provide guidance for application of the methods in the Sahel and identify limitations to their use. Effects of treatment with Green Muscle on two non-target grasshoppers, Pyrgomorpha cognata and Poekilocerus bufonius hieroglyphicus, were examined in 2003. Immediate effects of microbial pesticide appliction as well as residual effects of treatments applied in 2001 and 2002 were examined. Preliminary results show that both non-target species can get infected by Green Muscle, and a residual effect was found after one year. In order to examine the survival of the fungus in the field and hence the length of exposure to potential non-target organisms, soil samples were collected from newly treated plots as well as from plots treated 1 and 2 years ago. No spores from the product has so far been recovered from any of the soil samples. Experiments on transmission of Green Muscle between grasshoppers are currently being undertaken. The Ph.D. project is funded by RUF, DANIDA.

F-23 **ISOLATION AND CHARACTERISATION OF NATURALLY OCCURRING BEAUVERIA BASSIANA FROM VEGETATION SHOW HIGH DIVERSITY**

Nicolai Vitt Meyling, Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40 DK-1871 Frederiksberg C, DENMARK*; Mette Lubeck, *Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40 DK-1871 Frederiksberg C, DENMARK*

Abstract: Beauveria bassiana was isolated frequently from leaves of common hedgerow plants throughout the growing season in a Danish agroecosystem. A quantitative investigation was carried out in 2003 and isolates were obtained from grasses, stinging nettle and hawthorn, with highest frequencies on the lower leaves of nettle. In early May it was possible to find B. bassiana on all plant categories and the presence continued through July until the final sampling in September. Selected isolates from the locality were characterised by Universally Primed PCR, which revealed that genotypes of B. bassiana from hedgerow vegetation were different from isolates obtained from field soil in the same agroecosystem. Furthermore, the genetic diversity was much greater among isolates from hedgerow vegetation and hedgerow soil than among isolates from field soil. The described isolation method provides a valuable tool for quickly obtaining a large collection of diverse indigenous genotypes of B. bassiana (and potentially other insect pathogenic fungi) to be screened as biological control agents. In addition, it adds significantly to our knowledge of the occurrence, population structure and dynamics of B. bassiana in the field.

F-24 **BAUVERIA BASSIANA MUTANTS OVERPRODUCING PROTEASES SHOW DIFFERENT PROTEASE-PROFILE THAN PARENTAL STRAIN**

Andrea Alcazar-Pizaña, Magdalena Iracheta-Cardenas, Luis Galan-Wong, Hugo Luna-Olvera, Benito Pereyra-Alferez, *Universidad Autonoma de Nuevo Leon, MEXICO*

Abstract: The entomopathogenic fungi Beauveria bassiana is an effective alternative to control insect crop pests. The general mode of action involves several physical and biological factors. It is presumed that entompopathogenic fungi penetrate the insect cuticle by mechanical means and enzymatic action. These enzymes include proteases, chitinases and lipases. Two extracellular cuticle-degrading proteases produced by B. bassiana in liquid medium, bassiasin I and Pr1 with estimated molecular weights around 32 and 36 kDa respectively, have been previously purified and characterized. In this work we report protease overproducing mutants with different protease profiles than parental strain. Protease overproducing mutants were generated using mutagenesis with ultraviolet light. Mutants were screened on agar medium containing skimmed milk (AC), and selected for their ability to produce large clearing zones around the colonies. Almost two hundred protease overproducing colonies were screened and genetic stability was determined by repeated alternate subculturing on Dextrosa Sabouraud Agar (DSA) and AC. Nine mutant strains, named M29, M39, M41, M7, M24, M25, M26, M36 and M82 were stable for at least five repeated alternate subculturing and their protease profile were determined using casein zymograms. Mutants M7, M36 and M82 presented different protease profile, no trypsin-like activity was observed in any of the strains tested, but all of them showed chymotrypsin and elastin-like activity. Bioassays against two lepidopera larvae species and against grasshopper were done and results will be discussed.

F-25 **INSECT PATHOGENIC FUNGI AND PARASITOIDS AS NATURAL CONTROL AGENTS OF THE APPLE APHIDS APHIS POMI AND DYSAPHIS PLANTAGINEA**

Karin Westrum, Ingeborg Klingen, *The Norwegian Crop Research Institute, NORWAY*

Abstract: Insect pathogenic fungi and parasitoids are important control agents of aphids. In a survey in one conventional and four organic apple orchards in Norway insect pathogenic fungi and parasitoids as natural enemies of Aphis pomi and Dysaphis plantaginea were studied weekly in the summer 2002 and 2003. Four species of insect pathogenic fungi in the order Entomophthorales were observed in both apple aphid species: Entomophthora planchoniana, Neozygites fresenii, Erynia neoaphidis and Conidiobolus obscurus. The fungus N. fresenii caused an epizootic on A. pomi in one organic location and seemed to decrease the aphid population during the summer 2002. The highest mortality caused by fungal infection of A. pomi was 39,6 % and 33,3 % of D. plantaginea. Mortality caused by parasitoids was more important in A. pomi than in D. plantaginea and the highest parasitation recorded in A. pomi was 30 %. Four species of primary parasitoids hatched from A. pomi: Binodoxys angelicae, Lipolexis gracilis, Praon sp. and Ephedrus sp. Hyperparasitoids that hatched from A. pomi were: Dendrocerus carpenteri, Alloxysta pleuralis, Phaenoglyphis villosa and Asaphes suspensus. Only one individual of D. plantaginea was parasitized and this parasitoid was Ephedrus persicae.

F-26 **THE EFFECT OF METHOD USED ON OBSERVED INFECTION LEVEL OF NEOZYGITES FLORIDANA IN A TETRANYCHUS URTICAE POPULATION IN STRAWBERRY**

Inger Nordengen, Ingeborg Klingen, *The Norwegian Crop Research Institute, Plant Protection Centre, NORWAY*

Abstract: The Entomophthoralean fungi Neozygites floridana is an important mortality factor of the twospotted spider mite, Tetranychus urticae and of several other tetranychid mites in many crops. The N. floridana infection level observed in a host field population of T. urticae is, however, dependent on the method used to estimate it. Several methods have been used by different authors to estimate the N. floridana infection

level in T. urticae and Mononychellus tanajoa; a)Mounting of fresh mites for observation of one or more capillaconidia attached to the hosts surface; b)Mounting of fresh mites in a mixture of Amman's Blue and Hoyer's mounting medium for observation of hyphal bodies, restingspores or capillaconidia; c)Mounting of dead mites in lactophenol-cottonblue (LPCB), Hoyer's mounting medium, lactophenol-aniline blue or acetoorcein for observation of hyphal bodies, restingspores or capillaconidia; d)Incubation of live mites; e)Direct counting of fungal infected mites visible on the leaf surface of the host plant. The use of method a) is by some authors said to overestimate N. floridana infection levels in a M. tanajoa host population. Method c) are, however, said to give an underestimatien of the N. floridana infection level in T. urticae. In our study, the N. floridana infection level in T. urticae in strawberry was estimated by three different methods in one strawberry field throughout the summer 2003. These were the methods used; 1)A work effective washing technique that is used for the estimation of the T. urticae population level in strawberry was used for the estimation of N. floridana infection level. Washed out T. urticae were kept in 80% alcohol before healthy looking adult females were mounted in LPCB and chequed for N. floridana hyphal bodies; 2)Direct observation in a compound microscope for N. floridana infected T. urticae in washed out alcohol samples; 3)An incubation method where live T. urticae females were incubated and observed for death and N. floridana growth and sporulation. Our preliminary results show that the different methods yields different results. Estimating the infection level by method 1) gave a higher N. floridana infection level than method 2), that again gave a higher fungal infection level than method 3).

F-27 **INTERACTIONS BETWEEN PANDORA BLUNCKII AND ZOOPHTHORA RADICANS ISOLATES IN PLUTELLA XYLOSTELLA POPULATIONS**

A. Guzman-Franco, *Plant and Invertebrate Ecology Division, Rothamsted Research, School of Biosciences, University of Nottingham, UNITED KINGDOM*; S. J. Clark, *Biomathematics Unit, Agriculture and the Environment Division, Rothamsted Research, UNITED KINGDOM*; P. G. Alderson, *School of Biosciences, University of Nottingham, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

Abstract: There are few studies quantifying outcomes when more than one pathogen species infect the same host individual. The diamondback moth is susceptible to many entomopathogens including the fungi Zoophthora radicans and Pandora blunckii. How these two species interact with each other and with the diamondback moth is ecologically interesting and economically very important. If we understand some of these relationships we will have vital information for the future development of these species in microbial control programmes. Characterization of some biological attributes of isolates of each species was done in order to have enough information to select a few isolates, with different biological attributes, for in vivo interaction experiments. These included temperature requirements for growth, virulence and in vitro competitiveness. There were significant differences among the isolates and between species in their growth at different temperatures. The optimum temperature for both species was between 20 and 25C. Zoophthora radicans generally showed better growth at 25C. In the in vitro interaction experiment, the growth of three isolates from each species were unaffected by the proximity of other isolates or species growing in the same Petri dish. Some isolates significantly inhibited the growth of other isolates. In general, this inhibition was not related to temperature. One inhibitory isolate and one isolate that was strongly inhibited were selected from each species for in vivo interaction experiments, which are currently in progress, to determine the outcome of interactions that may occur in P. xylostella populations. The virulence of these same isolates was assessed in dose response assays against third instar P. xylostella larvae at 20 and 25C. The two P. blunckii isolates were more virulent than the Z. radicans isolates. The most inhibitory isolates of each species were also the most virulent against P. xylostella. Based on the partial sequence of the ITS region from the ribosomal DNA from the selected isolates, species-specific primers were designed, and these primers will be used to detect each species in the in vivo interaction experiments when identification from sporulation is not possible.

F-28 **EFFECT OF FUNGAL INFECTION ON THE REPRODUCTIVE POTENTIAL OF APHIDS AND THEIR PROGENY**

Jason Baverstock, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*; H. E. Roy, *Department of Life Sciences, Anglia Polytechnic University, UNITED KINGDOM*; S. J. Clark, *Agriculture and the Environment Division, Rothamsted Research, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

Abstract: Pandora neoaphidis (aphid-specific) and Beauveria bassiana (generalist) are commonly found species of entomopathogenic fungus that are being developed as biological control agents against aphids. These species require successful infection of a host to complete their life-cycle and to propagate. The time taken from initial infection until death of the host is dependent on the abiotic conditions and the species of both the pathogen and host. When infecting the pea aphid, Acyrthosiphon pisum, at 18oC, P. neoaphidis required approximately 4 days from initial infection until death of the host, which is followed immediately by sporulation. In contrast, at this temperature B. bassiana required 5-6 days until death of the host and a further 2 days until sporulation. The host aphid reproduces during the infection process of both species of fungus and continues to produce apparently healthy progeny until immediately prior to death. Potentially, the nymphs produced during the infection process may reduce the impact of these pathogens when used as biological control agents. However, body reserves that are used for reproduction may be utilised by the fungus during host-colonisation, and this could have a negative effect on either the reproductive potential of the host or the fitness of their progeny. Experiments were done to assess the effect of infection by P. neoaphidis and B. bassiana on the reproductive potential of A. pisum. Infection by both P. neoaphidis and B. bassiana reduced the number of nymphs produced within 24 hours of inoculation, with the total number of nymphs produced over the infection period being significantly lower than that of uninfected control aphids. Subsequent experiments indicated that infection by either P. neoaphidis or B. bassiana for 24 or 72 hours did not alter the intrinsic rate of increase of the host aphid's progeny. Fungal infection therefore appears to have a direct negative effect on the host aphid but no indirect effects on the fitness of the host's progeny. Implications for the use of P. neoaphidis and B. bassiana as biological control agents are discussed.

F-29 **EFFECT OF DIFFERENT CONIDIAL CONCENTRATIONS OF THE FUNGUS, VERTICILLIUM LECANII (ZIMM.)VIEGAS ON THE NET REPRODUCTIVE RATE (R0) OF THE PEA APHID, ACYRTHOSIPHON PISUM (HARRIS)**

S.A. Safavi, Aziz Kharazi Pakdel, G.R. Rasoulian, *Department of Plant Protection, Faculty of Agriculture, University of Tehran, IRAN*; H. Askari, *Research Institute of Forests and Rangelands, Tehran, IRAN*

Abstract: Effect of entomogenous fungus V. lecanii was studied on the net reproductive rate (R0) of the pea aphid, Acyrthosiphon pisum. Vertalec, a commercial product of fungus, was evaluated under controlled conditions. Second instar nymphs of aphid were placed on cutted alfalfa stems and were sprayed with a series of conidial suspensions varying in concentration from 104 to 108 conidia/ml 104, 105, 106, 107 and 108 conidia/ml and sterile distilled water (containing wetting agent) as control, and then caged. This units incubated at 231 oC and a photoperiod of 16:8 (L:D). Moist sponge maintained humidity at %973. Each treatment consisted of 30 aphids that was replicated three times. Four days after beginning the experiments, adults appeared and began to produce nymphs. The number of offspring was recorded daily for 12 days and removed from units everyday. R0 values were calculated from the equation Ro = lx. mx, where lx is the probability of surviving from day x to day x + 1 and mx is the average number of offspring produced by an individual on day x. R0 values decreased from 28.155.38 in control to 5.151.81 at 108 conidia /ml. Results of analyses of variance of R0 data showed a significant difference among concentrations (P<0.0001). There was a good linear relationship between log10-transformed R0 data and log10-transformed concentrations (R2=0.92). Slope and intercept of this regression line were 0.168 and 2.04, respectively. Although increment of concentrations resulted in significant decadence of net reproductive net (R0) in pea aphid, but in the used range of concentrations, R0 values didn't decrease to 1 or less and this showed that in long-time, fungus only regulates the aphid population increment to decrease it.

F-30 **COMPARISION OF TWO PROPAGULES TYPES OF BEAUVERIA BASSIANA AGAINST TRIALEURODES VAPORARIORUM.**

Javad Karimi, Aziz Kharazi Pakdel, Ali Mirshekar, *Dept. plant protection,Tehran univ., IRAN*

Abstract: Beauveria bassiana invades their host initially through yeast bodies, a obligatory parasitic cycle among most entomopathogenic fungi. virulence of B.bassiana (ATCC 74040) which cultured on SDAY in Yeast-like cycle toward third nymphal stage of Trialeurodes vaporariorum were investigated. The conidia of fungus in this phase developed by budding from germinating conidia after 24h incubation. Average size of cells were 5-10 μm. Colony of fungus were initially circular some later were cover with mycelia. On the basis of isolate type,timing and growth rate of yeast inoculum productin varied. Conidia and yeast-like had similar virulence against Trialeurodes vaporariorum , and average mortality were 65.4 % with 106 cells/ml. and 78.5 % with 10 7 cells/ml.,respectively. The results of bioassay indicated that the Yeast-like bodies are effective inoculum for B. bassiana against insects rather than conidia against some pests. Production of well-defined yeast phase culture may be essential work for using

mycoinsecticides.

F-31 **IN VIVO PATHOGENICITY OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE ON CHROTOGONUS TRACHYPTERUS(ORTH.:PYRGOMORPHIDAE).**

Ali Mirshekar, Aziz Kharazi Pakdel, *Dept. plant protection,Tehran univ., IRAN*; Mehran Ghazavi, *Plant Pest & Diseases Research Institute,Tehran, IRAN*; Javad Karimi, *Dept. plant protection,Tehran univ., IRAN*

Abstract: The Sugarcane grasshopper (Chrotogonus trachypterus) is one of the most important and harmful pest of field crops in Sistan region of Iran. Concerns over the environmental and human health impacts of chemical control of grasshoppers have led to considerable interest in developing of alternative control methods e.g. using entomopathogenic fungi. Effects of two native isolates of the fungus, Beauveria bassiana, a native isolate of Metarhizium anisopliae and Green Muscle, a commercialized formulation of Metarhizium anisopliae var. acridum , were investigated on the Chrotogonus trachypterus. After doing pathogenicity and bracketting tests, serial dilution for three isolates were prepared as followed : 102, 3/2102, 103,3/2103 ,104 spore/insect and 10,3/210,102,3/2102,103 spore/insect for native isolates of B.bassiana and M.anisopliae, respectively; finally, 104, 3/2104, 105, 3/2105 and 106 spore/insect were logarithmic doses for formulated product of M.anisopliae. Comparison between LD50 and LD95 off all isolates were demonstrated that native isolate of Metarhizium anisopliae had the lowest LD50 ; Other isolates had high pathogenicity rather than commercialized product .These results indicate that more studies in order to isolation of native strains of entomopathogenic fungi and determination of their effectivness on insect pests are essential

F-32 **ARE OLIGOPHAGOUS LABOULBENIALES SPECIES ACTUALLY SPATIALLY MONOPHAGOUS SPECIES?**

Larry Huldén, *Finnish Museum of Natural History, FINLAND*

Abstract: Laboulbeniales are ectoparasitic fungi, worldwide in distribution, occurring mainly on the adult stage of insects and a few other arthropodes. Spores of the parasite are transmitted by means of direct contact between two individuals of the hosts. The parasite can only exist on host species which have overlapping successive generations. In most cases they cause little or no detectable harm to the host. A number of Laboulbeniales species are known from only one host species, but many are known from several, usually closely related hosts. Polyphagous Laboulbeniales may be questional because of taxonomic problems.
Thorough studies of some oligophagous species unveil close dependance on one host species, although they may be found on several hosts in a specific locality. A particular main host seems to be a prerequisite for the prevailing of the parasite population in a single locality. Two cases of oligophagous parasites occurring on the beetle families Carabidae and Gyrinidae respectively were analyzed.
Laboulbenia fasciculata occurs on 4 species of Patrobus (Carabidae) in southern Finland. The four Patrobus species have quite different distribution patterns, but L. fasciculata coincides with the distribution of Patrobus atrorufus where the host is common in southern Finland. L. fasciculata has been found on the other Patrobus species only when P. atrorufus is present. Laboulbenia fennica was found on 8 species of Gyrinus (Gyrinidae) in southern Finland. G. aeratus seems to be the main host in Finland. The other Gyrinus species were infested only within populations of G. aeratus. In these cases the parasite species do not reach the northern range of the main hosts. The limiting factor is probably some shifts of the life cycle of the host. Successive generations of the host are possibly not overlapping or the host populations are too small north of a certain latitude. It is also possible that the main host may be replaced by another host in Central Europe.

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 6

Contributed Papers (Division of Viruses)

virus / contributed papers session 2

Chair: D. Lynn; J. Burand

15:00 **LEPIDOPTERAN CELL LINES AFTER LONG-TERM CULTURE IN A COMMERCIAL SERUM-FREE MEDIUM: COMPARISON OF GROWTH RATES AND BACULOVIRUS REPLICATION.**

Dwight Lynn, *USDA, Insect Biocontrol Laboratory, USA*

Abstract: Several manufacturers of cell culture media began marketing serum free medium (SFM) formulations for the growth of lepidopteran cells in the 1980's to capture the potentially lucrative market of using insect cell lines for production of proteins by the baculovirus expression vector. The first of these was Ex-cell 400 produced by JR Scientific (currently JRH Biosciences). In an effort to simplify maintenance of multiple cell lines in my laboratory, I tested Ex-Cell 400 for support of growth of over 20 insect cell lines. Of those tested, several grew poorly or not at all on the SFM (such as TN-368 and IPLB-TN-R2 from the cabbage looper) while others grew much better than on their normal medium (such as IPLB-Tcon and IPLB-Tex2 from Trichogramma wasps, and IPLB-Ld652Y, IPLB-LdEp, and IPLB-LdEIta from the gypsy moth). In general, when cell growth was as good or better on SFM, I subsequently maintained them on Ex-Cell 400 while if growth was poorer, cell line maintenance was continued on their original medium. In a few cases, cell lines were maintained on both SFM and serum-supplemented modified TC-100 (which is a modification of Grace's medium). Three of these lines, IPLB-LdFB from gypsy moth fat body, IPLB-LdEIta from gypsy moth embryos, and UFL-Ag286 from velvetbean caterpillar embryos have been maintained concurrently for over a year on both media. Cells grown in each medium were tested for susceptibility to and productivity of various nucleopolyhedroviruses (NPVs). LdFB and LdEIta were tested with Lymantria dispar NPV; LdEIta and Ag286 with Autographa californica NPV; and Ag286 also to Anticarsia gemmatalis NPV. The three lines chosen for these experiments fall into three categories of relative growth in SFM vs. TC100. LdFB cells grew similarly in each medium; LdEIta grew better Ex-Cell than in TC-100, while Ag286 grew better in TC-100 than in Ex-Cell. Even though disparity exists in growth rates between the two media for the different cell lines, endpoint assays suggest that cells grown in serum-containing medium are more susceptible to virus infect than their SFM counterparts. Alternatively, optimal virus productivity was consistently higher (15-30%) in each line that had been grown in SFM compared with the same cells in TC-100. The virus productivity results are consistent with an earlier study in my lab in which I compared virus replication in long- and short-term passage of the LdEIta and IPLB-Sf-21 cell lines, although in that case, both of the cell lines that were tested grow faster in SFM than in serum-containing medium. The contradictory nature of the results on susceptibility and productivity may simply indicate that, while it takes greater amounts of input virus to initiate an infection in the SFM-adapted cells, once a cell is infected it appears to produce more viral occlusion bodies.

15:15 **ALTERATION OF THE REPRODUCTIVE TISSUES OF HELICOVERPA ZEA FEMALES INFECTED WITH HZ-2V**

John Burand, Weijia Tan, Woojin Kim, *University of Massachusetts, USA*

Abstract: Productive replication of the virus Hz-2V in female Helicoverpa zea leads to malformation of the host's reproductive tissues and sterility of the adult moth. In addition, infected female moths produce 5 to 7 more times sex pheromone and attract twice as many male moths in flight tunnel experiments than do healthy females. This alteration of the development of reproductive tissues and the increased production of the pheromone in infected females creates conditions which favors virus replication in the insect host and the transmission of virus between individuals in the field. Analysis of the Hz-2V genome revealed the presence of a carboxylesterase (ORF-7) that codes for a 120 amino acid region which is homologous to the functional domain of the drosophila juvenile hormone esterase (JHE) gene. Upon examination of the level of JHE in female reproductive tissues during their development it was found that although JHE titers in tissues from healthy and infected insects followed the same pattern of decreasing as insect matured the JHE titers in the reproductive tissues of last instar, agonadal females where significantly lower than those in tissues from healthy females. This is surprising since we had also found that JHE levels in hemolymph, midguts and fat body from healthy and infected last instar females was the same. An examination of the reproductive tissues from these last instars showed that alterations in the development of these tissues had already started in last instar larvae.

15:30 **STU** **REPLICATION OF A NOVEL PICORNA-LIKE VIRUS OF THE GENUS IFLAVIRUS**

Juliette Ongus, Dick Peters, Just M. Vlak, *Wageningen University, THE NETHERLANDS*; Eberhard Bengsch, *Centre de Biophysique Moléculaire, CNRS, Orléans,, FRANCE*; Monique M. Van Oers, *Wageningen University, THE NETHERLANDS*

Abstract: Aggregations of 27-nm virus-like particles were observed in electron microscopy images of sectioned Varroa destructor mite tissue. The scattered occurrence and accumulation of the virus particles in lattices in the cytoplasm gave an apparent indication that the virus was replicating in the mite. The virus-like particles were isolated and purified. Sequencing of a 3' portion of the RNA genome revealed that this region encodes the putative non-structural proteins RNA-dependent RNA polymerase, protease and helicase, with a high sequence similarity to members of the genus Iflavirus. Phylogenetic analysis showed that the virus was most closely related to Deformed wing virus (DWV) and Kakugo virus (KV) with an overall RNA genome identity of 84% and polyprotein identity of 95%. 1455 bases in the 5' segment from the start of the open reading frame encoding two of the structural proteins showed the greatest diversion from DWV and KV having an RNA identity of 79%. This region is translated into a 485 amino acid sequence with an identity of 90%. Both, DWV and KV infect the honeybee Apis mellifera. The name of the new virus is tentatively proposed to be Varroa destructor virus 1 (VDV-1). To determine whether VDV-1 replicates in mites, a selective RT-PCR was done to detect the presence of the negative-sense RNA strand. Our virus isolate and the closely related DWV virus were discriminated by two sets of primers, each set specific to one virus. The results obtained showed that both viruses replicate in this mite species. The biological properties of VDV-1 in bees are under study.
Juliette.Ongus@wur.nl

15:45 **THE BIOLOGY AND CHARACTERISATION OF AN ASCOVIRUS (ASCOVIRIDAE) FROM AUSTRALIA**

Ian Newton, *University of Queensland, AUSTRALIA*

Abstract: Ascoviruses (Ascoviridae) are a group of enveloped DNA viruses that cause a chronic and lethal disease in insects. Most of the described ascoviruses are hosted in the larval stage of the Noctuidae (Lepidoptera). Unpublished reports indicated that an ascovirus was found at high incidence (>50%) in populations of Helicoverpa armigera (Hübner) and Helicoverpa punctigera (Wallengren) in southeast Queensland, Australia. These pathogens were thought to be vectored by the braconid parasitoid wasp Microplitis demolitor (Wilkinson). I have formally identified and characterised this ascovirus by examining; the genetic relationship to other known ascoviruses (using RFLPs, Southern blot hybridisations and sequencing the polymerase gene), the host range of the virus and the histopathology. The Australian ascovirus was similar to the Heliothis virescens (Fabricius) ascovirus (HvAV) and the Trichoplusia ni (Hübner) ascovirus (TnAV) from the USA. The Australian ascovirus isolated from H. armigera, was found to replicate, primarily in the fat body of Helicoverpa and Spodoptera hosts. To further understand the biology of the Australian ascovirus, I studied its transmission and interaction with hymenopteran parasitoids; The Australian ascoviruses were found to be vectored by the parasitoid M. demolitor. By using PCR and sequencing, ascovirus was detected on the hymenopteran parasitoids Heteropelma scaposum (Morley) and Netelia producta (Brullé). The virus can be vectored by mechanical transmission from a contaminated ovipositor (or even a pin), meaning any larval parasitoid that probes an ascovirus infected host could vector the virus. Ascovirus has an antagonistic effect on M. demolitor, by preventing development of the parasitoid larvae within the ascovirus infected host. I examined other alternative modes of transmission and its field prevalence in Australia. The Australian ascovirus appears to be an opportunistic pathogen; it may rely on multiple hosts, multiple vectors and multiple modes of transmission for dissemination and persistence in the field.

16:00 **A STUDY OF SINGLE NUCLEOCAPSID NUCLEOPOLYHEDROVIRUS ENVELOPE PROTEIN P74 REQUIRED TO THE INFECTION OF HOST MIDGUT**

Lun-Guang Yao, Wen-Ke Zhou, Feng Yan, Hua Xu, Yong Zheng, Yi-Peng Qi, *Wuahn University, CHINA*

Abstract: In order to study the function of envelope protein P74 of HaSNPV and AcMNPV, two p74Cnull recombinant baculovirus, rHa-gfpp74 and rAc-gfpp74, was constructed by inserting gfp driven by AcMNPV polyhedrin promoter into the p74 locus of HaSNPV and AcMNPV genome respectively. The resulting p74-inactivation occlusion-derived viruses (ODV) failed to infect their natural host larvae, Spodoptera exiga or Helicoverpa armigera, per os. However, when fed with 106 mixture virus OBs (wtHaSNPV: rHa-gfpp74 or wtAcMNPV: rAc-gfpp74=1:9), almost all the tested host larvae were infected and died, and the infected larvae turned green 3 d. p.i and liquefied in the end. Feeding the purified homologous P74 proteins along with the BVs or p74 deletion mutant OBs respectively to their host larvae resulted in the recruitment of oral infectivity in a dose dependent manner, and the mortality went higher with the increase in the concentration of P74 protein solution, but the heterogenous P74 cant rescue their oral infectivity. After dipped the midgut of S. exiga or H. armigera in the homologous p74 protein solution , the midgut surface formed holes and became more illegible under the electronic microscopy, but those treated with heterogenous P74 remained smooth and clear. In addition, the specific and saturable binding of P74 proteins to their host Brush Border Membrane Vesicles (BBMV) was involved in the invasion of virus. The binding tests by ELISA showed that the homologous P74 proteins could bind their host midgut BBMV, but not bind heterogenous BBMV. Further investigation showed an about 30 KD protein in BBMV was involved the specific binding by pull-down assay. The P74 protein was expressed in-frame with GFP to create a P74-GFP chimera for studying the localization of P74. The GFP portion of the chimera facilitated visualization of the trafficking of P74 in cells. When infected Hz-AM1 cells, the green fluorescence appeared mostly in the cytosol at 24h p.i.. then it was mostly concentrated in the nucleus at 36 h p.i., after this it emerged mainly in the intranuclear ring zone of nucleus at 48 h p.i. and finally the fluorescence protein accumulated into vesicles in the intranuclear ring zone, forming a green ring in the nucleus. These results demonstrated that P74 protein is essential for oral infectivity of ODV and play a role in binding with putative receptor at epithelial cell of midgut.The specific effect of P74 indicated it was a host-range determing factor.

16:30 **WSSV INTERACTION WITH FRESHWATER CRAYFISH**

Pikul Jiravanichpaisal, Kenneth Söderhäll, Irene Söderhäll, *Department of Comparative Physiology, Evolutionary Biology Centre, Uppsala university, SWEDEN*

Abstract: The signal and nobel freshwater crayfish, Pacifastacus leniusculus and Astacus astacus respectively, were found to be susceptible to infection with the white spot syndrome virus (WSSV). Histopathological observations of various tissues of virus-infected crayfish showed similar symptoms to those from WSSV-infected penaeid shrimp with an appearance of orange spots on the cuticle in some infected crayfish. The effect of water temperature on the development of white spot disease in crayfish was also studied. Crayfish were exposed to different temperatures after WSSV injection or oral exposure and the mortalities were recorded over a period of 45 days. No mortality was observed when crayfish were held at 4 2 C or 12 2 C and reached 100% when these crayfish transferred to 22 2 C. The mortalities of nearly moribund crayfish at 22 2 C with WSSV could be delayed after transfer to temperatures below 16 C. These results clearly show that low temperature affects the WSSV pathogenicity in crayfish. Haemocyte counts, phenoloxidase activity, mRNA levels of prophenoloxidase (proPO) and the lipopolysaccharide and beta-1,3-glucan binding protein (LGBP) and cell proliferation of haemopoietic tissues in crayfish exposed to various water temperatures were also studied. Only proliferation of the haematopoietic tissues was found to be significantly different at various temperatures, it was higher at high temperatures which may support replication of WSSV, and also explain the high mortality of crayfish with WSSV infection at high temperature. Moreover, replication of WSSV was investigated in haematopoietic stem cell (HSC) cultures from the crayfish. WSSV replicated in the cells, as evidenced by in situ hybridization. Similar to in vivo study, the infectivity of WSSV is dependent on temperature. At higher temperature WSSV replicated more rapidly than at lower temperature. Detectable WSSV-infected cells were present as early as 48 h post-inoculation by using in situ hybridization after incubation temperature was increased to 25 C.

16:45 **DENSOVIRUSES (DNVS) WITH AN AMBISENSE GENOME ARE HIGHLY DIVERSIFIED IN THEIR MODE OF EXPRESSION**

Max Bergoin, Yi Li, Adly Abd-Alla, *Laboratoire de Pathologie Comparée, Université Montpellier II, FRANCE*; Gilles Fédière, *Institut de Recherche pour le Développement, Faculty of Agriculture, Cairo University, EGYPT*; François Cousserans, Elizabeth Baquerizo, Françoise-Xavière Jousset, *Laboratoire de Pathologie Comparée, Université Montpellier II, FRANCE*; Peter Tijssen, Mohamed El-Far, *INRS-Institut Armand-Frappier, Université du Québec, CANADA*

Abstract: According to their genomic organization and terminal structure, densoviruses (DNVs) are clasified within the subfamily Densovirinae into 3 genera: Densovirus, Iteravirus and Brevidensovirus. Members of the genus Densovirus have a linear single-stranded genome 5.5-6 kb in length, long inverted terminal repeats (ITRs) and an ambisense organization, i.e. sequences coding for structural (VP) and non structural (NS or Rep) proteins occupy each the 5' half of one strand. Despite these similarities, our recent studies on transcription modalities of 5 members of this genus revealed a great variety in the way their genes are transcribed and their messenger RNAs are translated. Based on this diversity, at least 3 subgroups emerge within the genus Densovirus that parallel the classification of their hosts. The first subgroup which includes DNVs isolated from lepidoptera is very homogeneous. As exemplified by JcDNV (1), GmDNV (2) and MlDNV (3), all these DNVs have 6kb genomes and similar organization of their genes. They share 80-90 % identity in their coding sequences and their capsid polypeptides are serologically related. The structural polypeptides are synthesized from a single unspliced mRNA generating 4 N-terminally extended isoforms by a leaky scanning mechanism. The 3 NS proteins are

translated from an unspliced mRNA for NS-3 (5' most ORF) and by alternative initiation from a spliced mRNA for NS-1 and NS-2. The second subgroup is less homogeneous and includes DNVs isolated from Orthoptera, Dictyoptera and possibly Homoptera possessing a 5.5 kb genome. The most striking feature of this subgroup, as exemplified by the Acheta domesticus DNV (AdDNV) is that the coding sequences for VPs are split into two ORFs, the 5' proximal containing the AUG initiation codon of VP1. As a consequence, synthesis of VPs in this subgroup results from a double mechanism of splicing and leaky scanning. The mechanism of expression of NS polypeptides is similar to that of subgroup one. The third subgroup has so far for unique representative the CpDNV isolated from the mosquito Culex pipiens (4). The 6 kb genome of this virus is characterized by the fact that the N-terminal and C-terminal coding sequences of both NS-1 and NS-2 are split into two ORFs. A splicing setting in frame the four ORFS allows the synthesis of NS-1 and NS-2. Precise transcription maps of the 3 subgroups including structure of promoters, transcription starts, polyadenylation sites, and splicing will be presented.
(1) Dumas et al., 1992, Virology, 191, 202-22, (2) Tijssen et al. 2003, J. Virol., 77, 10357-65 (3) Fedière et al. 2004, Virology, 320, 181-9, (4) Jousset et al;,2000, Virus Res., 67, 11-16

17:00 **SPLTMNPV BLOCKS SEMNPV-INDUCED APOPTOSIS IN A SPODOPTERA LITURA CELL LINE**

Mei Yu, Kai Yang, Lei Lv, Lijing Pan, Yi Pang, *State Key Laboratory for Biocontrol & Institute of Entomology,Zhongshan University, CHINA*

Abstract: Inoculation Spodoptera exigua derived Se301 cells with SeMNPV (S. exigua multiple nucleopolyhedrovirus) resulted in successful infection as shown by the presence of occlusion body (OB). The same result can be seen when Sl-zsu-w cells, which derived from Spodoptera litura, inoculated with SpltMNPV (S. litura multiple nucleopolyhedrovirus). Here we report that inoculation of Se301 cells with SpltMNPV resulted in no OB producedhowever, Sl-zsu-w cells infected with SeMNPV show some characteristics of apoptosis, including detaching from the culture flasks, losing cytoplasmic extensions, becoming round and the plasma membrane blebbing. Molecular change such as nuclear fragmentation and increased Caspase-3-like protease activity had also been detected in Sl-zsu-w cell infected with SeMNPV. Sl-zsu-w cells were infected with SeMNPV at different input m.o.i. of 1,5,10 or 20 and the caspase-3-like protease activity at 48 h p.i. showed a linear increase from m.o.i. 0.1 to m.o.i. 10, reached the climax at m.o.i. 10. Oligomeric DNA laddering was detectable by 24 h p.i and clearly observed at 48 h p.i and increased up to 96 h p.i. Infection of Sl-zsu-w cell with SpltMNPV at least 24 hours prior to infection with SeMNPV can prevent apoptosis-like cell death. The cells infected with SpltMNPV prior to SeMNPV showed less fragmented DNA compared to the cells infected with SeMNPV alone, and caspase-3-like protease activity was also lower than that of SeMNPV-infected cells. These results provided evidences for the hypothesis that apoptosis is a factor of anti-viral system of insects and SpltMNPV have some factors can block apoptosis in Sl-zsu-w cells. Three antiapoptotic types of genes, iap, p35 and p49, were found in baculovirus and SpltMNPV posed p49 and iap. Functional analysis with Sf-9 cells transiently expression SpltMNPV-p49 further showed that SpltMNPV P49 exhibited clear apoptosis suppressing activity. It is possible that the accumulation of SpltMNPV P49 in Sl-zsu-w cells can block the SeMNPV-induced apoptosis and make SeMNPV replicated in the non-permissive cell.

17:15 **THE ANTICARSIA GEMMATALIS NUCLEOPOLYHEDROVIRUS (AGMNPV) GENOME**

Jose Luiz Caldas Wolff, *Laboratrio de Virologia Molecular, Nucleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes Mogi das Cruzes, SP, BRAZIL*; Bergmann Morais Ribeiro, *Departamento de Biologia Celular, Universidade de Brasilia, Brasilia DF, BRAZIL*; Alejandra Garcia-Maruniak, James Maruniak, *Entomology & Nematology Department, University of Florida, Gainesville, FL, USA*; Flavio Moscardi, *Embrapa/CNPSO, Londrina, PR, BRAZIL*; Marlinda Lobo de Souza, Maria Elita Batista de Castro, *Embrapa/CENARGEN, Brasilia, DF, BRAZIL*; Paolo M. de A. Zanotto, *Instituto de Ciencias Biomedicas, USP, Av. Lineu Prestes, Sao Paulo, SP, BRAZIL*

Abstract: The Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV) is the most important and widely applied biological control agent in Brazil. Currently it is used regularly in 1.7 million hectares of soybean crops, with an estimated demand for 4 million additional hectares. Given its economic importance for the Brazilian agriculture, studies have been undertaken on the genetic and genomic stability of temporal and geographical isolates and on the characterization and function of several individual genes of the AgMNPV. Nevertheless, for a better understanding of its biology, particularly of factors involved with its interaction with its host, the complete genome sequence of the AgMNPV (isolate 2D) was done. A previously determined physical map for 7 restriction enzymes (RE) was useful for planning the sequencing strategy and genome assembly. A combination of approaches of shotgun cloning, transposon library, subcloning of RE fragments and primer walking were used in the sequencing project. The overall genomic organization of the AgMNPV was similar to other Group I NPV. Moreover, it was found that AgMNPV genome shared extensive syntenic regions with Choristoneura fumiferana defective nucleopolyhedrovirus (CfDEFNPV) and, to a lesser degree, to Epiphyas postvitanna nucleopolyhedrovirus (EppoMNPV). However, a few glaring differences were noteworthy, like the absence of the genomic regions coding for the chitinase and cathepsin, which are common Group I NPVs genes. The genome sequence is now being used for transcriptome and genetic diversity studies of the virus. * This research was supported by a grant from FAPESP

17:30 **TOWARDS A COMPREHENSIVE PHYLOGENY OF LEPIDOPTERAN SPECIFIC BACULOVIRUSES**

Martin Lange, *Laboratory of Biotechnological Crop Protection, Agricultural Service Center Palatinate, GERMANY*; Hualin Wang, Zhihong Hu, *Joint Laboratory of Invertebrate Pathology, Wuhan Institute of Virology, P.R. CHINA*; Johannes A. Jehle, *Laboratory of Biotechnological Crop Protection, Agricultural Service Center Palatinate, GERMANY*

Abstract: Baculoviruses form a large and diverse group of DNA viruses, which are pathogenic for insects of the orders Lepidoptera, Hymenoptera and Diptera. Baculoviruses contain a double-stranded DNA genome of 80-180 kbp encoding for about 90170 genes. To date, more than 500 baculovirus species have been isolated from lepidopteran host insects. The present classification of these viruses is based on (i) morphological traits (NPV vs. GV) and (ii) virus-host associations. These criteria, however, do not result in a distinctive nomenclature in cases, where the same baculovirus is isolated from different hosts or where different baculoviruses are found to infect the same host species. Based on extensive genome sequence comparisons we have identified three target genes which can be used as taxonomic markers. By using degenerate primer pairs we have developed a PCR based method for the detection and taxonomic identification of lepidopteran specific baculoviruses. Highly conserved DNA sequences within the coding regions of the polyhedrin, lef-8 and lef-9 genes were targeted for amplification followed by sequencing. Sequence comparison and phylogenetic analyses of more than 130 baculovirus isolates will be presented and the necessity of an unambiguous nomenclature will be discussed.

17:45 **DETERMINATION OF THE PROMOTER REGION OF THE CHILO IRIDESCENT VIRUS DNA POLYMERASE GENE**

Remziye Nalçacioglu, Just M. Vlak, *Wageningen University, THE NETHERLANDS*; Zihni Demirbag, *Karadeniz Technical University, TURKEY*; Monique M. Van Oers, *Wageningen University, THE NETHERLANDS*

Abstract: Chilo iridescent virus (CIV) is a member of the family Iridoviridae and occurs in insects. The DNA genome (212,482 base pairs) is entirely sequenced, but very little is known about viral gene regulation, expression and function. We investigated the promoter region of the CIV DNA polymerase gene (DNApol). Previous work has shown that DNApol is a delayed early gene and that its transcription start site is located 35 nt upstream of the translational start codon. To determine which DNA promoter sequences are required for DNApol gene expression a region extending 282 bp upstream of the translational start site (ATG) was linked to the coding sequence of firefly luciferase. A series of increasing deletions were made, starting at the 5'end of this upstream region and extending towards the RNA start site. The effects of these mutations were examined in a luciferase reporter gene system in Bombyx mori cells transfected with promoter plasmids and infected with CIV. A gradual reduction in luciferase expression occurred as the deletions extended from 86 to 19, relative to the mRNA start site. A further 5' to 3'deletion of 3 bp reduced luciferase expression to almost zero. Site directed mutagenesis is being performed to confirm the importance of three adenines located between 19 and 15 for promoter activity.
This research is supported by grants from Tubitak (2002), a 2004 IAC-grant and a scholarship from the Wageningen Graduate School PE&RC.
E-mail: remziye.nalcacioglu@wur.nl

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 1

Symposium (Division of Fungi)

Insect-fungal associations

Chair: Fernando Vega; Meredith Blackwell

15:10 **PHYLOGENETICS OF THE INSECT PATHOGENIC FUNGUS BEAUVERIA**

Stephen Rehner, *USDA, ARS, Insect Biocontrol Laboratory, USA*

Abstract: The entomopathogen Beauveria is a cosmopolitan genus of haploid ascomycetes that has figured prominently in basic and applied investigations of fungal entomopathogenesis. Despite nearly 200 years of research on Beauveria, details of the evolution, natural history and reproductive biology of species in this genus remain poorly understood. A multi-locus phylogenetic analysis for Beauveria will be presented which demonstrates that the main lines of evolution within Beauveria correspond closely to traditionally accepted morphological species, although several morpho-species are in actuality cryptic complexes of phylogenetic species. An unexpected finding of the phylogenetic analysis is that B. bassiana is polyphyletic and consists of two unrelated, morphologically indistinguishable clades. A second major finding is that Cordyceps sexual states (e.g., C. bassiana, C. scarabaeicola, C. staphylinidicola) are placed within Beauveria, corroborating recent reports based on culturing studies that Beauveria and Cordyceps are directly linked to one another. Subsequent characterization and evolutionary analyses of MAT, the locus determining sexual mating type in ascomycetes, demonstrates that both mating type idiomorphs (MAT-1 and MAT-2) are each present throughout the genus, suggesting that all lineages are capable of sexual reproduction. Indeed, population genetic analyses of microsatellite markers in B. bassiana reveal random patterns of allele associations that are consistent with a recombining (i.e., sexual) reproductive mode. Together these phylogenetic and population genetic insights suggest most species of Beauveria reproduce sexually and hence may be amenable to manipulation through conventional genetic approaches.

15:35 **CRYPTIC SPECIES AND RECOMBINATION IN THE INSECT PATHOGENIC FUNGUS, METARHIZIUM**

Michael Bidochka, Cherrie-Lee Small, *Brock University, CANADA*; Michael Spironello, *University of Toronto, CANADA*

Abstract: The genetic relationships and recombinational potential of Metarhizium strains are particularly relevant in the evaluation of field applications and subsequent tracking of strains. Here we will show by using phylogenetic recombinational analysis based on polymorphic sequences of genes encoding subtilisin-like protease, neutral trehalase, calmodulin, actin, chitin synthase as well as ITS the absence of recombination between strains of two sympatric groups. However, the lack of concordance among gene genealogies among strains within a group was evidence that recombination had occurred. We argue that these non-recombining groups represent cryptic species within the genus Metarhizium.

16:00 **INTERACTIONS AMONG INSECT PARASITOIDS, ARTHROPOD PREDATORS AND ENTOMOPATHOGENIC FUNGI**

Michael Furlong, *University of Queensland, AUSTRALIA*; Judith K. Pell, *Rothamsted Research, UK*

Abstract: Complex multitrophic interactions between herbivores, predators, parasitoids and diseases contribute to arthropod community structure. Many studies examine individual host-parasitoid, predator-prey and host-pathogen relationships and several represent landmarks in the theory and understanding of population ecology and biological control. However, herbivores are frequently simultaneously exploited by several different natural enemies but until relatively recently studies of the relationships between these organisms, which are often phylogenetically distinct, were uncommon. Much of the current research on arthropodfungus interactions has focused on natural enemies that are candidate biological control agents. Studies have assessed associations between organisms that have co-evolved together and new associations which are the consequence of the introduction of one or more natural enemies into a particular agro-ecosystem. These studies provide a basis for predicting the consequences of introducing a new natural enemy into an existing co-evolved community and of elevating populations of one natural enemy above natural levels. The outcome of any intra-guild interaction is co-existence or exclusion of one or more species. However, this is within the context of spatial and temporal scale, and outcomes can be further affected by prevailing environmental variables and by the behaviour of the species involved. For example, even when natural enemies have co-evolved, their interactions in geographic regions which are environmentally distinct from their area of origin may result in profoundly different outcomes. In some co-evolved systems competition has led to the selection of mechanisms by which competition can be avoided and competing natural enemies may undergo niche differentiation so that their populations become spatially or temporally segregated. The interactions within a given system will probably need to be considered on a case by case basis and should be examined both at the level of the individual and the population. Continued research in this area will contribute greatly to our understanding of insect community structure and aid the development of effective biological control strategies.

16:25 **ECOLOGY AND EVOLUTION OF FUNGAL ENDOPHYTES AND THEIR ROLES AGAINST INSECTS**

A. Elizabeth Arnold, *Duke University, USA*; Leslie Lewis, *USDA Agricultural Research Service, USA*

Abstract: Fungal endophytes associated with foliage comprise a diverse group, primarily of ascomycetous fungi, which are considered ubiquitous, having been recovered from nonvascular plants, ferns, conifers, and both monocotyledonous and dicotyledonous angiosperms. Fungal endophytes represent an important but cryptic component of Earth's fungal biodiversity, and comprise myriad but poorly known interactions with other organisms. Through the hosts they inhabit, endophytes have the opportunity to interact closely with herbivorous insects, against which some may act antagonistically via direct antagonism, mosaic-type defenses, or as entomopathogens. Work with B. bassiana has shown that this entomopathogen can be harbored as an endophyte in a variety of hosts, including both agronomic and weedy species. In contrast to the constitutive mutualism embodied by other grass endophytes in the Clavicipitaceae, B. bassiana is transmitted among hosts by infected herbivores and by liberation of propagules from senescent tissues by rain and other disturbances. Moreover, it persists as an infective reservoir within living plant tissues. The endophytic symbiosis of B. bassiana with Z. mays blurs some of the general boundaries among major types of endophytic symbioses, and thus represents a model system for understanding general aspects of the ecology and evolution of endophytism, and the roles of endophytic fungi with regard to insects. We suggest that the especially high diversity of horizontally transmitted endophytes in tropical forests represents a particularly useful resource for seeking novel entomopathogens among plant symbionts, and anticipate that such research could generate new and interesting insect pathogens for systematics, agriculture, and biological control research.

16:50 **EVOLUTIONARY DYNAMICS OF THE MUTUALISTIC SYMBIOSIS BETWEEN FUNGUS-GROWING TERMITES AND TERMITOMYCES FUNGI.**

Duur K. Aanen, Jacobus J. Boomsma, *Biological Institute, University of Copenhagen, DENMARK*

Abstract: The 'agricultural' symbiosis between termites (subfamily Macrotermitinae, Isoptera) and fungi (genus Termitomyces, Basidiomycotina) is one of the most spectacular examples of mutualistic symbiosis. The 'fungus-farming' termites cultivate their crops in special fungus gardens. Those are continuously provided with externally derived plant material (e.g. wood, dry grass, leaf litter), while the older parts, consisting of partially degraded plant material and fungal mycelium, are consumed. The large colonies formed by many species have significant effects on carbon and nitrogen fluxes in savannah ecosystems. Recent work has shown that, as in humans, the transition to agriculture in termites has been irreversible. Moreover, the domesticated termite fungi belong to a single lineage, with no free-living descendents. The symbiosis between termites and fungi is therefore symmetrical in that both partners have a single origin with no reversals to non-symbiotic states and both are obligatorily interdependent. Furthermore, mutualistic interactions at higher taxonomic levels show considerable specificity, but at lower levels host switching has been frequent. The fungus-growing termites have evolved into approximately 330 species, and have independently moved 'out of Africa' into Asia at least four times. Interestingly, their fungal crops have probably colonized Asia several times independently of the termites. In this talk we summarize recent advances in our understanding of the major macroevolutionary developments that have shaped the symbiosis between the fungus-growing termites and their fungal symbionts and place these changes in an ecological context.

17:15 **FUNGAL BIOTROPHIC PARASITES OF INSECTS AND OTHER ARTHROPODS**

Alex Weir, *Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York, USA*; Meredith Blackwell, *Department of Biological Sciences, Louisiana State University, USA*

Abstract: Necrotrophic fungal parasites proliferate on the dead cells and tissues of the hosts they kill, a trait that suggests great potential for biological control. By comparison, biotrophic parasites require living cells, and the most successful among them do not kill their hosts outright. Obviously, there is less interest in the biotrophic parasites of insects for biological control, but these fungi have great biological and evolutionary appeal. Fungal biotrophs are morphologically simple and reduced in size, and for these reasons they are poorly known by both mycologists and entomologists. Sizes range from less than a millimeter for Laboulbeniales and Termitaria

at the larger end of the spectrum to the smallest size of 30-50 mm for dispersal states of Basidiobolus and Pyxidiophora, previously considered independent fungi. Current understanding of phylogenetic relationships has come not only from molecular methods, but also from highly informative life history studies. Laboulbeniales is the most diverse group in terms of morphology and taxa with about 2 000 species described. Laboulbeniales, now firmly linked to filamentous ascomycetes, is one of the few fungal groups that has lost the ability to reproduce asexually. Loss of sexual reproduction is the more common loss among other arthropod parasitic and fungi in general. Other fungi (Termitaria, Mattirolella, and Termitariopsis; Antennopsis, Hormiscium, Muiogone, Muiaria, Chantransiopsis) and several idiocentricities are even less well known among mycologists and seldom seen by entomologists. Recent findings suggest inclusion of additional taxa within Laboulbeniomycetes, and indicate that certain fungal biotrophs do not share morphological features with their closest non-arthropod-associated relatives. Examples are sister taxa with mycelium present (Pyxidiophora, Termitaria) and absent (Laboulbeniales, Kathistes) and reproduction asexual (Termitaria) and sexual (Kathistes). Intricate morphological character state modifications and innovations in life histories (e.g., loss of sexual or asexual state, dramatic host shifts), now can tracked in well-founded phylogenetic studies.

Monday, August 2^{nd}, 2004
Time: 15:00 - 18:00, Lecture Room 12

Symposium (Division of Microbial Control)

Bringing pathogens from the laboratory to the field

Chair: Vince d'Amico

15:00 **THE GYPSY MOTH, LYMANTRIA DISPAR, NUCLEOPOLYHEDROVIRUS PRODUCT GYPCHEK:**

John D. Podgwaite, *USDA Forest Service, Northeastern Research Station,, U.S.A.*; Vincent D'Amico, *USDA Forest Service, Northeastern Research Station, Department of Entomology and Applied Ecology, University of Delaware, U.S.A.*

Abstract: In the early 20th century, several decades after the accidental introduction of the gypsy moth, Lymantria dispar, into the United States, widespread disease epizootics began to impact larval populations of the pest. The disease, then referred to as 'wilt' due to the flaccid appearance of larval cadavers, became the subject of keen interest and its etiological agent soon was identified as a virus linked to the presence of polyhedral bodies found inside dead larvae. At the time, workers suggested that this virus, i.e., the gypsy moth nucleopolyhedrovirus (LdNPV), might have some practical value in control of the pest; results of field tests in the early 1900's supported this view. However, in the years to follow chemical pesticides were the gypsy moth control agents of choice until concerns over their deleterious effects on the environment became paramount. In response to those concerns the U.S. Forest Service initiated a program of research in the late 1950's that was directed toward the development of environmentally 'soft' pesticides and studies were begun to assess the potential of LdNPV as a biopesticide. The rationale for this was twofold. First, the virus was naturally occurring and responsible for wholesale collapses of gypsy moth populations. Second, all evidence indicated that the virus was 'specific' for gypsy moth and thus a logical choice for use in gypsy moth-infested areas where concerns for the environment were dominant. Research was conducted through a wide-reaching collaboration between government, academia and industry. The goal was registration with the U.S. Environmental Protection Agency (EPA) of a safe and efficacious biopestide. The research was focused on finding and characterizing a virulent LdNPV isolate, assessing its safety for man and wildlife, developing cost effective methods for its mass production and formulation, and testing various ground and aerial systems for its delivery to the target pest. The product of this research, Gypchek, a wettable powder produced from LdNPV-killed larvae and mixed prior to use with a lignosufonate-based formulation, was registered with EPA in 1978. Since its registration Gypchek has been used to treat 40,000 ha of forested lands and research continues to focus on improving the product through the development of an in vitro produced viral strain of enhanced potency and a ready-to-use formulation that will ensure extension of viral activity following application. Substantive improvements in potency and persistence along with reduced production costs are necessary to move production from government to the commercial sector.

15:30 **DEVELOPING A MICROBIAL: CHOOSING THE RIGHT FUNGAL STRAIN**

Ann Hajek, Thomas Dubois, Jennifer Lund, Charlotte Nielsen, *Dept. Entomology, Cornell University, USA*; Leah Bauer, *USDA, Forest Service, U.S.*; Michael Smith, *USDA, ARS, U.S.*; Zengzhi Li, *Dept. Forestry, Anhui Agric. Univ., CHINA*

Abstract: The literature is full of studies in which numerous strains of entomopathogenic fungi are tested against a pest to find the most virulent strain or strains. This is exactly how our group initially approached working on control of the Asian longhorned beetle (ALB), Anoplophora glabripennis. This cerambycid beetle is native to China where it is a major pest, killing numerous species of trees. In North America A. glabripennis was first found in the New York City area in 1996 and has since also been found in Chicago, New Jersey and Toronto and efforts to eradicate this beetle in North America have been intensive. B. brongniartii is sold as cultures grown in non-woven fiber bands that are placed around orchard trees in Japan for control of cerambycid adults that self-inoculate during prematurational wandering and our goal has been to develop this novel methodology for ALB control. Numerous isolates of Beauveria brongniartii, B. bassiana and Metarhizium anisopliae, including the commercially available B. brongniartii from Japan, were tested against ALB and the most promising isolates were tested using fungal bands in the field in China. Although the commercial product from Japan was virulent against A. glabripennis and a treatment effect was documented in the field, we could not confirm that that fungal species is native to North America; could we register this strain for control in the U.S.? Without substantial financial backing, the costs for conducting toxicological testing and registering a new strain of an entomopathogenic fungal species in the U.S. would be prohibitive for development of this mycoinsecticide destined for a niche market. Recently, M. a strain of M. anisopliae (F-52) was registered for outdoor use in the U.S. and the focus of our program has turned to this isolate. In summary, while studies to compare virulence of fungal isolates are valuable, if a means for control is seriously needed testing strains that are already approved by regulatory agencies should be the first step.

16:00 **ENTOMOPATHOGENIC NEMATODES: FROM LABORATORY STUDIES TO USE IN THE ORCHARD**

Lawrence Lacey, *USDA-ARS-YARL, USA*; David I. Shapiro-Ilan, *USDA-ARS-Byron, USA*; Robin Stuart, *University of Florida, USA*; Joel Siegel, *USDA-ARS-Parlier, USDA*

Abstract: Basic studies on the behavior and ecology of a diverse group of entomopathogenic nematode species have enabled their development for use against pest insects in a wide variety of soil and cryptic habitats. Selection of the appropriate nematode species and strain for specific insect targets, temperature ranges and habitat types has optimised nematode efficacy and persistence. A multitude of insects attack tree fruit and nuts and many of these that are found predominantly in soil and cryptic habitats are good targets for EPNs. Promising results on the use of EPNs against plum curculio (Conotrachelus nenuphar), codling moth (Cydia pomonella), navel orangeworm (Amyelois transitella) and root weevils in citrus (Pachnaeus spp. and Diaprepes abbreviatus) have led to further development of nematodes for control of these pests. Large scale operational control of the Diaprepes root weevil in soil was possible after the discovery that Steinernema riobrave was highly efficacious against this species. The use of irrigation for application of infective juveniles (IJs) of S. riobrave to orange groves faciltated effective delivery to targeted sites. There are still obstacles for large scale application to cryptic habitats. Formulation and application improvements are needed for more effective delivery and maintenance of mositure that enables the survial of IJs until the host insect can be penetrated.

16:30 **BRINGING SERRATIA ENTOMOPHILA FROM UNKNOWN BACTERIUM TO A COMMERCIAL BIOPESTICIDE**

Trevor Jackson, *AgResearch, NEW ZEALAND*

Abstract: Bacteria of the genus Serratia are commonly found in soil throughout the world and are occasionally isolated as insect pathogens. In the early 1980s, a novel Serratia spp. was found associated with a disease condition of the New Zealand grass grub (Costelytra zealandica, Coleoptera: Scarabaeidae). Pathogenic strains of bacteria were isolated and categorized as a new species, S. entomophila. Selected strains of bacteria could be cultured, applied to healthy field populations and induce disease outbreaks. S. entomophila was registered in New Zealand as a microbial control agent and has been marketed as a grass grub control agent for more than a decade. Registration of a novel bacterium required extensive safety testing, but the registration pathway was simplified by the highly specific nature of the insect/bacteria interaction. Scaling up to commercial release has required implementation of an effective quality control system

to ensure consistent product performance. Problems in developing and maintaining the bacterium as a commercial product have included strain stability, bacteriophages, farmer perception and continuity of suppliers. User uptake of the original liquid bacterial concentrate was limited by the need for low temperature storage and specialized application equipment. To overcome these limitations, a thermostable, dry granular formulation has recently been released on the market. The pathway for development of S. entomophila as a biopesticide has faced biological, organizational and commercial challenges which will be discussed in this presentation.

17:00 **FROM BASIC RESEARCH TO FIELD APPLICATION WITH GENETICALLY ENGINEERED BACTERIAL INSECTICIDES**

Brian A. Federici, Hyun-Woo Park, Dennis K. Bideshi, Yuko Sakano, Margaret Wirth, *Department of Entomology, University of California, UNITED STATES*

Abstract: Insecticidal hacteria have been one of the success stories of invertebrate pathology, with over 100 commercial formulations available worldwide. Despite this considerable success, insecticides based on wild type bacteria remain expensive to produce and use in comparison to many synthetic chemical insecticides. In addition, the success of Bt transgenic crops has reduced the need in several commodity crops for both chemical and bacterial insecticides, as well as other control agents such as pheromones, parasitic wasps, and insect predators. Several technical possibilities exist for making bacterial insecticides more competitive, the most promising being the use of recombinant DNA technology. In fact, several excellent recombinant bacteria that use either Bacillus thuringiensis or Pseudomonas fluorescens as host strains have been developed and commercialized. Unfortunately, although these had good to excellent insecticidal properties, the markets for these have not been strong owing to the advent of Bt crops and the development of new types of chemical insecticides. The situation is considerably different with bacteria directed against nuisance and vector mosquitoes. Against these, bacteria have been increasingly accepted as replacements for chemical insecticides, especially in environmentally sensitive habitats. Nevertheless, there is a need for improved strains for mosquito and vector control, and recombinant DNA technology has provided excellent tools to develop both basic knowledge and reagents for creating recombinant bacteria that have better insecticidal properties than their wild type relatives. In this presentation, we describe the basic and applied research used to develop several commercially promising recombinant strains of B. thruingiensis subsp. israelensis and B. sphaericus 2362. In these strains, we have recombined mosquitocidal toxins from these and other bacterial species to produce individual strains that produce three or more endotoxins. These strains are from 8-10 fold more potent than their wild type relatives, and have built-in resistance management properties conferred by inclusion of the Cyt1Aa protein. To move these strains forward in the commercial development process, permission for field testing had to be obtained from the U.S. as well as state environmental protection agencies. In addition, intellectual property issues had to be resolved and industrial partners identified to assist in commercial development. Examples of the types of scientific, regulatory, and commercial hurdles that must be overcome to develop a successful commercial product will be provided.

Monday, August 2^{nd}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Symposium (Division of Bacteria)

Risk assessment and non-target effects of Cry toxins in sprays and transgenic plants

Chair: Brian Federici; Juan Ferré

16:00 **THE MAMMALIAN SAFETY OF BACILLUS THURINGIENSIS SPRAYS, WITH AN EMPHASIS ON THE HUMAN EXPERIENCE**

Joel Siegel, *USDA/ARS, UNITED STATES OF AMERICA*

Abstract: Bacillus thuringiensis (Bt) is the most widely used microbial pest control agent (MPCA) and Bt products are used in agriculture, forestry and for vector control. Toxicity and infectivity studies of commercially produced Bt isolates were conducted on designated mammalian species as part of the registration process. The emphasis of these studies was on direct effects, typically assessed in one-month laboratory studies, although long-term feeding studies were also conducted. Initially, one of the main concerns about the safety of Bt was its close relationship to Bacillus anthracis a mammalian pathogen, although Bt does not possess the plasmids that contain the genes for B. anthracis toxins and the capsule that enables B. anthracis to evade the mammalian immune system. Recently, some researchers have suggested that both Bt and B. anthracis are subspecies of Bacillus cereus, based on chromosomal similarity. The close relationship between Bt and B. cereus raises additional concerns because B. cereus produces emetic and diarrheal enterotoxins, and genes coding for these enterotoxins have been discovered in numerous Bt isolates. However, there is no evidence that B. cereus enterotoxins are present in commercial Bt products and there has been no increase in the incidence of diarrhea during large-scale Bt spray campaigns. There are three commonly cited reports associating human infection with Bt dating back to the early 1980's, and in these cases the isolates recovered produced Cry proteins. Additional reports of human infection in the late 1990's are problematic because the reports did not distinguish between B. cereus and B. thuringiensis. These researchers regarded simple recovery of Bt from humans as evidence of infection, although the isolates were not commerciall y produced nor were they linked to tissue damage. Furthermore, Bt spores can remain viable in mammalian tissue without causing damage and may have been simple contaminants in the sample. Several large-scale epidemiology studies following spray campaigns in Canada, New Zealand, and the United States will be discussed in detail.

16:25 **EMETIC TOXIN AND ENTEROTOXINS A POTENTIAL RISK OF USING B. THURINGIENSIS PRODUCTS?**

Hansen Bjarne Munk, Niels Bohse Hendriksen, *Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, DENMARK*

Abstract: B. thuringiensis based products have been used in insect control programmes for decades without identification of significant undesired effects on humans and environment. Similar results have been obtained from numerous experiments where animals and environments have been exposed to B. thuringiensis products and/or B. thuringiensis spore-crystal mixtures. However, during the last 10 years risk assessments and research programmes have focused on the potential risks of traits normally found in the close relative, B. cereus. B. cereus is normally considered being an opportunistic human pathogen primarily causing gastro-intestinal diseases, but also somatic infections are significant. Two types of gastro-intestinal diseases are caused by B. cereus: emesis and diarrhoea. Emetic symptoms are caused by cereulide, a cyclic dodecadepsipeptide. The cereulide is primarily produced during storage of processed food under insufficiently refrigerated conditions, and the toxin is extremely resistant to heat, acid, alkali and enzymatic activity. The genetic basis for synthesis of the emetic toxin is still unknown. The diarrhoeal symptoms are caused by a number of toxins expressed in the gut after germination of ingested spores. The toxins involved in diarrhoea are primarily the hemolytic (HBL) and the non-hemolytic enterotoxic complexes (NHE) but also products like phospholipase C, sphingomyelinase, proteases and cytolytic activities are expected to participate. Genetic and phenotypic investigations have shown that all B. cereus traits, which seems to be involved in diarrhoea including their regulatory functions, are present in B. thuringiensis. Toxicity analysis with Vero cells have shown that B. thuringiensis has the same toxicity level as B. cereus isolated from cases of human diarrhoea. Thus, with the present knowledge from B. cereus strains causing diarrhoea, it must be concluded that B. thuringiensis has the potential to cause diarrhoea. However, cases where commercial B. thuringiensis strains have caused diarrhoea have not been described, which might be explained by the fact that physicians never look for crystal production in B. cereus isolated from patients. But as long as we are unable to predict whether a specific B. cereus isolate is able to cause disease in humans, we have to search for an understanding of the potential B. thuringiensis pathogenesis.

16:50 **MULTIYEAR FIELD EVALUATIONS OF BT COTTON AND CORN INDICATE NO BIOLOGICALLY SIGNIFICANT IMPACTS ON NON-TARGET INSECTS**

William Moar, Micky Eubanks, Barry Freeman, *Auburn University, UNITED STATES*; Sam Turnipseed, *Clemson University, UNITED STATES*; John Ruberson, *University of Georgia, UNITED STATES*; Galen Dively, *University of Maryland, UNITED STATES*; Graham Head, *Monsanto, UNITED STATES*

Abstract: Field studies were conducted in 2000-2002 to compare arthropod populations between Bollgard and conventional cotton season-long in Alabama, Georgia, and South Carolina. For each region, three or four paired fields were evaluated weekly, with each pair consisting of a Bollgard field and a conventional cotton field (treated for lepidoptera when necessary). Insect species sampled included Heliothis virescens, Helicoverpa zea, Spodoptera frugiperda, Spodoptera exigua, Trichoplusia ni, Pseudoplusia includens, phytophagous stink bugs and plant bugs, cotton aphids, Solenopsis invicta, Geocoris spp., Orius spp., spiders, parasitic wasps, green and brown lacewings, and Nabis spp. Most results show that there were no significant differences for any arthropod population between a particular Bollgard field and the corresponding conventional cotton field. However, the most dramatic significant differences occurred in South Carolina when

comparing conventional cotton sprayed with at least several applications of insecticides to control primarily H. zea, and Bollgardthat was not treated for H. zea. In these instances, there were significantly more non-target arthropods in the Bollgard fields than in the conventional fields. These results demonstrate that the use of Bollgardusually has no impact on non-target arthropod populations, and when compared with cotton treated with insecticides for lepidoptera, results in significantly more non-target arthropods. Similar trials were conducted in Bt corn in Maryland. Again, when compared to conventional corn plots treated with insecticides, there were significantly more non-target arthropods in the Bt corn fields than in the conventional fields.

17:15 **PRECAUTIONARY PRINCIPLE AND THREE YEARS OF FIELD TRIAL EXPERIENCE IN BT-MAIZE MONITORING: IMPLICATIONS FOR A FUTURE RISK ASSESSMENT**

Achim Gathmann, Ingolf Schuphan, *Biology V, RWTH Aachen, GERMANY*

Abstract: The precautionary principle is part of the Cartagena Protocol on Biosafety and it is now implemented in the EU regulations on GMOs. One of the most universal description of the precautionary principle is that it seeks to impose early preventive measures to ward off even those risks for which we have little or no basis on which to predict the future probability of harm (Wiener 2001) However there are a lot of various interpretations regarding the release of GMOs. They range from if there is doubt, do not or doing nothing to uncertainty should neither be used as an excuse for government inaction nor as a justification to prevent a regulatory response. Different interpretations are presented and how this principle could be usefully considered in a future risk management of GMOs will be discussed. In the second part of the talk we present results of a field trial evaluating the impact of growing Bt-maize on non target arthropods. Besides the results of non target organisms of different trophic levels, we focus on methodology problems. In particular we address interactions between different herbivore species, identification of monitoring organisms, impact of differences between transgenic and isogenic varieties and statistical analysis. Implications for a future risk assessment of Bt-crops are given.

Monday, August 2^{nd}, 2004
Time: 18:30 - 20:30, Main Building, Lehtisali

Helsinki University reception

Chair: Hannele Niemi, *Vice Rector, Helsinki University*

Monday, August 2^{nd}, 2004
Time: 20:00 - 22:00, Lecture Halls 1, 12, 6, 10

Division meetings: V, B, N, Ms

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 10:00, Lecture Room 12

Contributed Papers (Division of Fungi)

fungus / contributed paper session 1

Chair: Cezary Tkaczuk; Richard Meadow

08:00 **CLIMATIC CONSTRAINTS FOR FUNGAL INFECTION OF TRIALEURODES VAPORARIORUM IN MEDITERRANEAN TOMATO GREENHOUSE**

Jacques Fargues, Thierry Boulard, Benoît Jeannequin, *INRA, FRANCE*

Abstract: One of the greatest challenges for improving fungi as microbial control agents is to identify and establish a hierarchy of pertinent environmental constraints and to develop ways to overcome them. Collaborative research was conducted in the South of France to assess the microbial control potential of Beauveria bassiana- and Lecanicillium lecanii-based formulations against Trialeurodes vaporariorum (Homoptera: Aleyrodidae) in Mediterranean tomato greenhouses. Because of expected climatic constraints, the greenhouse climate was manipulated to optimize mycoinsecticide efficacy by closing the ridge vents 2 h more at night-time. Thus, the daily period at high humidity (>90% RH) was two or three times longer in the "humid" greenhouse compartment than in the "dry" one. In spite of this differential, mycoinsecticide treatments reduced numbers of surviving whitefly larvae by >85% in the "humid" compartment as expected as favorable, as well as in the "dry" compartment, expected as unfavorable. The climatic heterogeneity was taken into account by comparing the fungus-induced mortality of nymphs located on lateral row plants to that of nymphs on center row plants. In spite of significant differences in air flows (0.7-1.2 and 0.3 ms-1, respectively) there was no difference in fungus efficacy. When comparing the influence of greenhouse equipments (sophisticated glasshouse vs polyethylene-covered greenhouse), the fungus was not affected in spite of significant differences in ventilation rates. Microclimatic investigations of the under leaf surface boundary layer and assays in microcosms (sandwich cells) strongly supported that the infection dynamics depends on the conditions prevailing in the habitat of the targeted whitefly larvae. The leaf transpiration activity could minimize greatly humidity constraints under ambient conditions expected as unfavorable. In contrast, leaf surface temperature is not really disconnected from that of the ambient greenhouse air.

08:15 **INFLUENCE OF TEMPERATURE PREFERENCE OF TWO RDNA-ITS LINEAGES OF PAECILOMYCES FUMOSOROSEUS ON THEIR CO-INFECTION PATTERN**

Jacques Fargues, *INRA, FRANCE*; Marie-Claude Bon, *EBCL/USDA-ARS, FRANCE*

Abstract: Influence of temperature preference of two rDNA-ITS lineages of Paecilomyces fumosoroseus on their co-infection pattern Jacques Fargues 1 and Marie-Claude Bon 2 1 Centre de Biologie et de Gestion des Populations, INRA, Montferrier, France & 2 European Biological Control Laboratory, USDA-ARS, Montferrier, France. In order to clarify the epidemiological potential of entomopathogenic hyphomycetes for insect pest control, the role of the temperature as one major environmental constraint was investigated on the pattern of co-infection of Galleria mellonella by two distinct rDNA-ITS lineages of Paecilomyces fumosoroseus. The distribution of conidial populations collected on cadavers of hosts co-infected under twenty temperature regimes, ranging from 13C to 35C, was examined. The temperature tolerance of both fungal isolates was based on their in-vitro colony growth and their in-vivo sporulation ability. The conidial populations were characterized by molecular markers based on restriction fragment length polymorphisms of the internal transcribed spacers (ITS-RFLP) and random amplified polymorphic DNA (RAPD) contrasting profiles in combination with the conidial size. This study allowed a temperature profile to be formed for each isolate. Under most temperature regimes, only one lineage was prevailing on the infected insect, whereas both lineages coexisted at 20-25C and 25-25C. When one haplotype dominated, the displacement of the other one depended on its temperature tolerance. When both lineages coexisted, molecular analyses strongly supported that there was no hybridization inside co-infected hosts. These results suggest that more consideration should be given to population genetics analysis for evaluating the adaptability of microbial control agents to targeted environments.

08:30 **EFFECT OF INITIAL HIGH HUMIDITY EXPOSURE ON THE EFFICACY OF LECANICILLIUM LECANII BLASTOSPORES AGAINST THE HEMLOCK WOOLLY ADELGID ADELGES TSUGAE ANNAND (HOMOPTERA: ADELGIDAE).**

William Reid, Vladimir Gouli, Svetlana Gouli, *University of Vermont, USA*

Abstract: A high level of relative humidity (RH) is the most important factor for efficiency of entomopathogenic fungi as microbial pesticides. We can surmise that high humidity is especially necessary during the first stages of interaction of the fungus with hosts. In this period the spores germinate and penetrate into insect body cavity. Experiments were conducted to determine the optimal minimum period of high RH for a productive relationship between the fungus and insect. Blastospores of a small-spore isolate of Lecanicillium lecanii was investigated for efficacy against the aestivating stage of Hemlock Woolly Adelgid (HWA). Insect-infested branchlets were field-collected, treated with blastospores and transferred to sealed glass tubes to maintain 95-100%RH. Tubes were unsealed at 24h time intervals to reduce total humidity from 22 to 30%RH. All mortality counts were taken 7d post-application. Mortality increased with respect to longer exposure periods of humidity, reaching >90% when 100%RH was maintained throughout the duration of the testing. Statistically significant increases in efficacy were observed after exposure periods 24h, 72h, 96h and 144h. Tested blastospores germinated in less than 24h, and since HWA is sessile, the punctuated increases in efficacy may be the result of infection from vegetative stage, and recycling of the fungus, which was observed in treatments that had been subjected to a minimum of 72h of 100%RH. These results suggest that the use of blastospores for management of HWA is reliant on continuous high humidity in order to achieve efficacy. Further field testing of blastospores should be conducted during periods of high humidity and incorporate the use of a humectants to maintain humidity at the microclimate.

08:45 **GERMINABILITY OF METARHIZIUM ANISOPLIAE AND BEAUVERIA BASSIANA CONIDIA IN THE PRESENCE OF COMMON SOIL AND PHYLLOPLANE FUNGI**

Richard Meadow, *Norwegian Crop Research Institute, NORWAY*; Linda Gordon Hjeljord, *Agricultural University of Norway, NORWAY*

Abstract: When applied prophylactically to soil or plant surfaces, Beauveria bassiana and Metarhizium anisopliae conidia must remain viable and able to infect target insects. In some applications the conidia are applied in nutrient formulations which they are intended to colonize, in order to increase the inoculum potential in situ. We investigated the ability of B. bassiana and M. anisopliae isolates to compete with conidia of fungi commonly isolated from soil and above-ground surfaces of strawberry (Fragaria X ananassa) plants in southern Norway, in preparation for field trials in the same geographic area. We determined the speed of germination of the isolates at various temperatures, and investigated the effects of inoculating various nutrient substrates with conidia of the insect pathogens, before or together with conidia of Cladosporium sp., Trichoderma spp., Verticillium sp., Botrytis cinerea, and Gliocladium sp. Results showed that the insect pathogens germinated as quickly as the environmental isolates only at temperatures >=25oC. Although germination speed of the insect pathogens varied between isolates, none germinated nor grew as quickly at 15 or 20oC as did the indigenous isolates. When coinoculated with conidia of the environmental isolates on a range of agar media at 20oC, growth of the insect pathogens was inhibited by 88 8% (on average for all isolates and media). In contrast, when the insect pathogens were inoculated alone (not mixed), their colonies inhibited mycelical growth of all the other fungi; M. anisopliae isolates produced clear antibiosis zones against Cladosporium sp. and B. cinerea. On nutrient-poor media, or when substrate nutrients were depleted, colonies of the insect pathogens were eventually overgrown by Gliocladium sp. and Trichoderma spp.. These results indicate that biotic and abiotic factors at the application site will affect the competitive potential of the insect pathogens, and suggest that formulations based on precolonized nutrient substrates may be more persistent than spray formulations.

09:00 **DOSE DEPENDENT ACQUISITION OF BEAUVERIA BASSIANA CONIDIA BY WESTERN FLOWER THRIPS, FRANKLINIELLA OCCIDENTALIS (PERGANDE).**

T.A. Ugine, *Cornell University, UNITED STATES*; S. P. Wraight, *USDA-ARS, UNITED STATES*; J.P. Sanderson, *Cornell University, UNITED STATES*

Abstract: Four-dose laboratory bioassays evaluated the efficacy of three preparations of B. bassiana (strain GHA) against second-instar western flower thrips, and one preparation against adult female thrips. Preparations included an unformulated technical powder (TP) suspended with 0.01% Silwet L-77, a clay-based wettable powder (BotaniGard 22WP) (females), and an emulsifiable oil (BotaniGard EO). Insects were exposed to treated bean leaf disks and mortality was assessed after 5d. Counts of conidia on thrips' bodies (stained with acid fuscin) conducted 24 h after initial exposure, were related to the dose applied (conidia/mm2 leaf disk) using Probit analysis to determine a true five day LD50 (conidia/insect). Counts of conidia deposited on Petri dish lids at the time of application were used to estimate the density of conidia to which insects were exposed and this data was used to generate LD50 and LC50 estimates. The acquisition rate defined as the number of conidia observed on the whole thrips body divided by the total number of conidia to which the thrips were exposed, decreased as the application rate increased. Additionally the slopes of LD regressions were lower compared to the slopes of LC regressions (LD 1.9 and 1.5 versus LC 1.3 and 0.7, second-instar and adult female, respectively). This difference was most evident in tests of adults. This finding supports the finding of an inverse relationship between dose and acquisition rate. In the regression analyses, it is assumed that conidial concentrations on the leaf disks are a reliable measure (at least a reliable relative measure) of the doses applied against the insects. There is obviously a problem if the conidia repel the insects or somehow become more difficult to pick up at higher concentrations (application rates). At the higher concentrations, doses are overestimated, resulting in underestimation of the regression coefficient (slope). This result suggests that the low slopes often obtained in fungal assays may arise, at least in part, as an artifact of unequal rates of dose acquisition at low versus high application rates. Greenhouse tests investigating the effect on increasing application rate on efficacy and the number of conidia acquired 24 h post application revealed no relationship between the number of conidia on the bodies of adult female thrips and application rate. Possible explanations for this result will be discussed, as will results of laboratory tests investigating a potential repulsion effect of B. bassiana conidia on thrips.

09:15 **STU** **REDUCING ADULT LIFE SPAN OF MALARIA (ANOPHELES GAMBIAE S.L.) AND FILARIASIS (CULEX QUINQUEFASCIATUS) VECTORS USING THE ENTOMOPATHOGENIC FUNGUS METARHIZIUM ANISOPLIAE: A FIELD STUDY IN TANZANIA**

Ernst-Jan Scholte, *Wageningen University and Research, THE NETHERLANDS*; Kija Ng'abi, *Ifakara Health, Research and Development Centre, TANZANIA*; Bart Knols, *International Atomic Energy Agency, AUSTRIA*; Willem Takken, *Wageningen University and Research, THE NETHERLANDS*; Salim Abdulla, Gerry Killeen, *Ifakara Health, Research and Development Centre, TANZANIA*

Abstract: Current indoor vector vector control in Africa depends on the use of insecticides and/or repellents. Although several biological control agents are effective in reducing larval mosquito populations, none are aimed at the adult stage. Following successful studies of the entomopathogenic fungus M. anisopliae on adult An. gambiae and Cx quinquefasciatus, we investigated the potential of this fungus as a biological control agent for adult malaria and filariasis vectors in the field. Two small scale field experiments of malaria and filariasis vector control were carried out using oil-formulated M. anisopliae-impregnations both on a) 3m2 cloths hung indoors, as b) on a part of the wall of local households in a holoendemic area of South-Eastern Tanzania. From the wild mosquitoes caught on the impregnated cloths 33.58% (181 specimen) of the An. gambiae s.l. and 10.0% (6 specimen) of the Cx quinquefasciatus were infected with the fungus. Comparison of survival curves and LT50 values showed that fungus-infected mosquitoes died significantly earlier than uninfected mosquitoes with overall effect (males and females pooled) of $p<0.001$; $F=178.9$ for An. gambiae s.l. and $p<0.001$; $F=16.30$ for Cx quinquefasciatus. LT50-values ranged between 3.70 and 3.49 days (fungus-infected males and females respectively) against 5.88 and 9.30 days (not infected, males and females) for An. gambiae s.l.. For Cx quinquefasciatus these were 12.02 (fungus-infected, males and females pooled), against 20.59 and 24.06 days (fungus-free males and females). An. gambiae s.s. exposed to conidia on the wall showed similar infection percentages and longevities. This study is the first fieldwork to determine the potential of the entomopathogenic fungus M. anisopliae against adult stages of Africotropical mosquito vectors. By resting indoors on impregnated cloths, wild mosquitoes were passively contaminated and died due to infection of this fungus. We argue that the proportion of mosquitoes that died due to infection of this fungus is high enough to have an impact on An. gambiae populations if used for longer periods and on larger scale.

09:30 **SELECTION OF BEAUVERIA BASSIANA STRAINS FOR CONTROL OF LYGUS POPULATIONS**

Michael McGuire, Jarrod Leland, *USDA-ARS, USA*

Abstract: In an attempt to determine the impact of Beauveria bassiana on Lygus hesperus (CA) and Lygus lineolaris (MS), field collections of adult bugs were made and held in the laboratory for sporulation. In California, collections were made throughout the San Joaquin Valley and at several spots in the Mississippi River Delta region of Mississippi at various times of the year. In California, at least a few adults from all collections were infected with B. bassiana and infection levels were as high as 65% in some fields. In Mississippi, adults were not as widely infected, but B. bassiana was found. Isolates from these collections were cultured and screened for a variety of factors including: pathogenicity (LC50 and LT50) against both L. hesperus and L. lineolaris, ability to grow in vitro at high temperatures, presence of beauvericin (with R. Plattner, ARS-Peoria, IL), activity against natural enemies, survival of spores in simulated sunlight, and potential for mass production (with S. Jaronski, ARS-Sidney, MT). In addition, 7 SSR markers were used to analyze the genetic relatedness of the isolates (with M. Ulloa and Y.H. Park, ARS-Shafter, CA). All tests were done in parallel with the commercial isolate (GHA). Currently, we are focusing on two new isolates, one from California and one from Mississippi. The isolates have approximately 10 fold higher and a 1 day faster activity than the commercial isolate, GHA. They grow at 350C, unlike GHA and beauvericin production is not different among the three strains. Activity against natural enemies is similar and spores survive in simulated sunlight the same as or better than GHA. Preliminary information indicated that the two new strains produce fewer spores than GHA under semi commercial production conditions. The SSR markers suggest that isolates from the SE US are genetically distinct from most of the California isolates. The GHA isolate was intermediate between the two groups. Both new isolates and GHA will be field tested in 2004 in alfalfa (CA) and pigweed (MS). Molecular markers will be used to distinguish applied isolates from natural infection.

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 09:30, Lecture Room 10

Symposium (Division of Microsporidia)

Can microsporidia be seriously considered as biological control agents?

Chair: Rudolf Wegensteiner

08:00 **MICROSPORIDIA IN MOSQUITOES: CONTROL VERSUS MANAGEMENT STRATEGIES**

James Becnel, *USDA/ARS/CMAVE, U.S.*

Abstract: The attraction of microsporidia for management of mosquitoes lies with their ability to cause larval epizootics, continuously cycle within a host population, and spread to new habitats. The idea of utilizing these natural enemies of mosquitoes as manipulative control agents was perhaps first raised by Kudo (1921) who suggested that larval sites might be contaminated with microsporidian infected mosquito tissues. He further suggested (unaware at that time of the mechanism of transovarial transmission) that infected adults could distribute the parasite to new sites that may escape our watchful eye by dying during oviposition,. The complex life cycles exhibited by microsporidian parasites of mosquitoes and the chronic nature of the infection precludes their use as biorational insecticides. The approach to utilizing microsporidia as part of a program to manage mosquitoes must rely on a thorough knowledge of the dynamics of the host-parasite relationship. It is crucial to look beyond short-term population reduction and instead, rely on the benefits of long-term abatement as part of an overall management strategy. The recognition and protection of seasonal epizootics would prevent disruption of the natural balance and control, thus maintaining the disease in the population. Additional relief could be expected due to a reduction in the survival, vigor and reproductive success of infected mosquitoes during other parts of the life cycle. Much of the information concerning the life cycles and evaluations of polymorphic microsporidia has been derived from studies on Amblyospora dyxenoides, Amblyospora connecticus and Edhazardia aedis. Incorporation of E. aedis as a classical biological control agents for Aedes aegypti will be discussed and the circumstances where this approach might be feasible. In contrast, understanding the dynamics of A. connecticus in the mosquito and intermediate host will be presented with respect to mosquito control practices that take into account how conventional control practices can be incorporated so as not to interfere with natural control by the pathogen. Utilizing microsporidia for the management of mosquitoes is proposed not as a sole method but rather as part of the natural complex of regulatory factors. This approach recognizes that eradication of the target mosquito is an unrealistic expectation but with a combination of physical, cultural, chemical and biological control methods, mosquito vectors and pests can be regulated.

08:25 **RHYME OR REASON: ISSUES FOR RELEASE OF EUROPEAN GYPSY MOTH MICROSPORIDIA INTO NORTH AMERICAN HOST POPULATIONS**

Leellen F. Solter, *Illinois Natural History Survey, UNITED STATES*; Michael L. McManus, *USDA Forest Service, NERS, UNITED STATES*

Abstract: Microsporidia might rightfully be considered as failures in biological control programs where they have been applied with the expectation that they would perform as microbial insecticides. The chronic nature of microsporidian infections, even of relatively virulent species, seriously limits their immediate impact on hosts, and certainly limits their effectiveness in pest-host systems characterized by low economic injury thresholds and stable pest populations. However in systems with higher thresholds, microsporidia have been shown to be effective natural enemies that share many characteristics typical of parasitoid/host interactions, and usually possess higher host specificity. A series of laboratory and field studies conducted over a period of years with several microsporidian species isolated from gypsy moth (Lymantria dispar) populations in eight European countries, suggest that these pathogens, in their role as a component of the natural enemy complex, do impact their host, contributing to declines in gypsy moth populations and a reduction in the frequency and amplitude of outbreaks; Additionally, they do not appear to impact non-target species. An overview of several projects will be presented and the characteristics of the microsporidia discussed within the framework of a petition to introduce these entomopathogens into North American gypsy moth populations.

08:50 **THE INTRODUCTION AND ESTABLISMENT OF PARANOSEMA (NOSEMA) LOCUSTAE IN GRASSHOPPERS (ORTHOPTERA: ACRIDOIDEA) OF ARGENTINA.**

Carlos Lange, *CEPAVE, CIC-UNLP-CONICET, ARGENTINA*; María Laura De Wysiecki, *CEPAVE, UNLP-CONICET, ARGENTINA*

Abstract: Paranosema locustae is a microsporidian of the adipose tissue of orthopterans that was developed in the USA as a microbial control agent of grasshoppers. When its development was well advanced but as early as two years before registration, a series of introductions into grasshopper communities began in Argentina that extended from 1978 to 1982. The short-term impact of the releases will remain unknown because reports were not produced and data on infectivity and host density reductions are not available. The long-term outcome was also unknown for years until the pathogen was re-isolated parasitizing three species of grasshoppers. Since then, monitoring activities are conducted whenever possible. Up to now, establishment of the agent was observed in two well-defined areas: Gualjaina in northwestern Patagonia, and an area in the western Pampas in the surroundings of three of the application sites. Infections were diagnosed in 16 species of grasshoppers, while 30 others, including some known to be experimentally susceptible and some occurring in sites where infection was present, were never found to be infected. Prevalences were normally much higher than in regions of the world where P. locustae is native, and epizootics were registered. Natural spore loads per individual were high and consistent with experimentally obtained spore loads. Although at the time of the introductions, P. locustae was used in a rather inundative manner, expecting some short-term effects, the case became an example of the colonization approach at using entomopathogens. It is also an example of new association classical (or neoclassical) biological control, in which an exotic agent is used to control a native pest. Given the levels of occurrence of P. locustae and knowing the negative effects on hosts, the pathogen must be acting as an additional control factor. The original concept for the use of P. locustae was to augment natural control factors for the long-term suppression and maintenance of grasshopper densities. Later commercial development obscured this initial concept, and false expectations were assumed by many, expecting rapid reductions of pest grasshoppers. P. locustae appears to be operating in Argentina very much like the way it was originally conceived.

Tuesday, August 3^{rd}, 2004
Time: 08:00 - 10:00, Lecture Room 1

Symposium (Cross-Divisional)

Oryctes virus - from discovery to classical microbial control agent

Chair: Trevor Jackson; Suzanne Thiem

08:00 **THE ORYCTES BACULOVIRUS: ITS DETECTION, IDENTIFICATION, AND IMPLEMENTATION IN BIOLOGICAL CONTROL OF THE COCONUT PALM RHINOCEROS BEETLE, ORYCTES RHINOCEROS**

Alois M. Huger, *Federal Biological Research Centre for Agriculture and Forestry,, GERMANY*

Abstract: In view of the increasing and devastating damage of Oryctes rhinoceros to coconut palms in the middle of the last century, many efforts have been made to find an efficient natural control factor against this pest that could not be controlled by pesticides. Basic procedures of these monitoring campaigns are outlined together with the final detection of a virus disease in an oil palm estate in Malaysia in 1963. In extensive laboratory studies, the virus was isolated and identified as the first free baculovirus of insects. Many infection experiments determined to clarify the pathology, histopathology, and virulence of the virus proved that the virus is extremely virulent to larvae after peroral application. These findings encouraged the first experiment of virus release in coconut plantations of Western Samoa in 1967. For this purpose, breeding sites were contaminated with virus. Surprisingly, the virus became established in the Samoan rhinoceros beetle populations and spread autonomously throughout the Western Samoan islands. As a consequence, there was a drastic decline of the beetle populations followed by a conspicuous recovery of the badly damaged coconut stands. This unexpected phenomenon only became understandable after it was clarified that the adult beetle of O. rhinoceros itself is a very active virus vector and thus was responsible for the efficient autodissemination of the virus. The functioning of the beetle as a 'flying virus factory' is due to its unique pathology developing after peroral virus infection. Pathological details of this process will be presented.

08:30 **REPLICATION, GENETICS AND MOLECULAR BIOLOGY OF ORYCTES VIRUS**

Allan Crawford, *AgResearch, NEW ZEALAND*

Abstract: This paper reviews the early work on the cell culture, replication and genomic studies of Oryctes virus. At the time this work was undertaken it was regarded as a non-occluded baculovirus but now forms part of a separate group of dsDNA viruses. Early molecular studies included the development of a physical map of the virus genome which was then used to examine the variation within the virus strains isolated from different regions of the world. A unique opportunity to study virus evolution was provided by the release of 3 different characterised strains into the Maldive Islands and the subsequent sampling of the genome variation that had occurred since release.

08:55 **THE INCIDENCE AND USE OF ORYCTES VIRUS FOR CONTROL OF RHINOCEROS BEETLE IN OIL PALM PLANTATIONS IN MALAYSIA**

Ramle Molsem, Norman Kamerudin, Wahid Mohd Basri, *MPOB, MALAYSIA*; Travis Glare, Trevor Jackson, *AgResearch, NEW ZEALAND*

Abstract: The rhinoceros beetle, Oryctes rhinoceros has emerged as a serious pest of oil palm since the prohibition of burning as a method for maintaining estate hygiene in the 1990's. The abundance of beetles is surprising given that the beetle is endemic to the region and that the Malay peninsula was the site of first discovery of the Oryctes virus, which has been used to good effect as a biological control agent in other regions. A survey of adult beetles was carried out throughout Malaysia using pheromone traps. Captured beetles were examined for presence of virus using both visual/microscopic examination and PCR detection methods. The survey indicated that virus was common in Malaysia but levels of infection were highly variable between populations. Viral DNA analysis using restriction digestion with HindIII indicated at least three distinct viral genotypes. Bioassays have been carried out to compare the viral strains and suggest that one strain (type B) is the most virulent against both larvae and adults of the beetle. Virus type B has been cultured and released into healthy populations where another strain (type A) forms the natural background. Capture and examination of beetles from the release site and surrounding area has shown the spread and persistence of the applied virus strain accompanied by a dramatic reduction in palm frond damage. Future use of Oryctes virus for management of rhinoceros beetle in oil palm plantations in Malaysia will be discussed.

09:20 **ORYCTES VIRUS TIME FOR A NEW LOOK AT A USEFUL BIOCONTROL AGENT**

Trevor Jackson, Travis Glare, *AgResearch, NEW ZEALAND*

Abstract: Release of Oryctes virus for control of the rhinoceros beetle, Oryctes rhinoceros (Coleoptera: Scarabaeidae), has been one of the major successes of classical biocontrol with a microbe. In the early part of the last century, rhinoceros beetle was spreading throughout the Pacific causing devastation to coconut palms in the outbreak areas. The discovery of the virus in Malaysia and its subsequent liberation to rhinoceros beetle infested Pacific Islands in the 1960's and 70's resulted in effective control of the pest throughout much of the infested area. Further releases have been made in affected areas in South Asia and islands of the Indian Ocean where the insect has become a pest. Maintenance of the virus depends on continued availability of insect hosts and the existence of virus reservoirs. Maintaining beetle breeding sites to favor virus transmission has been the basis of beetle management in some regions. In recent years, however, there have been new reports of high levels of rhinoceros beetle damage to palms. This has been especially intense in SE Asia following the introduction of no-burn polices for land clearance and replanting, but outbreaks have also been reported from the Pacific Islands where control seems to have diminished over time. SE Asian studies show that there is considerable genetic variation among endemic Oryctes virus isolates and studies in new island release areas have shown rapid evolution of the virus. The consequences of such genetic variation are in need of further study. Molecular genetics can also assist in management of the virus. Visual/microscopic diagnosis of the infection can be problematic and relies on an experienced observer, but a PCR detection system has been developed to provide unambiguous information on infection. In the laboratory, Oryctes virus has a host range beyond rhinoceros beetle but the extent of natural infections and the possibility for use against other pests is not known. Oryctes virus has achieved wide success in the past without the benefit of molecular analysis and identification techniques. In order to fully take advantage of this unique pathogen a renewed, coordinated effort centred on genetic analysis, selection, formulation, application and population analysis is required

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Tuesday, August 3^{rd}, 2004
Time: 08:00 - 10:00, Lecture Room 6

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 1

Chair: Juan Ferré; P. Caballero

08:00 **CRY1AC INTERACTION WITH THE HELIOTHIS VIRESCENS CADHERIN-LIKE RECEPTOR**

Meibao Zhuang, Ruiyu Xie, Linda Ross, Sarjeet Gill, *Department of Cell Biology and Neuroscience, University of California, USA*

Abstract: Bacillus thuringiensis Cry protein exerts its toxic effect through a receptor-mediated process. For the Cry!Ac toxin on lepidopteran insects both aminopeptidases and cadherin-like proteins are identified as putative receptors. Support for the role of these receptors has come from RNAi inhibition and insect resistant studies, respectively. To further analyze these interactions we have evaluate the action of the Cry1Ac toxin on cells expressing the cadherin receptor. We therefore generated a stable cell line Flp-InäT-REXä-293/Full-CAD (CAD/293) that expressed the H. virescens cadherin. As expected, the cadherin-like protein was mainly localized in the cell membrane. While toxin application affected cell morphology only low levels of cell death was observed at relevant Cry1Ac toxin levels. We also analyzed the expression of the cadherin in the midgut of Heliothis larvae. Immunohistochemistry showed the cadherin-like proteins are present in the midgut apical membranes, which are the target site of Cry toxins. This subcellular localization is distinct from that of classical cadherins, which are usually present in cell-cell junctions.

08:20 **RESISTANCE TO CRY2AB IN HELICOVERPA ARMIGERA**

Ray Akhurst, Karen Olsen, Lisa Bird, Rod Mahon, *CSIRO Entomology, AUSTRALIA*

Abstract: Transgenic cotton expressing the Cry1Ac and Cry2Ab toxins of Bacillus thuringiensis (BOLLGARD II) being introduced into Australia is expected to reduce the risk of the evolution of resistance by H. armigera to these and similar toxins. This cosmopolitan pest has demonstrated its capacity for developing resistance to synthetic chemical insecticides and to the Cry1A toxins, such as the Cry1Ac produced in the first generation

of transgenic cotton. Investigation has rapidly confirmed the capacity for H. armigera to develop resistance to Cry2Ab. Five lines of H. armigera, including a field derived line obtained from an F2 screen, with various levels of resistance to Cry2Ab have been established in our laboratory using several approaches. Some lines also have some resistance to Cry1Ac. Characterisation of resistance in these lines will be discussed.

08:40 **INHERITANCE OF CRY-RESISTANCE AND CROSS-RESISTANCE IN CULEX QUINQUEFASCIATUS SELECTED WITH TOXINS FROM BACILLUS THURINGIENSIS ISRAELENSIS**

Margaret Wirth, Jeffrey Johnson, *Dept. of Entomology, University of California, USA*; Brian A. Federici, *Dept. of Entomology & Interdepartmental Graduate Program in Genetics, University of California, USA*; William Walton, *Dept. of Entomology, University of California, USA*

Abstract: The underlying genetic basis for insecticide resistance toward microbial toxins provides information necessary to predict the dynamics of resistance alleles in nature and to facilitate the development of appropriate resistance management strategies. Furthermore, genetic studies can promote the identification of resistance mechanisms. Here we report the patterns of inheritance of mosquitocidal Cry toxin resistance and cross-resistance in Culex quinquefasciatus selected for resistance to B. t. israelensis Cry toxin combinations. Reciprocal mass-crosses were prepared between the selected colony and a laboratory susceptible colony and backcrosses were performed using F1 offspring with the appropriate parental colony. In the C. quinquefasciatus colony selected with Cry4A + Cry4B, inheritance of resistance to Cry4A + Cry4B was intermediate in resistance phenotype in the F1 offspring and offspring of the backcross did not fit a monofactorial model, suggesting involvement of 2 or more loci. Similar results were obtained when inheritance patterns of cross-resistance to Cry11B from B. t. jegathesan were evaluated. However patterns of inheritance of cross-resistance toward Cry11A from B. t. israelensis fit a monofactorial model. Using an in vivo binding assay with FITC-labeled Cry11A crystal proteins, binding was observed in susceptible mosquito larvae whereas no binding of Cry11A was observed in the Cry4A + Cry4B resistant larvae. The implication of these results is that mosquitoes under long-term selection pressure with Cry4A + Cry4B evolved resistance at several loci, one locus of which is responsible for cross-resistance to Cry11A. Furthermore, the in vivo binding tests suggest that Cry11A cross-resistance in this colony results from alteration in the binding site for Cry11A and point to shared binding sites among B. t. israelensis Cry toxins.

09:00 **RESTORATION OF ANTI-BACTERIAL ACTIVITY OF A CRYPTIC ORF (CYT1CA) FROM B. THURINGIENSIS ISRAELENSIS BY SITE-DIRECTED MUTAGENESIS**

Mark Itsko, Robert Manasherob, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

Abstract: Insecticidal crystal proteins (ICP) of different Bacillus thuringiensis subsps. are classified to two unrelated families: receptors-specific Cry toxins that permeabilize the membrane of midgut insect cells and Cyt toxins that lyse a broad range of cells, bacteria included, via direct binding to phospholipids. A new cyt-like gene, cyt1Ca encoding a 60 kDa protein has recently been discovered in B. thuringiensis subsp. israelensis. Its predicted product displays a two-domain fusion protein: N-terminal half resembling the common Cyt toxins, and C-terminal half similar to the receptor binding domain of several unrelated ricin-like toxins. Neither larvicidal activity of cyt1Ca expressed in Escherichia coli nor hemolytic effect of His-tagged purified Cyt1Ca was found. This inactivity was attributed to four amino acid differences between its Cyt-like (N-terminal) moiety and Cyt1Aa [1]: the non-polar amino-acids (three Gln and a Gly) in the former compared to charged ones (two Lys, a Glu and an Asp) in the latter. In attempts to obtain toxic variant(s) of Cyt1Ca and dissect the dual actions of Cyt1Aa on E. coli cells [2], the 3'-end of cyt1Ca was truncated (removing the C-terminal domain), and four single bases in the remaining domain were appropriately site-directed mutagenized to replace the non-polar by charged amino acids according to Cyt1Aa. The modified Cyt1Ca versions were lethal upon expression in E. coli, with varied anti-bacterial activities among them. Their impacts on cell divisions, cell and nucleoid morphologies were characterized. [1] Ward, E.S., Ellar, D.J. & Chilcott, C.N., 1988. J. Mol. Biol. 202: 527-535 [2] Manasherob, R., Zaritsky, A. et al., 2003. Microbiology 149: 3553-3564
Contributed Student Paper

09:20 **A NOVEL BACILLUS THURINGIENSIS INSECTICIDAL PROTEIN TOXIC TO MEMBERS OF SEVERAL FAMILIES FROM LEPIDOPTERA AND COLEOPTERA.**

Ruiz De Escudero, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*; Anna Estela, *Departamento de Genética, Universidad de Valencia, SPAIN*; M. Porcar, F. J. Pérez-Llarena, J. A. Oguiza, C. Martínez, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*; Baltasar Escriche, Juan Ferré, *Departamento de Genética, Universidad de Valencia, SPAIN*; Primitivo Caballero, *Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN*

Abstract: To date, more than 200 cry genes encoding specific Cry proteins toxic against a number of insect pests have been identified from a variety of Bacillus thuringiensis strains. We report the characterization of a new protein in the Cry1I-class which is produced and secreted into the media during the vegetative growth phase of the HU4-2 strain of B. thuringiensis serovar aizawai. By PCR, an ORF of 2200 bp was identified which encodes for a new protein with a predicted molecular mass of 80.9 kDa. The deduced sequence of the protein presented a homology of 96.1% with Cry1Ia1, 92.8% with Cry1Ib1, and 89.6% with Cry1Ic1. In line with current classification criteria, the new protein was named Cry1Ia7. The novel cry1a7 gene was subcloned into the pET Easy Vector and overexpressed in Escherichia coli BL21DE3. Cry1Ia7 showed insecticidal activity against Lobesia botrana (Lep.), Plutella xylostella (Lep.), and Leptinotarsa decemlineata (Col.) with LC50 values of 8.55, 12.16, and 10.00 g/ml, respectively. However, toxicity was not detected towards Spodoptera exigua and Helicoverpa armigera. Using 125I-labeled Cry1Ab toxin, we observed that this protein does not compete with Cry1Ia7 for a common binding site on midgut cells. Since Cry1Ab is the most active Cry protein in bioinsecticide products used to control L. botrana and P. xylostella, we discuss the potential use of Cry1Ia7 in case resistance appears to Cry1Ab in pest populations.

09:40 **IFICATION AND CHARACTERIZATIONS OF A NEW BACILLUS THURINGIENSIS VIRULENCE FACTOR : THE INHA2 METALLOPROTEASE**

Myriam Hajaij, *Unité Génétique Microbienne et Environnement, INRA, FRANCE*; Michel Gohar, *Unité Génétique Microbienne et Environnement, INRA, Unité Microbiologie et Génétique Microbienne, INRA, FRANCE*; Sinda Fedhila, *Unité Génétique Microbienne et Environnement, INRA, FRANCE*; Didier Lereclus, Christina Nielsen-LeRoux, *Unité Génétique Microbienne et Environnement, INRA, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*

Abstract: The main insecticidal activity of Bacillus thuringiensis (Bt) is due to the larval ingestion of the insect specific Cry toxins. However, strains of both crystal minus Bt and of B. cereus are known to produce other factors contributing to the overall virulence of these bacteria toward insects. The importance of the Bt pleiotrophic PlcR regulator was demonstrated by reduced mortality in larvae of the greater wax moth Galleria mellonella infected with spores from a Bt 407 cry- plcR mutant (Salamitou et al., 2000). PlcR governs many putative virulence factors (phospholipases, enterotoxins, hemolysins, proteases etc.), and recently the putative PlcR-controlled zinc protease InhA2 was discovered to be important for pathogenesis via the oral route (Fedhila et al. 2002, 2003). InhA2 may interfere with intestinal barriers (peritrophic membrane and/or intestinal midgut cells). InhA2 is found as a 72 kDa polypeptide in the secretomes of Bt 407 cry- cultures in early stationary phase. InhA2has 66% homology with InhA (inhibitor A) which degrade some insect antimicrobial peptides. Purification of InhA2 was performed in order to characterize its enzymatic activity and specificity and to correlate this with the possible mode of action of InhA2 during the larval infection process. Since InhA2 is lethal for E. coli, purification was obtained from supernatants of a recombinant Bt 407 cry- plcR mutant transformed with the plasmid [pHT315 Omega (papha3-inha2)] where inhA2 is placed downstream of the constitutive promotor of apha3 resulting in high level expression. Following precipitation by 85% ammonium sulphate, InhA2 was fully purified by anion-exchange chromatography in the presence of Ca2+ which is required for stability. Using azo-casein as a colorimetric substrat for measuring the enzymatic activity, InhA2 was found to be active in a large range of temperatures, from 25 to 55C, with an optimum at 45C, and in a rather acid and neutral pH spectrum, from pH 6 to 8. Enzymatic activity was inhibited by several protease inhibitors,both specific metallo and serine inhibitors as well as EDTA and EGTA chelators and by high concentrations of zinc. Besides its activity on casein, InhA2 was also found to degrade albumin, collagene, gelatin, and actin. No direct larvicidal activity was observed when pure InhA2 was ingested by Galleria mellonella;Further investigations related to cellular targets in the larvae are under process.
Salamitou S. et al., 2000 The regulon PlcR is invooved in the opportunistic

properties of Bacillus thuringiensis and Bacillus cereus in mice and insects. Microbiology 146 :2825-2832 Fedhila, S., Nel, P., Lereclus,D., 2002. The InhA2 metalloprotease of Bacillus thuringiensis strain 407 is required for pathogenicity in insects via the oral route. J. Bacteriol. 184: 3296-3304. Fedhila, S., Gohar, M., Slamti, L., Nel, P., Lereclus,D., 2003. The Bacillus thuringiensis PlcR-regulated gene inhA2 is necessary, but not sufficient, for virulence. J. Bacteriol., 185: 2820-2825.

Tuesday, August 3^{rd}, 2004
Time: 10:15 - 12:00, Lecture Room 1

Society General Meeting

Chair: Harry Kaya

Tuesday, August 3^{rd}, 2004
Time: 12:00 - 14:30, Solvalla

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5 k Fun Run

Note: Departure at 12:15 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 13:00 - 18:00, Nuuksio

Excursion 1: Nuuksio National Park (off-path)

Host: Larry Huldén
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 13:00 - 18:00, Nuuksio

Excursion 2: Nuuksio National Park (easy)

Host: Lena Huldén
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 13:00 - 18:00,

Excursion 3: Marimekko factory outlet

Host: Ingeborg Menzler-Hokkanen
Note: Departure at 13:00 by bus from UH Main Building

Tuesday, August 3^{rd}, 2004
Time: 19:00 - 24:00, Tolkkinen

BBQ

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 10

Contributed Papers (Division of Microsporidia)

microsporidia / contibuted paper session 1

Chair: Rudolf Wegensteiner; Regina Kleespies

09:00 **THE DIVERSITY OF MICROSPORIDIA IN FRESHWATER AMPHIPODS: HOST-PARASITE INTERACTION DURING INVASIONS**

Johanna Slothouber-Galbreath, Judith Smith, Rebecca Terry, *School of Biology, University of Leeds, UNITED KINGDOM*; James Becnel, *USDA/ARS, Center for Medical, Agricultural and Veterinary Entomology, UNITED STATES*; Alison Dunn, *School of Biology, University of Leeds, UNITED KINGDOM*

Abstract: Parasitism may moderate invasion success. The 'enemy release' theory predicts that parasite prevalence and diversity may be reduced during invasion events by virtue of selection against hosts with reduced fitness due to parasitism. Successful invasive amphipods and their microsporidia provide an informative model to examine host-parasite interactions during invasion events. Fibrillanosema crangonycis (Slothouber-Galbreath et al. 2004) is a vertically transmitted (VT), sex ratio distorting microsporidium described from invasive European populations of the North American freshwater amphipod Crangonyx pseudogracilis. VT microsporidia may have little direct impact on host fitness but they may utilize sex ratio distortion by feminization to increase transmission success. We predict that VT microsporidia will not be lost by invading hosts and may be selectively retained. Additionally, by increasing host population growth rate, feminizing microsporidia may increase host establishment success. Over 17 species of horizontally and vertically transmitted microspordia have been attributed to at least 14 species of European amphipods but only one other microsporidium had been characterized from North America hosts. We examined archived specimens and re-sampled source populations to establish the diversity of and characterize microsporidia in North American amphipods. Additionally, we surveyed candidate sites in North America and Europe to determine the origin and spread of the invasive C. pseudogracilis and F. crangonycis. We demonstrate that F. crangonycis is widespread in Europe. Both the host and parasite exhibit low genetic diversity. This may indicate a single invasion event or low genetic diversity in the source population(s). No additional invasive microsporidia were found. This implies that the VT F. crangonycis may have been selectively retained. We summarize data on the diversity of microsporidia present in North American amphipods and show that the occurrence and prevalence of these microsporidia and their hosts have changed significantly over the previous 30 years. This may be due to change in land use and climatic factors. We discuss how these factors may impact parasite prevalence and diversity in a manner similar to invasion events.

09:20 **MICROSPORIDIAN PARASITES OF AUSTRALIAN FRESHWATER CRAYFISH, CHERAX DESTRUCTOR AND CHERAX SETOSUS (DECAPODA: PARASTACIDAE)**

Elizabeth Moodie, *University of New England, AUSTRALIA*

Abstract: Three new microsporidia that infect Australian freshwater yabbies (Cherax destructor and Cherax setosus), have been characterised. Cell morphology and ultrastructure, patterns of development and ribosomal DNA sequence (rDNA) data are described. Molecular phylogenies of the three species, based on SSU rDNA data, are presented. Thelohania parastaci, Thelohania montivirulorum and Vairimorpha cheracis target the muscle tissue of host crayfish. Infections progress slowly, leading to death of the host. T. parastaci was found in wild and cultured populations of C. destructor from NSW, Victoria and Western Australia. A coastal population of Cherax setosus was also infected by T. parastaci. A population of C. destructor from a highland stream near Armidale, NSW, was co-infected by T. montirivulorum and V. cheracis. Ultrastructural features, patterns of spore production and SSU rDNA sequence similarities indicated T. montirivulorum and T. parastaci were congeneric with the crayfish pathogen T. contejeani, from Europe. Shared ultrastructural features and SSU rDNA similarities with other Vairimorpha species indicated that V. cheracis was best placed in this genus. Molecular phylogenetic analyses of a wide variety of microsporidia from different lineages within the phylum Microsporidia placed Thelohania species from crustacean hosts in a sister clade to that containing the Vairimorpha/Nosema group of species. PCR assays based on SSU rDNA were developed for the detection of T. parastaci, T. montirivulorum and V. cheracis in crayfish tissues. Assays based on the ITS region of the rDNA of T. parastaci and T. montirivulorum were also developed. PCR methods proved to be more sensitive in detecting the presence of microsporidia than microscopic examination for spores in the same tis-

sues. Means for improving the sensitivities and specificities of the assays are suggested. Prevalence of infection of T. montirivulorum and V. cheracis in the highland stream-dwelling population of Cherax destructor in which the parasites were endemic, was investigated over a period of 26 months. Patterns of prevalence in relation to stream habitats are described. Future research directions are discussed.

09:40 **MICROSPORIDIA SUPPRESS MELANIZATION REACTION AND PHENOLOXIDASE ACTIVITY OF THE HAEMOLYMPH OF THEIR INSECT HOSTS**

Yuriy Tokarev, *All-Russ. Inst. Plant Protection, Dept. Microb. Control, RUSSIA*; Ya.L Vorontsova, *Inst. Anim. Syst. Ecol., Lab. Insect Pathol, RUSSIA*; Yulia Sokolova, *Inst. Cytol., Lab. Cytol. Unicell. Org., RUSSIA*; V.V. Glupov, *Inst. Anim. Syst. Ecol., Lab. Insect Pathol, RUSSIA*; R. Entzeroth, *Dresden Techn. Uni., Lab Parasitol, GERMANY*

Abstract: Melanization, mediated by phenoloxidase (PO) system, is a principal defense reaction of insect haemolymph, and many parasites suppress or avoid action of POs. We have shown previously, that microsporidia (M) Paranosema (=Nosema) grylli suppress PO in haemocytes of cricket Gryllus bimaculatus at the acute stage of microsporidiosis (Sokolova et al., 1999, 2000). The goals of the present work were to demonstrate effect of M on host PO activity and melanization in three parasite-host systems: G. bimaculatus - P. grylli; locust Locusta migratoria - P. locustae; and wax moth Galleria mellonella - Vairimorpha ephestiae, and to find out possible effects of host PO system on M. We measured insect haemolymph melanization and PO activity in haemocytes and haemolymph after peroral infection or injection of spores. Additionally, melanized M spores were stained using DAPI and Calcofluor, and cricket haemocytes were coincubated with M spores under in vitro conditions. It was found that M spores suppress PO activity both in plasma and in haemocytes. Whole haemolymph uptaken from crickets and wax moth larvae heavily infected with M spores did not show any signs of melanization, in contrast to insects without M or at the early stage of the disease. PO activity in plasma and in haemocytes of wax moth started to decrease after 3 days of V. ephestiae infection. Injection of V. ephestiae and P. locustae spores into body cavity of larvae significantly reduced the quote of PO-positive (PO+) haemocytes as well. In crickets, the quote of PO+ haemocytes was also reduced at the acute phase of the disease. Co-incubation of P. grylli and P. locustae spores with cricket haemocyte monolayers caused 3-fold and 30-fold decrease in the quote of PO+ haemocytes, respectively. DAPI and Calcofluor staining showed, that melanized nodules in crickets and locusts contained 6-10 times higher quotes (as compared to non-melanized tissues) of teratospores - enlarged spores with aberrant nuclear apparatus. The obtained results showed that suppression of PO activity took place in all studied systems, though it may vary in extent and patterns, and bring some evidence that melanin deposits are toxic for M development and may lead to formation of teratospores. Ability to suppress PO synthetic pathway might be considered an adaptation of insect M to survive inside the host. Supported by a DAAD stipend to Y. Tokarev # 325 PKZ A/03/01385, and by Russ. Found. Basic Res. #03-04-49629.

10:00 **TRANSMISSION OF THE MICROSPORIDIAN, NOSEMA FUMIFERANAE, IN SPRUCE BUDWORM POPULATIONS**

Christina Campbell, Sandy Smith, *University of Toronto, CANADA*; Kees Van Frankenhuyzen, *Canadian Forest Service, Natural Resources Canada, CANADA*

Abstract: The spruce budworm (Lepidoptera: Tortricidae) is a major forest pest throughout North America. During outbreaks, extensive feeding on spruce and fir foliage results in massive tree mortality and economic loss. A parasitic protozoan, Nosema fumiferanae (Microsporida: Nosematidae), is often found at high levels during a budworm outbreak and delays budworm development and reduces fecundity. This sublethal pathogen is thought to be involved in the collapse of budworm populations. Nosema is transmitted via per os (horizontally) and transovarially (vertical) in budworm populations. We have put forth a third transmission mode, self transmission, occurring during the early instars. During the early instars, larvae remain in feeding shelters and a vertically infected larva is potentially exposed and ingests self-egested spores. The subsequent re-infection may result in a higher spore a yield which possibly corresponds to more efficient horizontal transmission and higher levels of infection in adult females. I explore the importance and applicability of self transmission. The incubation period of Nosema and spore production for various instars is examined at differing levels of infection and temperature. Implications and impacts of these findings are discussed in relation to horizontal transmission. I explore the rate of horizontal transmission in the greenhouse. Rates will be at determined various budworm densities. These ratios will provide information on how the pathogen spreads and can reach high infection levels during budworm outbreaks. My work will expand our current state of knowledge about insect-pathogen population dynamics in general. Most insect literature focuses on lethal pathogens and the involvement of sublethal pathogens in the system has largely been ignored.

10:20 **STRUCTURE AND DEVELOPMENT OF THELOHANIA SOLENOPSAE IN FIRE ANTS**

Yulia Sokolova, James Fuxa, *Louisiana State University Ag.Center, USA*

Abstract: Thelohania solenopsae Knell Allen and Hazard 1977 (T.s.) has become established intermittently in populations of red imported fire ants, Solenopsis invicta Buren, in the southeastern United States. Successful experimental releases of T.s. have demonstrated its potential for biological control of fire ants. Yet the life cycle of this microsporidium is largely unknown. T.s. produces at least four types of spores: (1) diplokaryotic spores, which develop only in brood, with a large posterior vacuole, thin wall, and few polar filament coils; (2) octospores developing in octets within sporophorous vesicles, the most prominent spore type in adults but never occurring in brood; (3) Nosema-like diplokaryotic spores developing in adults; (4) large megaspores with thick walls and numerous polar filament coils, occurring occasionally in larvae and adults of all castes. The fat body was previously identified as the prime site of infection. All attempts to experimentally infect ants per os with any kind of spores have failed. So called cysts, conglomerates of octospores, are sporadically produced in adults. The current research has revealed further aspects of the T.s. life cycle by light and electron microscopic analysis of infected workers and queens. Nosema-like spores function in autoinfection of adjacent adipocytes. Eventually meiosis occurs, and the diplokaryotic sequence switches to the octospore sequence, resulting in production of large numbers of octospores and occasional formation of a cyst from a hypertrophic adipocyte. Presumably, when certain tissues are infected, megaspores develop after meiosis instead of octospores from the same type of sporoblast. These megaspores are found in muscles and in layers of fat body underlying ovarioles in reproductive females. The data strongly suggest that placing this species in the genus Thelohania is not correct and that a new genus may be necessary. We are continuing further research of the T.s. life cycle.

10:40 **UNIKARYON DUPLICATI AS COMMON PATHOGEN OF IPS DUPLICATUS ATTACKING SPRUCE**

J. Holusa, *Forestry and Game Management Research Institute, CZECH REPUBLIC*; Jaroslaw Weiser, *Heralecka 964, 140 00 Praha 4, CZECH REPUBLIC*; Z. Zizka, *Electron Microscopy, Inst. of Microbiology, Acad. Sci., CZECH REPUBLIC*

Abstract: The Northern spruce bark beetle, Ips duplicatus Sahl. (Col.: Scolytidae) appears as major pest in spruce stands in NE Moravia, usually in association with Ips typographus. Both beetles have in common the infection with Gregarina typographi and the microsporidian Chytridioposis typographi, which attacks and destroys in 2 to 10 % the epithel of the midgut of adult beetles. Another microsporidian , Unikaryon montanum infects the muscularis of the midgut, the fat body and the ovaries of 0.5 % adults and only in Ips typographus. In Ips duplicatus a new microsporidian Unikaryon duplicati is present in a rather high level infestation in 10-70% of adults in all inspected localities. The infection is localized only in the muscularis of the midgut of adult beetles, and does not invade other tissues. The pathogen is not transmited to Ips typographus living in the lower part of the same tree. The microsporidian fills the circular and longitudinal muscles of the muscularis, only limited development is in the columnar cells of the epithel. The spores do not leave the infected tissues in watermount and the frequency of the infection is reported only from dissected beetles with extracted midgut. In larval progeny of infected parent beetles, in pupae and yellow freshly hatched animals, the infection is not present. The parasite causes evidently reduced peristaltics of the midgut. Further characteristics of the infection will be investigated. The microsporidian does not infect Ips typographus and Pityogenes chalcographus living in association.

11:00 **THE CYST LIKE SPOROPHOROUS VESICLE OF CHYTRIDIOPSIS TYPOGRAPHI**

Rudolf Wegensteiner, *Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU - University of Natural Resources and Applied Life Science, AUSTRIA*; Jaroslaw Weiser, *Emeritus, Institute of Entomology, Academy of Sciences of the Czech Republic, CZECH REPUBLIC*

Abstract: Chytridiopsis typographi is a common pathogen of the spruce bark beetle, Ips typographus, in Europe, usually it attacks 2-3 % of adult beetles, but in some localities it infects more than 30% beetles. Furthermore, this microsporidium infects several other bark beetle species living associated with I. typographus on Norway spruce. The infection is transmitted with persistent spores which are enclosed in a resistant sporophorous vesicle which is a typical structure of the genus Chytrid-

iopsis. The origin of the structure is in the early sporont and is formed during the division of the sporogonial plasmodium into the usual 16 sporoblasts. The structure of the plasma membrane of the sporoblasts and of the sporophorous vesicle is identical during the first period. There is no surface deposit, typical for sporoblasts in other microsporidia, but instead of this the sporophorous vesicle is incrustated by electron dense deposits and grows in a solid smooth cystic structure. The sporoblasts are closed inside the vesicle in a thin plasma membrane and in the interspace is a fine granular deposit. The wall of the vesicular structure is composed of two layers which are resistant to fixation and the cyst is constricted after fixation in the same way as it is in young spores of other microsporidia in the crenate stage. Details of the cyst in Chytridiopsis typographi are compared with analogous structures in other Chytridiopsidae. The role of persistent spores and spores for primary infection is discussed in the infection with Chytridiopsis typographi.

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 12

Workshops (Division of Viruses)

Genome analysis methodology -workshop

Chair: Johannes Jehle

09:00 **GENOME SEQUENCING AND ANALYSIS**

Claudio L Afonso, Gerald F. Kutish, *Plum Island Animal Disease Center, Agricultural Research Service, U.S.A.*

Abstract: Determination of the entire viral genome sequence can lead to a better understanding of virus biology. Assembly of complete genomic DNA sequence allows comprehensive prediction of genome structure, coding capacity, transcriptional regulation of gene expression, and protein function. Presence of genes of known function allows prediction of virion structure, virus replication strategies, and putative viral-host interactions. Complete genome phylogenetic analysis of nucleic acid and protein sequences allow more accurate evolutionary predictions. Available molecular biology and bio-informatics tools allow selection of multiple strategies and methodologies for genome sequencing, assembly and analysis. Discussion will include brief descriptions of strategies, steps and methods for sequencing, genome assembly and analysis applicable to small and large viral DNA genomes. Criteria and methods will be presented for whole genome comparison, gene finding, identification of gene families, and functional prediction. A general introduction to free and commonly available UNIX, PC or server based DNA analysis tools and available databases will be presented. Logistics, timelines, pitfalls, and bottlenecks will be discussed.

09:30 **A FEW SIMPLE AND QUICK STRATEGIES FOR USING WHOLE GENOME SEQUENCE INFORMATION FOR SIMILARITY-BASED CLUSTERING**

Paolo M. de A. Zanotto, *Instituto de Ciencias Biomedicas II, Universidade de São Paulo, USP, Sao Paulo, SP, BRAZIL*; Ricardo Pereira, *Instituto de Matemática e Estatística - IME, Universidade de São Paulo USP, Sao Paulo, SP, BRAZIL*

Abstract: Complete genome sequences allowed the advent of the comparative genomics and genome systematics. A key issue is that of devising methods that will take genome content information and consider the ancestral relationships among homologous and paralogous genes together with overall genomic architecture within an integrated framework. Useful and statistically sound optimality criteria such as Bayesian and maximum likelihood methods are available for phylogenetic inference. They use explicit and testable evolutionary models and allow for significance testing based on the explanatory power of competing hypotheses. However, data on the lack or absence of genes or their order in the genome are hard to integrate with inferences based in gene alignments. Basically quantitative gene content analysis can be expressed as either adimensional quantities or by parsimonious reconstructions. This amounts to a character weighting in the absence of a explicit evolutionary model. However, genetic distances relate to observed rates of change along sites and are proportional to evolutionary time. Gene-based systematics can be extended to complete genomes once the shared set of aligned genes are and treated, either independently or as a single concatamer (proteon) in a given order. Then, incongruencies may inform about gene transfer, etc. However, at low levels of synteny this entails aligning complete genomes or sets of orthologues with the loss of partially shared traits. Alternatively, we may do pairwise comparisons among complete genomes and build distribution from scores of shared genomic features. The distribution moments, the integration differences or the Kullback-Leibler or Chernoff distances are used for clustering genomes. We will show individual clusters for some viral families and compare to phylogenies from complete genome alignments. An advantage of this approach is the speed of computation and the lack of constraints imposed by the size of the dataset.

10:00 **GENOME PHYLOGENIES**

Elisabeth Herniou, *Imperial College London, UK*

Abstract: Phylogenetic trees provide invaluable information about the evolutionary relationships of genes and of species. They are now part of the molecular biologist tool kit. However, often lack of skill prevents the full exploitation of the data. The current spurt of complete genomes sequencing has led to the creation of the field of phylogenomics. Beyond the reconstruction of species trees based on complete genomes, the application of phylogenetic methods to the study of genomes can provide insights on gene function, which might in the future shorten the time required to elucidate biological mechanisms.

10:30 **APPLICATIONS OF DNA MICROARRAYS FOR THE STUDY OF BACULOVIRUS TRANSCRIPTIONAL REGULATION**

Gary W. Blissard, *Boyce Thompson Institute, Cornell University, U.S.A.*; Erik D. Burnett, *Boyce Thompson Institute, Cornell University, Lawrence Livermore National Laboratory, U.S.A.*; Warren F. Lamboy, *Center for Agricultural Bioinformatics, USDA-ARS, Cornell Univ.,, U.S.A.*

Abstract: The analysis of whole genome expression profiles using DNA microarrays is an attractive methodology for studying highly and moderately complex genomes, including those of large viruses such as baculoviruses. Baculovirus genomes are moderate in size, encoding app. 90-180 open reading frames that are closely packed within the genome. In the genome of the best studied baculovirus, AcMNPV, most open reading frames do not overlap others, but genes are closely spaced with little distance between adjacent reading frames. Also genes are oriented in both directions on the genome and from genes that have been studied in detail, it is known that adjacent transcripts may overlap in some cases. Thus because the genome is compact and has the potential for overlapping adjacent transcripts, this presents challenges in the design and interpretation of microarray data. Issues relevant to the selection of the array design and methods, cost, and methods of analysis will be discussed. In addition, strengths and limitations of various approaches will be considered in relation to their use for the analysis of baculovirus gene expression.

11:00 **USE OF GENOME DATA FOR TAXONOMY AND CLASSIFICATION**

David Theilmann, *Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, CANADA*

Abstract: There has been a recent rapid increase in the number of baculovirus genomes that have been completely sequenced and characterized. This data has identified hundreds of potential genes some of which are highly conserved and others that are specific to a virus or group of viruses. Whole genome and single gene phylogenetic analyses have identified natural clusters of genomes that are more highly related. This new wealth of molecular data along with biological data is providing new tools for determining the evolutionary relatedness and hence the taxonomic structure of virus groups. Baculovirus taxonomy provides an excellent example of how this data is forcing changes in the family structure and definition of a baculovirus species.

Wednesday, August 4^{th}, 2004
Time: 09:00 - 12:00, Lecture Room 1

Symposium (Cross-Divisional)

Fungi and nematodes under unfavorable conditions

Chair: Solveig Haukeland-Salinas; Ingeborg Klingen

09:00 **IMPROVEMENT OF THE DESSICATION AND TEMPERATURE TOLERANCE OF HETERORHABDITIS BACTERIOPHORA**

Ralf-Udo Ehlers, Olaf Strauch, Jesko Oestergaard, *Institute for Phytopathology, Department for Biotechnology and Biological Control, Christian-Albrechts-University Kiel, GERMANY*

Abstract: Foliar application of EPN against lepidopteran pests, leaf miners, thrips and white flies are currently tested under greenhouse conditions to investigate the potential for commercial use. After spraying EPN are exposed to low humidity and high temperature. An enhancement of the desiccation and heat tolerance can increase the performance of commercial EPN products. Nematodes are able to adapt to desiccation stress by the production of several protective substances like glycerol, trehalose and proteins. Prior to the selection process, the optimal adaptation conditions were determinated. Nematodes were dehydrated in polyethylenglycol (PEG) with defined water activities (Aw-values). Decreasing water activity causes increasing dehydration. The highest desiccation tolerance was observed in populations adapted at an Aw-value of 0.96 for 72 h. The variability of the desiccation tolerance within a population increased during adaptation. The proportion of the genetic variability on the phenotypic variability (heritability - h) for adapted was h=0.46 and for not adapted h=0.48. By selection the mean tolerated Aw-value could be reduced from 0.89 to 0.81 including the adaption to low humidity. No reduction of the mean tolerated water activity could be obtained for non-adapted populations. The heritability for the heat tolerance was h= 0.68. After 4 selection steps the mean tolerated heat tolerance was increased from 38.5 up to 39.2 degrees Celcius. By selection for cold active infective juveniles the mean tolerated temperature was continuously reduced from 7.3 down to 5.2 degrees Celcius during the first 5 steps. Afterwards, however, the mean tolerated temperatures increased to 6.7 degrees Celcius. A screening among isolates of Photorhabdus luminescens resulted in the identification of strains which were growing at lower temperature.

09:25 **EFFICACY OF ENTOMOPATHOGENIC NEMATODES UNDER COLD CONDITIONS**

Haukeland Salinas Solveig, *Norwegian Crop research Institute, NORWAY*

Abstract: The use of entomopathogenic nematodes against some field pests is restricted by low temperature, often temperatures below 10-12oC. In early 1990 one of the first European scientific networks, Cost Action 812 ("Cold active lines of insect parasitic nematodes"), addressed this issue and many achievements, including several enterprises starting nematode production, were made. Until fairly recently however, no nematode products have been available on the market especially directed for use under cold conditions. Several studies have shown that the vine weevil (Otiorhynchus sulcatus) can be controlled successfully with entomopathogenic nematodes at favourable temperatures, however at temperatures below 10-12oC effective control is more difficult. The vine weevil larvae feed actively on plant roots from about 6oC, rendering them protected from nematode attack until soil temperatures rise. This means plant damage is done before the nematodes are able to effectively kill the larvae. Thus control strategies using cold active nematodes are still needed for vine weevil control both in temperate climates, with its cold spring and autumn temperatures, and in more southern climates where the vine weevil larvae are active through most of the winter months. Research on the use of entomopathogenic nematodes against vine weevil is currently being conducted within a national research project entitled Reduced use of pesticides in field grown strawberries. One of the objectives is to investigate the efficacy of Norwegian entomopathogenic nematode species at low temperatures. Results from some of the work conducted so far will be presented.

09:50 **PHASMARHABDITIS HERMAPHRODITA TO CONTROL SLUGS UNDER COLD CONDITIONS**

M. J. Wilson, *University of Aberdeen, UNITED KINGDOM*

Abstract: Slugs, and in particular the field slug Deroceras reticulatum are pests in cool damp areas such as north west Europe. D. reticulatum is active under cold conditions and can cause feeding damage to crops at temperatures as low as 2C. The nematode parasite Phasmarhabditis hermaphrodita is sold as a biological control agent for slugs and thus it will need to be active at low temperatures. This presentation will review the thermal biology of this nematode. P. hermaphrodita also appears to be active at low temperatures. Furthermore, the optimum temperature for host and parasite are remarkably similar.

10:15 **HOW TO FIND FUNGI IN EXTREME ENVIRONMENTS**

Marilena Aquino de Muro, Julian Smith, Paul Cannon, *CABI Bioscience, UNITED KINGDOM*

Abstract: Fungi constitute the second most diverse major organism group on Earth, and are crucially important in decomposition. Species are active in a wide range of extreme environments, including high and low temperature, arid, oxygen-deficient and polluted sites. Species are present even in the dry valleys of the Antarctic. Many are enzymatically highly competent, leading to applications in bioremediation. Except in low oxygen environments where anaerobic respiration is dominant, fungi play a much greater role in nutrient cycling than bacteria. Numbers of species recoverable from environmental samples can often run into the hundreds. There are numerous challenges in the detection, extraction, separation and characterization of fungi from extreme environments, although the relatively species-poor guilds of fungi in these conditions, actually simplifies the task in many circumstances.

Direct observation techniques are time-consuming and inaccurate, and many species must be cultured in order to identify them. This is often problematic as many do not produce spores which are critical for identification. A particular challenge is to separate slow-growing species from faster-growing weedy species that frequently overgrow colonies in mixed isolation plates. Methods will be described for minimising the risk of this occurring.

Molecular methods for assessment and characterization of fungal diversity in samples from extreme environments hold particular province, and standard bacterial techniques such as D/TGGE and T-RFLP are starting to be applied to fungi with some success. However, interference by pollutants in the amplification process and distinction between actively growing species, those present only as surviving propagules, and dead material are concerns which apply to the sampling.

10:40 **INSECT PATHOGENIC FUNGI COPING WITH THE COLD**

Charlotte Nielsen, Susanne Harding, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Edda Sigurdís Oddsdóttir, Gudmundur Halldórsson, *Iceland Forest Research, Mogilsa, IS 116, Reykjavik, ICELAND*; Tróndur Leivsson, *Forestry Service of the Faroe Islands, Hvítanesvegur 3, P.O Box 1174, FO-110, FAROE ISLANDS*; Niels M. Schmidt, Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*

Abstract: A range of naturally occurring insect pathogenic fungi infects and regulates insect populations all over the world. In most studies, attention has been given to the natural occurrence of infection in pest insect populations in the temperate zone during the summer months, and knowledge concerning the natural occurrence of insect pathogenic fungi in the subpolar and polar regions is much more limited.

The objective of this presentation is to give an overview of our present knowledge on the natural occurrence of insect pathogenic fungi in cold temperate, subpolar and polar regions in Iceland, Faroe Island and Greenland based on surveys of aphids and flies as well as Galleria and Tenebrio baiting of soil samples. From soil samples we were able to document fungi from the following hyphomycete genera: Beauveria, Metarhizium and Paecilomyces. In dipterans and aphids the survey in Iceland documented entomophthoralean species belonging to the following seven genera: Pandora, Strongwellsea, Entomophthora, Entomophaga, Erynia, Conidiobolus and Neozygites. Under cold conditions, the ability of the fungi to survive for extended periods outside its living host insect during winters is critical. Furthermore, the ecosystem in Iceland is very fragmented due to soil erosion and forest destruction. Spreading between geographically distinct populations in a fragmented landscape must be a major challenge for the fungi. These challenges will be exemplified and discussed further for entomophthoralean fungi infecting aphids.

11:05 **EFFECTIVENESS OF ENTOMOPATHOGENIC FUNGI AS BIOLOGICAL CONTROL AGENTS UNDER DRY CONDITIONS**

Italo Delalibera Jr, *Department of Entomology, Plant Pathology and Zoology, ESALQ-University of São Paulo, BRAZIL*; Ann Hajek, *Department of Entomology, Cornell University, USA*

Abstract: Environmental factors such as humidity, temperature, and UV light, are important in determining fungal survival. High relative humidity is widely recognized to be a critical requirement for sporulation of entomopathogenic fungi and is a crucial constraint to their recycling ability

in the environment. Secondary cycling of the fungus may contribute significantly to classical biological control but is less important to the effectiveness of mycoinsecticides. High relative humidity may not be critical for germination of infective propagules on the host cuticle and growth of some pathogens within the body because adequate moisture to promote infection can be found on the cuticle and within the body of the arthropod host. Therefore, high humidity appears not to be the most crucial climatic constraint for use of fungi as bioinsecticides which supports the testing of pathogens such as Beauveria bassiana as control agents against pests in dry conditions such as in semi-arid areas and storage environments. Some fungi use different strategies to survive under dry conditions enabling them to explore dry habitats unsuitable for many fungi. Strategies to meet the moisture needs include the ability to sporulate at low relative humidity and to efficiently use brief periods of high humidity to complete the vulnerable process of infection. Very few fungal species are able to sporulate at relative humidities lower than 50%. In fact, even fungi able to sporulate at broad ranges of relative humidity prefer moist environments. Other fungal species depend on moderate to high relative humidity for production and germination of spores, but they can use the limited moisture available to complete this part of the life cycle in only a few hours. A typical example of this strategy is Neozygites tanajoae, one of the most important natural enemies of the cassava green mite, Mononychellus tanajoa, in semi-arid regions of the northeastern Brazil. Mite cadavers dry out during the day, protecting the fungus from environmental conditions on the abaxial side of the leaf, and readily adsorb water necessary for sporulation at night. Rainfall may not be essential for N. tanajoae because saturation at the leaf surface is usually reached during night time. N. tanajoae survives the dry and hot climate of semi-arid places, and when conditions are favorable, can respond to cause epizootics in populations of M. tanajoa.

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Fungi)

fungus / contributed paper session 2

Chair: Ann Hajek; John Vandenberg

13:30 **PCR-BASED STRATEGY FOR THE IDENTIFICATION OF BEAUVERIA BASSIANA ISOLATES**

Emma Ormond, Fiona Kussy, Helen Roy, *Anglia Polytechnic University, UK*; Judith K. Pell, *Rothamsted Research, UK*; Alison Thomas, *Anglia Polytechnic University, UK*

Abstract: The entomopathogenic fungus Beauveria bassiana is a commercially important biocontrol agent and is used world-wide in the management of agricultural pests. To assess the effect of applications of B. bassiana on the soil community and beneficial insects it is important to be able to track the movement of particular isolates. Random Amplified Microsatellites (RAMs), also known as inter simple sequence repeat (ISSR) markers, have been used to detect genetic variation in both plants and fungi, with the technique being highly reproducible. The aim of this study was to determine whether this method was useful in producing isolate specific fragment profiles, which could be used to identify particular B. bassiana isolates. Preliminary work involved the use of five RAM primers and twelve isolates of B. bassiana. Three primers were each capable of distinguishing all twelve isolates by a unique banding pattern and all demonstrated reproducibility. Further work is ongoing with the inclusion of more isolates in the test sample and with the final aim of conducting a clustal analysis to determine the phylogenetic relationships among the different B. bassiana isolates.

13:45 STU **BIOCHEMICAL, MORPHOLOGICAL AND PATHOGENICITY VARIATIONS IN BEAUVERIA BASSIANA ISOLATES**

Reza Talaei Hassanloui, Aziz Kharazi Pakdel, *Dep. Plant Protection, College of Agriculture, University of Tehran, IRAN*; Mark Goettel, *Lethbridge Research Centre, CANADA*; Javad Mozaffari, *Genetic Dep., Seed and Plant Improvement Institute, IRAN*

Abstract: An investigation was conducted to assess biochemical, morphological and pathogenicity variations among ten isolates of the most common entomopathogenic fungus, Beauveria bassiana, obtained from diverse geographical and biological origins. There is considerable interest in research on intraspecific variations for beneficial exploitaion of this fungus. A biochemical profile was generated by API 50 CH strips which revealed a high degree of variation among isolates on the basis of different carbon source oxidation. Variation in morphological characteristics of isolates was measured by studying germination characteristics on SDAY plates and PDA broth and radial mycelial growth. A split-plot factorial GLM showed significant differences among isolates. Micrometry of over a thousand conidia by IMAGE PRO and ANOVA of area(polygon), diameter(mean); average length of diameters measured at two degree intervals and passing through the conidial centroid, and size(length and width) were also studied as morphological markers. There were significant differences among isolates for conidial measurements. Compared means indicated that the least virulent isolate for CPB was within the highest group of diameter size LSD grouping. Virulence of these isolates was determined against the second instar larvae of diamondback moth, Plutella xylostella and Colorado potato beetle, Leptinotarsa decemlineata. Significant differences in mortality were found among isolates with ranges of 14.5 - 53.73 % for DbM and 25.05-74.078% for CPB. No correlation between the infectivity of the isolate and relatedness of the origin of the host to the assayed insect was observed except for one case in DbM. Spraying of 200 ul suspended conidia on larvae resulted in different Cumulative Time Mortality indices among isolates varying between 1.2603 7.3853 and 1.1103 5.4933 on DbM and CPB, respectively. Experiments are currently underway to further define the germination characteristics and molecular biology of isolates. The relationship between these biochemical and morphological markers of isolates and their virulence against DbM and CPB will be discussed.

14:00 **VIRULENCE TO COLORADO POTATO BEETLES AND GENETIC STABILITY OF BEAUVERIA BASSIANA PARASEXUAL RECOMBINANTS**

L. A. Castrillo, *Department of Entomology, Cornell University, UNITED STATES*; Michael H. Griggs, John D. Vandenberg, *USDA-ARS, US Plant, Soil & Nutrition Laboratory, UNITED STATES*

Abstract: We wished to determine the potential for recombination between strains of Beauveria bassiana and whether recombination could result in new strains with altered virulence or host range. We co-inoculated Colorado potato beetle larvae with vegetatively compatible strains ARSEF 5813 and ARSEF 6986 and recovered spore progeny for bioassay and molecular analysis. By using nitrate reductase mutants, recombinants were readily detected by their prototrophic growth compared to sparse growth produced by parent strains or any non-recombinant spore progeny on minimal medium. Sampling among the recombinant spore progenies revealed isolates with altered virulence. Bioassays using third instar CPB larvae showed isolates more virulent (12-10, 15-1 and 15-2), less virulent (18-5, 18-8 and 18-9), or of comparable virulence (19-6, 19-7, 19-10) to their parent strains. To determine whether this phenotypic change is stable, we conducted a serial passage study of representative strains 12-10, 18-5, and 19-6 using CPB larvae. Since repeated passage through a susceptible host has been shown to enhance virulence in some B. bassiana strains, the parent strains were also included in this study. Preliminary analysis of our data indicates that changes in virulence we observed among recombinants is stable and that repeated passage through CPB larvae did not alter virulence of neither parents nor recombinants. Additional assays are underway. We are also conducting molecular analysis of parent strains and recombinant spore progenies to determine if recombinant strains are genetically stable after serial passage in vivo.

14:15 **GENETIC VARIATION IN THE GYPSY MOTH FUNGAL PATHOGEN ENTOMOPHAGA MAIMAIGA FROM NORTH AMERICA AND ASIA**

Charlotte Nielsen, Michael G. Milgroom, Ann Hajek, *Cornell University, USA*

Abstract: Entomophaga maimaiga is a naturally occurring fungal pathogen specific to larvae of the gypsy moth, Lymantria dispar. The gypsy moth was introduced into the eastern US from France in 1868 and has become the most important defoliator of broadleaved trees in the northern United States. E. maimaiga was originally described from Japan and is thought to be native to Asia where it causes epizootics among gypsy moth populations, suppressing outbreak populations. Although E. maimaiga was released in the US in an effort to control gypsy moths in 1910-1911 (from Tokyo) and 1985-1986 (from Ishikawa), no fungal infections were recovered as a result of the field releases and the fungus was not observed again until 1989. Since 1989 this fungus has spread throughout the range of the gypsy moth in the US. Nevertheless, the origin of E. maimaiga in the US is still unknown. Several hypotheses for its origin and establishment have been proposed. One hypothesis propose that the fungus presently in the US originated from one of the purposeful introductions and since then has been present at undetectable level until a more aggressive strain arose through natural selection. Another hypothesis propose that E. maimaiga was only recently successfully introduced to the US by accident. The two objectives for this study were to compare the genetic diversity of North American and Asian populations of E. maimaiga, and to determine the origin of the North American population. We used AFLPs to assay the genetic diversity of E. maimaiga isolates collected in the US and Asia (Japan, China and far East Russia). Among 14 US isolates, we found only 10 polymorphic AFLP loci, whereas 56 loci were polymorphic among 16 Asian isolates and 29 loci were polymorphic among the 12 isolates from Japan. Average gene

diversity was 0.2230.005 for Asia (including Japan), 0.1310.006 for Japan only, and 0.0410.006 for the US. Thus, native populations from Asia were more genetically diverse than the introduced US population. These results are consistent with what would be expected when a population is founded from a source population by a small number of individuals. Both distance and parsimony analyses of AFLP data formed five distinct clusters that correlated to geographic regions of origin. Among the Asian isolates one cluster consisted of isolates collected near Tokyo (Chiba, Ibaraki), whereas another cluster included isolates from the rest of Japan (Ishikawa, Iwate and Hiroshima). On the Asian mainland, one cluster was formed by the 3 Chinese isolates, and the one isolate available from Russia was distinct from all other isolates. All 14 isolates from the US were included in one cluster that is most similar to the Japanese cluster of isolates from Ishikawa, Iwate and Hiroshima. These results plus further analysis support the hypothesis of an undocumented introduction of E. maimaiga into North America from Japan.

14:30 **A SINGLE GENE MUTATION IN THE OPPORTUNISTIC FUNGUS ASPERGILLUS FLAVUS RESULTS IN INSECT-HOST SPECIALIZATION**

Lisa Scully, Michael Bidochka, *Brock University, CANADA*

Abstract: Aspergillus flavus is an opportunistic fungus capable of infecting a wide variety of hosts including plants, insects, and animals, although with low virulence. Here we report the derivation of an A. flavus strain 6982 that exhibited characteristics of an obligate insect pathogen. This insect-dependent strain was unable to conidiate on a variety of agar media but was able to infect insects from different orders and to conidiate on the surface of the infected insects. However, unlike the parental strain, it was unable to infect various plant species, indicating a diminished host range. Because of its dependency on the insect host for growth and conidial production, this strain was designated Af6982conins. Extensive biochemical characterization revealed that Af6982conins was a cysteine/methionine auxotroph. A spontaneous revertant (frequency was 1 in 2x106) of Af6982conins displayed full recovery of growth and conidiation on artificial media as well as virulence toward the waxworm larvae (Galleria mellonella) and alfalfa. We argue that the role of nutrition in the host-pathogen relationship may be a general mechanism of host restriction and specialization toward obligate pathogenesis.

14:45 **BIOLOGICAL PROPERTIES OF A NEW ENTOMOPATHOGENIC FUNGUS ASCHERSONIA MARGINATA**

Svetlana Gouli, Bruce Parker, Vladimir Gouli, *University of Vermont, USA*

Abstract: Entomopathogenic fungus Aschersonia marginata was isolated from elongated hemlock scale (EHS), Fiorinia externa and circular scale, Nuculaspis tsugae, collected in different districts of the New England. The identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics between EHS populations in the New York and Massachusetts States. Scale mycosis has specific unusual signs representing relatively big irregular accumulation of the melanistic mass of mycelia. Numerous microscopic analyses of EHS cadavers did not reveal any fungal morphological structures connected with spore formation. A. marginata forms polymorphic colonies on different nutrient media. The fungal colonies grow radially and produce different pigments from white-pink to intense black. Development of the pathogen on the Sabouraud dextrose agar and yeast (SDAY,) media is connected with formation of numerous black hemispherical or pulvinate stromas 1-2 mm wide. Fungal conidia develop inside the stroma and have an elliptical form with acute ends, 5-10 x 21μm. Differentiation of A. marginata from other species belonging to the genus Aschersonia is easy because only this species has the black stromata. A preliminary investigation showed that A. marginata grows well on different liquid and dense nutrient media including small grains. Submerged culture on SDAY1/4 contains the mass of mycelia as rounded relatively stable formations. In case of two-stages of cultivation when fungal propagules after submerged cultivation are moved to solid nutrition substratum for sporulation, the fungus can form sclerotia or conidia. This development depends from media composition. Grain substratum gives possibility to produce 5.21.3 x 1010 conidia per gram, two-stages of cultivation 1.40.3 x 109 conidia per gram medium. Fungi of the genus Aschersonia are tropical and subtropical species and some are well-known whitefly pathgens. Manifestation of an epizootic caused by the fungus from the genus Aschersonia in the northeastern US is very unusual. We could not find any information about A. marginata as an insect pathogen. We believe this fungus should be studed as an active potential biological means for control of F. externa, N. tsugae and probably other scale species.

15:00 **ADHESION OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA TO SUBSTRATA**

Diane Holder, Nemat Keyhani, *University of Florida, U.S.*

Abstract: The kinetics of adhesion to hydrophobic and hydrophilic surfaces of the entomopathogenic fungus Beauveria bassiana was quantified using fluorescein isothiocyanate labeled conidia and blastospores. Results indicated complex interactions between fungal cells and substrata with B. bassiana conidia and blastospores displaying differing adhesive qualities. Whereas conidia adhered rapidly to hydrophobic surfaces and poorly to hydrophilic substrata, blastospores bound only poorly to hydrophobic surfaces and rapidly to hydrophilic surfaces. Atomic Force Microscopy (AFM) was used to investigate the adhesion forces between the fungal cells and substrata, as well as to visualize fungal surface features. A rodlet (hydrophobin) layer could be distinctly visualized on conidia, but appeared to be absent in blastospores. These data are consistent with the view that hydrophobins play a role in fungal adhesion to hydrophobic surfaces. The gene encoding for the B. bassiana hydrophobin was cloned and sequence homology alignments appear to indicate that it belongs to the type I class of hydrophobins. The variation in the adhesive qualities of B. bassiana conidia and blastospores may help to explain the differential susceptibility of various arthropod species to the two forms of the fungus.

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 6

Contributed Papers (Division of Microbial Control)

microbial control / contributed paper session 2

Chair: Vladimir Gouli; Justin Hatting

13:30 **DIVERSE ENVIRONMENT-DEPENDENT COSTS OF RESISTANCE TO CRY1AC IN DIFFERENT STRAINS OF THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA**

Ben Raymond, Ali Sayyed, Denis Wright, *Imperial College London, UK*

Abstract: Resistance to pathogens, parasitoids and other challenges can incur fitness costs for insects. These fitness costs can be cryptic and/or variable and may only be suffered under moderately stressful environmental conditions. Different investigations have revealed varying stability and costs of resistance to Bacillus thuringiensis and its Cry toxins in several insects. This variability may be the result of both variation in the nature of fitness costs between strains as well as the variation in environmental conditions in different laboratories. We investigated the nature of the costs of resistance under diverse environmental conditions in two strains of diamondback moth, Plutella xylostella, originating from Malaysia. In one strain, Serd4, fitness costs, in terms of reduced survival, were only imposed under conditions of larval competition. Long term culture cage experiments revealed that under conditions of low larval competition resistance to Cry1Ac was stable over six generations. In contrast, under high larval competition, resistance declined over the same period. In a second strain of P. xylostella, Karak, costs of resistance did not change with larval competition. However, fitness costs varied with culture plant species. When grown on Pei-Tsai Chinese cabbage, Brassica pekinensis, which has little or no natural resistance to diamondback moth, Karak Cry1Ac resistance imposes a small fitness cost. In contrast, when grown on a variety of Brassica oleracea showing moderate levels of natural resistance to DBM, fitness costs in terms of survival and larval growth rate increased markedly.

13:50 **RELATIONSHIP BETWEEN BT FORMULATION, TORTRIX VIRIDANA L. (LEPIDOPTERA, TORTRICIDAE), AND PUPAL PARASITOIDS IN OAK CONSORTIONS**

Anatoly Ivashov, Andrei Simchuk, Irina Peletskaya, *V.I. Vernadsky National University, UKRAINE*; Vladimir Gouli, *University of Vermont, USA*

Abstract: Represented research is focused on possible interference of Bt and pupal parasitoids with Tortrix viridana in the individual oak consortiums. The study was done in the natural population of T. viridana on southern coast of the Crimean peninsula. Nine model trees of pubescent oak, Quercus pubescens Willd., were chosen as models. The crown of each tree was divided into the two parts, one of which was spread with Bt formulation and that another served as control. The Bt was applied against third-instar of larvae as a water suspension, 108 spores/ml. After pupation, the insects were colleted from each tree, and material was used for hatch-

ing of adults and parasites in laboratory. Four major parasite species were taken into account, including Itoplectis maculator F., Phaeogenes invisor Thunb., Brachimeria intermedia Nees, and Cyclogastrella deplanata Neus. (Hymenoptera; Ichneumonidae). When adults and parasites were hatched, the insect sex was detected. Level of T. viridana mortality was 40%. The mortality of males and females had significant difference (.2 = 13.3 and 10.9; d.f.=2; P<0.01 in control and experiment respectively). Also there was the difference in the contribution of parasites for total pest mortality (2 = 12.4; d.f.=3; P<0.01). In experimental variants the efficiency of specialized parasite - Ph. invisor was higher then in control variants, but only for males (2 = 5.68; d.f.=1; P<0.02). The most common species - I. maculator showed the opposite trend (2 = 5.45; d.f.=1; P<0.02). The control and experimental variants were differed for the pest mortality from parasites (or unknown factors) only in case of males (2 = 4.19; d.f.=1; P<0.05). In experimental variants pests mortality from unknown cause were increased in case of insects with parasites, while in control variants mortality from parasites prevailed. The application of Bt against larvae affected only for pupae males, as a result was decreasing the role of parasites in total insect mortality. Only efficiency of specialized species Ph. invisor varied significantly among the control variants (2 = 14.3; d.f.=7; P<0.05). Application of the Bt formulation erased these differences. At the same time, males showed significant variation in total parasitism (2 = 10.1; d.f.=2; P<0.01) and mortality from unknown cause (2 = 25,5; d.f.=2; P<0,001) among experimental localities. It is interesting that these mortality factors are negatively related between themselves in all the studied variants (control + experiment) (r =-0.88; d.f. = 9; P<0.01). This may be an evident of competitive interaction between parasites and pathogen. Thus peculiarities of individual oak tree could influence interaction between herbivore and its natural enemies.

14:10 **COMPARATIVE EFFECTIVENESS OF BASIC METHODS FOR MASS-PRODUCTION OF ENTOMOPATHOGENIC FUNGI**

Vladimir Gouli, Svetlana Gouli, *University of Vermont, USA*

Abstract: The biological properties of entomopathogenic and antagonistic fungi preclude the use of traditional microbiological fermentation equipment for mass-production of mycological pesticides. In connection with this circumstance the cottage industry scale production is progressed. All cottage industry methods provide the optimal parameters for fruiting of fungi. There are two principal methods for the cottage industry scale production of fungi. The first method employs solid substrata both for growing mycelia masses and for final sporulation. The second method involves two discrete stages: - traditional fermentation equipment for the development of vegetative fungal propagules, and then maintenance of the optimal physical conditions for fungal sporulation. The first method has numerous modifications because based on different local materials and non-standard equipment, but inoculation of the substratum can be realized with conidia and blastospores separately or together. In case of the first method, optimal conditions provide the following harvests of conidia per gram of substratum: Beauveria bassiana 1.2-7.5x1010 on millet (Sikura, Primak, 1970); 1.5-3.0x109 on corn (Telenga, 1959); 5.0-9.0x1010 wheat grain with bran; Metarhizium anisopliae 5.0-8.5x109 and Verticillium lecanii 6.0-9.5x109 wheat grain with bran (S.Gouli et al. 1997). The second method can provide the following conidia production per one milliliter of medium: B. bassiana 0.8-1.4x109 on corn molasses (Primak, 1968), 7.3x107 on potato-peptone medium (Sikura, 1970). Our research for estimating inoculum concentration and depth of fungal liquid material from shaker on conidia productivity shows following results: B. bassiana, strain ERL 932, 2.10.4 (from 0.6 to 5.5)x108; M. anisopliae, strain 1080, 1.40.5 (from 0.6 to 2.6)x108, and V. lecanii, strain SPTR-151, 0.80.2 (from 0.3 to 1.5)x108 per ml of medium containing neopepton (0.25%), dextrose (1.0%) and yeast extract (0.25%). The two stage method of the cottage industry scale production of entomopathogenic fungi has significantly lower conidia productivity in comparison with the first method, but this method is characterized by same advantages, including low energy and labor expenditures; ability to mechanize the industrial processes; ability to prepare conidial material without any debris from the solid nutrient substratum.

14:30 **THE PERFORMANCE OF METARHIZIUM ANISOPLIAE VAR. ACRIDUM (GREEN MUSCLE) AGAINST MIXED GRASSHOPPER POPULATIONS IN ETHIOPIA UNDER FIELD CONDITION**

Emiru Seyoum, Merid Negash, *Addis Ababa University Department of Biology, ETHIOPIA*

Abstract: We studied the field performance of Metarhizium anisopilae var. Acridum against mixed grasshopper species in range lands under field conditions in two consecutive field trials. The performance of the pathogen was compared with the chemical insecticide Fenithrithion 95 % ULV, which is currently in use to control grasshoppers in Ethiopia. In both trials, the fungus killed more grasshoppers than the control. The post treatment mortality of experimental animals treated with Green Muscle(R) in days after treatment ranged from 0 to 19.78 % in the first trial and 20 to 70.91% in the second trial Mortality was proved to be due to mycosis for 37.91% and 37.71% of the grasshoppers died from the fungus treated plots showed external sporulation of conidia following incubation in the first and second trials, respectively. The fungus as opposed to the chemical insecticide found to be target specific for none of the non-targets checked showed sign of infection/fungal external sporulation. The result suggests that Green Muscle(R) can cause infection which could be sufficient to suppress grasshopper populations below that which could cause economic injury level when applied under field conditions. Discussion will attempts to surface the possibilities and associated practical limitations based on the current results and experience.

14:50 **EMPLOYING A NOVEL BIOASSAY METHODOLOGY FOR COMPARISON OF THE RELATIVE SUSCEPTIBILITY OF TWO RUSSIAN WHEAT APHID CLONES TO BEAUVERIA BASSIANA (HYPHOMYCETES)**

Justin Hatting, *ARC-Small Grain Institute, SOUTH AFRICA*; Stephen P. Wraight, *ARS-USDA, USA*

Abstract: Since its appearance in South Africa in 1978, the Russian wheat aphid, Diuraphis noxia (Kurdjumov) has become the principal pest of wheat produced under dryland conditions in the summer rainfall region. As part of an integrated control approach, entomopathogenic fungi are being evaluated as biological agents against D. noxia and other secondary cereal aphid species. From a commercial point of view, the quantitative expression of virulence (i.e., median lethal concentration or LC50) of these agents is a crucial step in the screening and quality control processes during development as mycoinsecticides. A new bioassay methodology was developed employing direct spray inoculation of aphids and subsequent incubation on live, untreated plants, thus limiting secondary-dose-acquisition post inoculation. Initially, four strains of the hyphomycete Beauveria bassiana (Balsamo) Vuillemin were assayed in a single-dose maximum-challenge test followed by two series of six-dose assays using the best isolate; each series comprising five assays. LC50's, fiducial limits and other regression parameters were used to evaluate the efficacy of the protocol and to investigate between-assay variability within two clones of D. noxia originating from the Free State (FS) and Western Cape (WC) provinces, respectively. Against the FS clone an average LC50 estimate of 83 (95% confidence interval: 58 120) conidia per mm2 for B. bassiana strain GHA was calculated. The data indicated high assay precision, reflected by an average coefficient of variation for slope of less than 20%, an average chi-square value (with 4 df) of 5.46 2.74 (n = 10 assays), and control mortality below 4%. A second set of assays was conducted against the WC clone for verification of assay precision and comparison of susceptibility to B. bassiana between the two D. noxia clones. Results will be discussed. This design will also accommodate the use of cereal-aphid species other than D. noxia and facilitate tritrophic studies on the effect of host-plant resistance on fungus-induced mortality of cereal aphids. Other measurable phenomena include cadaver distribution (e.g., host plant substrate versus soil surface) as well as pre-mortem behaviour of infected aphids.

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 1

Symposium (Division of Bacteria)

Genomics and pathogenesis of invertebrate pathogens

Chair: R. Aroian; D. Ellar

13:30 **IDENTIFICATION OF NOVEL BACILLUS CEREUS VIRULENCE GENES BY APPLICATION OF IN VIVO EXPRESSION TECHNOLOGY IN AN INSECT INFECTION MODEL**

Sinda Fedhila, Didier Lereclus, *Unité Génétique Microbienne et Environnement, Institut National de la Recherche Agronomique, Groupe Génétique et Physiologie des Bacillus Pathogènes, FRANCE*

Abstract: Bacillus cereus and Bacillus thuringiensis are closely related species sharing numbers of opportunistic properties. Indeed, as for B. thuringiensis, B. cereus cells are highly virulent when coingested with Cry toxins. This phenomenon, referred to as synergism, is related to the production of non specific virulence factors. In order to identify genes that are specific to infection we applied the previously reported IVET genetic system (Salamitou et al. 1997. Gene, 202: 121-126). This system is relying on site specific recombination as a reporter of transient gene activation. The genetic recombination is promoted by the site-specific recombinase, TnpI. The insertion of an active promoter upstream of tnpI results in the

acquisition of an antibiotic resistance marker by the bacterium through a resolution event mediated by TnpI. We successfully adapted this system for use in B. cereus strain ATCC 14579 and demonstrated that this strategy can be used in this bacterium to positiveley select conditionally expressed genes. In order to identify bacterial genes that are specifically induced during host infection, a B. cereus genomic DNA library was constructed by cloning partial digested chromosomal fragments upstream of tnpI coding sequence. The genomic library was next screened for genes expressed strongly in vivo during oral infection of the insect host Galleria mellonella larvae, but minimally or not expressed at all in vitro. 100 clones were selected for DNA sequencing of the inserts located upstream of tnpI. This revealed genes encoding proteins of different functional classes: DNA repair and replication, cell metabolism, transport system, regulation, virulence and adaptation to environmental stress. Sequences encoding unknown functions were also trapped. Clones harbouring genes belonging to the three latter classes were individually analyzed for resolution in vitro and in vivo in order to quantitate their activity. A gene encoding an orthologue of Listeria monocytogenes InlA, known to promote the internalization of Listeria by the mammalian host-cells, was identified as specifically and higly expressed during host infection. Work is underway to determine the role of this gene in virulence and the regulatory mechanisms controlling its expression.

13:55 **GENOME ANALYSIS OF PHOTORHABDUS LUMINESCENS, AN ENDOSYMBIONT OF ENTOMOPATHOGENIC NEMATODES**

Eric Duchaud, *Atelier de Bioinformatique, 12 rue Cuvier, 75252 Paris Cedex 05, FRANCE*; Alain Givaudan, Noël Boemare, *Laboratoire de Pathologie Comparée,r II, 34095 Montpellier Cedex 05, FRANCE*; Frank Kunst, *Laboratoire GMP, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, FRANCE*

Abstract: The genus Photorhabdus belongs to the family Enterobacteriaceae that comprises intestinal bacteria living in symbiosis with entomopathogenic nematodes (EPNs) of the genus Heterorhabditis. Most of these bacterial species are orally toxic or pathogenic for insect larvae when injected into the hemocoel. While most of the insect symbionts are endocytobionts and not culturable, Photorhabdus has the advantage to grow on standard media. Symbionts of EPNs encounter two different situations in their life cycle: they survive in the gut of their nematode host, and once inoculated they multiply in the body cavity of insects, killing the insect host due to septicaemia. These bacteria are now recognized as potentially important since Photorhabdus genes encoding entomotoxins may be useful to create transgenic plants for crop protection. We have recently completed the genome sequence of P. luminescens (Duchaud et al., 2003. Nat. Biotechnol. 21:1222). The analysis of the genome sequence revealed the presence of a large number of repeated sequences, including insertion sequences, putative transposons or their remnants and ERIC-like sequences (enterobacterial repetitive intergenic consensus). The most striking result of the analysis of this genome is its capacity to encode a large number of toxins, virulence factors and proteins involved in the interaction with the host (insect and nematode), representing families of paralogous genes. Examples of these gene families are: 1) eight tc (toxin complex) loci, including 30 genes, several of which encode insecticidal proteins; 2) four rhs genes that may serve as recombination hot spot or may encode toxins; 3) four complete genes (and four truncated copies) probably encoding Rtx toxins; 4) eight genes encoding hemolysin-, hemagglutinin- or adhesin-related proteins secreted by the two-partner secretion pathway; 5) putative virulence genes including a type III secretion system; 6) 82 genes, clustered in 11 loci, probably involved in the biogenesis of pili; 7) at least 57 genes involved in the biosynthesis of antibiotics such as peptide and polyketide synthetases. Finally, we identified a gene, possibly encoding a homologue of juvenile hormone esterase, which has been shown to possess mosquitocidal activity. The genome analysis will allow to highlight the particularly interesting properties of this bacterium for fundamental and applied research, such as the studies of host-bacterial interactions (symbiosis and pathogenesis), the mechanisms of enzyme secretion and production of specific metabolites.

14:20 **THE TOXIN-CODING PLASMIDS OF BACILLUS THURINGIENSIS AND THEIR HOST BACTERIA: PHENOTYPIC REGULATION AND STRAIN IMPROVEMENT**

Colin Berry, Katherine Gammon, Brian Dancer, *Cardiff School of Biosciences, Cardiff University, UNITED KINGDOM*

Abstract: The majority of Bacillus thuringiensis Cry and Cyt toxins are encoded extra-chromosomally on large plasmids. In addition to genes encoding the toxins, proteins involved in their accumulation (eg P19 and P20 helper proteins) and the transposases that may facilitate toxin gene movement, these megaplasmids contain a large number of other coding sequences that may have significant effects on the phenotype, and possibly the virulence, of the host bacterium. A set of three germination genes (ger) were identified in the sequence of the toxin-coding plasmid pBtoxis from B. thuringiensis israelensis (Bti). Southern blotting experiments indicated that there are no homologous genes on the chromosome of Bti and the role of the plasmid-borne genes was, thus, investigated. Bti 4Q5 (carrying only pBtoxis) was found to germinate more readily than Bti 4Q7 (plasmidless). Recovery of the germination phenotype could be achieved by transforming Bti 4Q7 with the shuttle vector pHT301 containing the three ger genes. This is the first demonstration that the presence of toxin-coding plasmids can alter the phenotype of their hosts (other than by conferring toxicity). An understanding of toxin-coding plasmid sequences and functions may also be valuable in strain improvement by the supplementation of toxin arsenals. Movement of pBtoxis to other hosts has proved possible and the potential for such plasmid transfer will be discussed.

14:45 **DISTINCT MAP KINASE PATHWAYS ARE IMPORTANT FOR DEFENSE AGAINST CRYSTAL TOXINS**

Danielle Huffman, *University of California at San Diego, Division of Biological Sciences, USA*; Roman Sasik, *University of California at San Diego, School of Medicine, USA*; Wayne Hsu, *University of California at San Diego, Division of Biological Sciences, USA*; Jacques Corbeil, *University of California at San Diego, School of Medicine, USA*; Raffi Aroian, *University of California at San Diego, Division of Biological Sciences, USA*

Abstract: Bacillus thuringiensis is an invertebrate-specific pathogen that produces a family of crystal toxins with insecticidal or nematicidal activities. The soil nematode, Caenorhabditis elegans, has proved useful for studying the genetics of resistance to such toxins. While these studies have identified a class of C. elegans genes whose products are required for proper toxin action, recently, our lab has characterized the genes involved in toxin defense. We have used Affymetrix gene chips to characterize the transcriptional response to the Bt toxin Cry5B following a three hour exposure time. The results of this analysis reveal that the animals respond rapidly and robustly with changes in gene expression that are distinct from those seen with heavy metal intoxication. Analysis of these toxin responsive genes led to the identification of two MAP kinase signaling pathways. The first of these pathways is a p38 type MAP kinase cascade. Although only the MAP kinase-kinase was trancriptionally induced upon Cry5B exposure, elimination of the gene product of any of the three kinases of this cascade results in increased sensitivity to Cry5B and Cry21A. This result demonstrates that this signaling pathway triggers an important defense mechanism against these toxins. To better understand such a mechanism, further microarray analysis was used to identify the transcriptional targets of this pathway in C. elegans. A total of 89 potential toxin-regulated p38 targets were identified. Using RNA interference, mutations in the candidate target genes were generated and the mutants were tested for increased sensitivity to Cry5B. The results of this screen will be presented.
The second MAP kinase identified in the gene chips is homologous to the JNK family of MAP kinases. Like the p38 pathway, deletion of this gene also confers decreased protection to Cry5B and Cry21A. Currently, genetic analysis is being used to dissect the relationship of these two signal transduction pathways to determine if and how they interact and why both are needed for wild type levels of Bt toxin susceptibility. Exploring host responses and defenses to Bt toxins is critical if we are to fully understand how these toxins kill their invertebrate hosts.

Wednesday, August 4^{th}, 2004
Time: 13:30 - 15:30, Corridor leves II and III

Poster Session 2: ALL other than fungi and bacteria

V-1 **DEVELOPMENT OF NOVEL AND EFFECTIVE SUBUNIT VACCINES AGAINST EAST COAST FEVER BASED ON INSECT CELL-DERIVED T. PARVA SPOROZOITE SURFACE PROTEIN P67**

Stephen A. Kaba, *Laboratory of Virology, Wageningen University,, THE NETHERLANDS*; Anthony J. Musoke, *International Livestock Research Institute, Nairobi,, KENYA*; Dick Schaap, *Intervet International BV, THE NETHERLANDS*; Vish Nene, *The Institute for Genomic Research, USA, USA*; Just M. Vlak, Monique M. Van Oers, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*

Abstract: East Coast fever (ECF) in cattle is caused by the tick-borne protozoan parasite Theileria parva. The major sporozoite surface antigen of T. parva (p67) is an important candidate for inclusion in a subunit vaccine. Recently, we reported the expression and production of different parts of p67 as fusion to either GFP or to the baculovirus GP64 envelope glycoprotein in insect cells, which resulted in enhanced levels of stable

proteins recognized by a monoclonal antibody specific for native T. parva p67. The immunogenicity of these fusion proteins was examined in out-bred mice and in cattle. In mice, the full-length p67 molecule without its signal peptide and transmembrane region, but fused to GFP (GFP:p67ÄSS) was the best immunogen followed by the C-terminus of p67 fused to GP64 (GP64:p67C). These two immunogens also provoked a high level of seroconversion in cattle when formulated in a water-in-oil or saponin-derived adjuvant. The vaccine-elicited antibodies efficiently inhibited the infectivity of T. parva sporozoites in in-vitro neutralization assays. Upon challenge with life sporozoites a clear correlation was observed between the in vitro neutralizing capacity and the reduction in severe ECF for individual animals. The mean protection against severe ECF in the immunized groups was 77% using only two inoculations and much smaller amounts (100 μg per dose) than needed for previous recombinant p67 constructs of bacterial origin (0.5 mg per dose with multiple doses). monique.vanoers@wur.nl

V-2 COMPETITIVE INTERACTION BETWEEN WILD TYPE AND RECOMBINANT HELICOVERPA ARMIGERA SNPV IN MIXED INFECTIONS IN INSECT LARVAE

Liljana Georgievska, Monique M. Van Oers, Wopke Van der Werf, Just M. Vlak, *Wageningen University, THE NETHERLANDS*

Abstract: An improved method for biological control of insects may involve recombinant baculoviruses for efficient insect pest suppression. Interactions between wild type and recombinant viruses sharing a host may have important consequences for the epidemiology and evolution of the virus. In this study we investigated the fitness of a recombinant Helicoverpa armigera nucleopolyhedrovirus (HaSNPV-CXW2) compared to wild type HaSNPV in larvae of the cotton bollworm (H. armigera). The recombinant HaSNPV-CXW2 has an improved speed of action compared to the wild type and is marked by the absence of the ecdysteroid UDP-glycosyl transferase (egt) gene and the presence of the Green Fluorescent Protein (GFP) gene and an insect selective neurotoxin from the scorpion Androctonus australis (AaIT). We have studied the changes in the composition of mixed-genotype infections of the wild type and the recombinant in laboratory (bioassay) experiments. The infectivity of both viruses was compared by infecting 4th instar H. armigera larvae in single- or mixed genotype infections. The replication dynamics of wild type and recombinant HaSNPV in mix infections was determined by challenging H. armigera larvae with different ratios (1:1, 9:1 and 1:9) of the two viruses. The hemolymph was collected from the infected larvae at 24, 48 and 72 hours post infection. The composition of progeny virus in terms of relative proportion of each genotype was determined by PCR. Experiments are currently under way to further detail the interactions between these two viruses. The results will be discussed in the light of the ecological fitness of recombinant baculoviruses.
This project is supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). Email: liljana.georgievska@wur.nl.

V-3 LIGHT AND ELECTRON MICROSCOPICAL INVESTIGATIONS ON A VIRAL DISEASE OF THE COMMON GREEN LACEWING, CHRYSOPERLA CARNEA

Regina G. Kleespies, *Federal Biological Research Centre for Agriculture and Foresty, Institute for Biological Control, GERMANY*

Abstract: In a laboratory rearing of an industrial company the common green lacewing, Chrysoperla carnea (Neuroptera: Chrysopidae), high mortality rates occurred inspite of appropriate rearing conditions. Dead and moribund larvae and adults were sent to the Laboratory for Diagnosis, Cyto- and Histopathology of Arthropod Diseases of the Federal Biological Research Centre for Agriculture and Forestry in Darmstadt. Investigations of various tissues showed that the death of C. carnea was due to a virus infection. It is a non-enveloped spherical virus with a diameter of about 70 nm infecting the midgut. Data on the symptomatology and pathology of the disease, and results of histo- and cytopathological studies, as well as on the morphology of the virus will be presented.

V-4 USING PHYLOGENIES TO DELIMITATE SPECIES

Elisabeth Herniou, *Imperial College London, UK*; Jenny Cory, *Wageningen University, THE NETHERLANDS*; Julie Olzewski, *Shippensburg University, USA*; David O'Reilly, *Syngenta, UK*; Tim Barraclough, *Kew Gardens, UK*

Abstract: The definition of species is one of the most important issues in biology. However, species concepts, such as the biological species concept are not universally applicable. Viruses are probably the most difficult organisms to classify. The phylogenetic species concept has been seen has as the answer to virus taxonomy. But, but recombination and horizontal transfers lead to gene phylogenies that do not reflect species phylogenies. Therefore the boundaries between species often seem blurred. The taxonomy of baculoviruses provides an excellent framework to test a new phylogenetic method that delimitsate clusters of individual sequences into species group. The nomenclature of baculoviruses, by using the juxtaposition of host name and virus morphology, has long had the advantage of being simple. However, as more virus strains are discovered, we have to face a number of problems. Several names can refer to the same virus, when this virus has a wide host range and the same name can refer to different viruses found in the same host. Molecular phylogenies based on individual genes or combined genes reveal the relationships between individual viral isolates. The branching patterns within trees give further information on the evolution of the viruses, which is reflected in part by changes in evolutionary branching rates during the history of the group. We developed a new method that detects variation of evolutionary ratesthe transition from between-species to within-population branching within phylogenies. One of the benefits of this approach is the definition of groups of individuals that evolve at similar rateswith shared evolutionary histories. These clusters of isolates can be interpreted as species groups. This method provides an objective way to delimitate species without a priori assumptions of host use.

V-5 SEQUENCING AND GENE ORGANIZATION OF THE OF CHORISTONEURA OCCIDENTALIS GRANULOVIRUS GENOME

Shannon Coppens, Hilary Lauzon, *Great Lakes Forestry Centre, CANADA*; Peter Krell, *Microbiology, University of Guelph, CANADA*; Basil Arif, *Great Lakes Forestry Centre, CANADA*

Abstract: Baculoviruses form a large family of occluded viruses composed of two genera: nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) that are differentiated by size, shape, and virion occlusion. Choristoneura occidentalis granulovirus (CoGV) genome was sequenced in both directions to give 8 times coverage. Analysis of the sequence showed that it consisted of 104,711bp and contained 117 open reading frames (ORFs) potentially encoding 50 amino acids or more. Of these, 57 (49%) were homologous to already sequenced r baculoviruses, 34 (29%) were granulovirus specific, and 5 (4%) were unique to CoGV. The A+T content was found to be 67.3% (second highest to another granulovirus CrleGV with 67.6%). The total area covered by ORFs is equal to 91.7%. The average amino acid identity of similar genes showed that CoGV is closest to CpGV with 51.7%, followed by CrleGV with 51.2%. Three copies of genes with BaculoPepN (polyhedron envelope protein) motifs were found (cogv17, cogv18, cogv19) along with three copies of fgf (fibroblast growth factors) (cogv60, 105, 117). To date, CoGV is the only granulovirus that contains the apoptosis preventing protein p35present in some NPVs. CoGV shares its closest homology to SpltMNPV with an overall 25.7% aa identity. Five homologous regions were found (without palindromes) which were not similar to NPV homologous regions. In CoGV, the homologous regions vary from 106bp to 288bp with 3-10 repeated sequences within the region. There is a large ORF, cogv39 that is 1145aa showed homology to other GVs and contained a large repeat area of approximately 1.8kb. Within this repeat, there are 27 leucine zippers. Because of the large repeat, there is significant rearrangement within the ORF. Significant part of the sequence analysis was carried out with the aid of Magpie, a software offered through the University of Calgary.

V-6 DISRUPTION OF SYSTEMIC VIRAL RESISTANCE IN GYPSY MOTH (LYMANTRIA DISPAR) BY CO-INFECTION WITH A BACULOVIRUS AND A POLYDNAVIRUS

James McNeil, Diana Cox-Foster, Mike Grove, Kelli Hoover, *Penn State University, US*

Abstract: The baculovirus Lymantria dispar nucleopolyhedrovirus (LdNPV) is a natural pathogen of gypsy moth caterpillars. Effectiveness of this biological control agent may be limited by a phenomenon referred to as intrastadial developmental resistance. This form of resistance is marked by an increase in resistance to viral infection as larvae age within a stadium. The most dramatic difference in susceptibility is observed between fourth instar caterpillars inoculated with virus immediately after molting (designated as 40) and those inoculated at 48-hours post-molt to the fourth instar (designated as 448). A dose that kills 80% of 40 larvae results in about 30% mortality in 448 larvae. We hypothesize that resistance of the older caterpillars is due to anti-viral defenses. To test this hypothesis, we examined the effect of immunosuppression by a polydnavirus (PDV) from the braconid wasp Glyptapanteles flavicoxis on age-related resistance to LdNPV. PDV virions are produced in the reproductive system of parasitic wasps in the families Braconidae and Ichneumonidae. PDV serve to protect the egg of the parasitoid by disrupting the host's immune response so that the developing parasitoid is not encapsulated and destroyed. In our study, two larval cohorts, 40s and 448s, were co-injected with LdNPV and G. flavicoxis PDV. All larvae that died were autopsied to confirm the presence of NPV occlusions. PDV significantly increased NPV-induced mortality in

448 larvae; although PDV also increased mortality in 40 larvae, it was not statistically significant. No death occurred in larvae inoculated with media or PDV only and all survivors successfully pupated. While the mechanism whereby PDV increased susceptibility to NPV has yet to be determined, this study indicates that PDV can serve as a useful tool for further exploring the hypothesis that intrastadial developmental resistance to NPV in the gypsy moth involves immune responses.

V-7 NEODIPRION SERTIFER AND NEODIPRION LECONTEI NUCLEOPOLYHEDROVIRUSES: COMPARATIVE GENOMICS AND EVOLUTION

Hilary Lauzon, *Canadian Forest Service, CANADA*; Paolo M. de A. Zanotto, *Instituto de Ciencias Biomedicas, BRAZIL*; Alejandra Garcia-Maruniak, *University of Florida, USA*; Basil Arif, *Canadian Forest Service, CANADA*; James Maruniak, *University of Florida, USA*

Abstract: The recent sequencing of the hymenopteran baculoviruses, Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) and Neodiprion sertifer NPV (NeseNPV) has provided further insight into the evolution and comparative genomics of baculoviruses. Both genomes are relatively small at 81,755 and 86,462 bp, respectively, have a low GC content and contain 89 (NeleNPV) and 90 ORFs (NeseNPV). They shared 67 ORFs (average amino acid identity = 52.7%) of which only 43 were identified baculovirus homologues. The number of conserved baculovirus genes is now 29 as both NeleNPV and NeseNPV lacked an ld130 homologue. Their 24 shared ORFs not found in other baculoviruses, included a trypsin-like serine protease, an ORF with a double stranded RNA binding motif, a zinc finger like protein, three ORFs similar to regulators of chromosome condensation proteins, a densovirus-like capsid protein and a phosphotransferase. Genes missing from both genomes or unique to the hymenopteran baculoviruses may play a role in host specificity and/or tissue tropism as hymenopteran baculoviruses are restricted to the midgut. Present baculovirus taxonomy separates its members into two genera: NPVs or GV. Phylogenetic analysis based on multiple conserved genes has so far been determined to be the best method for studying the evolution of baculoviruses. Previous analyses of baculovirus genomes have shown that the dipteran Culex nigripalpus NPV is a large evolutionary distance from the lepidopteran baculoviruses. Phylogenetic analysis of NeseNPV using DNA polymerase and NeleNPV using concatamers of 29 conserved baculovirus genes from 24 genomes supports a separate grouping of the Lepidoptera, Diptera and Hymenoptera baculoviruses and suggests the need for new baculovirus genera. In this study both the NeleNPV and NeseNPV genomes were included in various methods of genomic analyses. These techniques included traditional methods based on concatamers of conserved genes as well as newer methods to derive similarity matrices from complete genomes using shared traits. The advantage of the newer methods is computational speed and the lack of constraints imposed on genome numbers and size. This work has supported the need for re-evaluation of the number of genera in the family Baculoviridae for better placement of the hymenopteran and dipteran baculoviruses.

V-8 BACULOVIRUSES ISOLATED FROM FOREST AND ORCHARD PESTS AND THEIR POTENTIAL AS PEST CONTROL AGENTS IN LATVIA

Liga Jankevica, Ivars Zarins, *Department of Experimental Entomology, Institute of Biology, University of Latvia, LATVIA*

Abstract: An outbreak of widespread pest species cause important loses in forestry and horticulture in Latvia. Baculoviruses cause diseases of insects and can control the pest populations. Since 1968 baculoviruses were isolated from the most dangerous pest species. Nuclear polyhedrosis viruses (NPV) were isolated from the fruit-tree pests Malacosoma neustria L., Operophthera brumata L, Orgyia antiqua L., Yponomeuta malinellus Zell., the forest pests Bupalus piniarius L., Eriogaster lanestris L., Gilpinia pallida Kl., Lymantria monacha L., Neodiprion sertifer (Geoffr.), Yponomeuta cognatella Hg and Y. evonymellus L. Granulosis viruses were isolated from the fruit-tree pests Cydia pomonella L. and Yponomeuta padella L. The main tasks were: 1) to obtain new isolates and to describe their morphological and biological characteristics, 2) to implement the methodology molecular characterisation of isolates and experimental strains, 3) to describe the natural occurrence of viruses in pest populations. New sensitive methods of pathogen detection are used for monitoring of occurrence and presence of pathogens in the insect populations. Virulence of natural isolates was determined. Experimental strains with high virulence were developed by selection and used as a basis of virus preparations. The virus preparations had high efficiency (70-100%) in the climatical conditions of Latvia. This work has been financially supported by the grants from the Latvian Council of Sciences.

V-9 DISRUPTION OF NEGATIVE GEOTAXIS IN GYPSY MOTH (LYMANTRIA DISPAR) LARVAE INFECTED WITH TRANSGENIC BACULOVIRUSES

Mike Grove, Brianna Reed, Ryan Bissot, *Penn State University, US*; Nancy Hayes-Plazolles, James Slavicek, *USDA Forest Service, USA*; Kelli Hoover, *Penn State University, USA*

Abstract: Baculovirus-infected lepidopteran larvae typically climb upwards to exposed positions immediately before dying (negative geotaxis); a behavior believed to benefit the virus by increasing dispersal of progeny occlusion bodies. Gypsy moth larvae also seek elevated sites and rest in an inverted position prior to molting. We hypothesized that the molting hormone, ecdysone, influences this behavior. Inactivation of ecdysone by the EGT gene product, an enzyme normally produced by baculoviruses, might induce negative geotaxis in infected larvae. To test this hypothesis, we orally inoculated insects with occlusions of one of the following viral constructs: (1) wild type Lymantria dispar nucleopolyhedrovirus [LdNPV, A21]; (2) LdNPV expressing lacZ under control of the hsp70 promoter from Drosophila with EGT-deleted [LdNPV-hsp70/lacZ/EGT(-)]; or (3) LdNPV-hsp70/lacZ containing the EGT gene [7H5]. Following inoculation, larvae were placed in screened plastic coke bottles and larval heights were recorded until all larvae either died or pupated. As anticipated, larvae infected with the EGT-deletion virus did not climb upwards prior to dying; however, neither did larvae infected with the EGT-positive virus (7H5). What these constructs have in common is the presence of the lacZ cassette. Experiments are underway using other lacZ-expressing viral constructs to determine if b-galactosidase expression per se is interfering with virus-induced negative geotaxis, or if insertion of the lacZ gene has specifically disrupted the viral gene(s) responsible for this behavior. Preliminary data thus far favor the latter hypothesis.

V-10 EFFICACY OF PERITROPHIC MEMBRANE FOR PREVENTING NUCLEOPOLYHEDROVIRUS INFECTION IN ADOXOPHYES HONMAI AND SPODOPTERA LITURA

Shohei Okuno, Jun Takatsuka, Takayoshi Ishii, Shigeyuki Mukawa, Madoka Nakai, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

Abstract: The stilbene-derived brightener Tinopal UNPA-GX enhanced the infectivity of a Spodoptera litura nucleopolyhedrovirus (SpltNPV) and Autographa californica NPV (AcMNPV) in S. litura (Lepidoptera: Noctuidae) larvae, but did not enhance the infectivity of Adoxophyes honmai NPV (AdhoNPV) or AcMNPV in A. honmai (Lepidoptera: Tortricidae) larvae. Addition of 1% Tinopal reduced LD50 value of SpltNPV and AcMNPV from 9500 to 4.4 occlusion bodies per larva (OBs/larva) (2200-fold) and from 8.3 x 106 to 3100 OBs/larva (2680-fold), respectively in forth inster S. litura larvae. However, LD50 value of the forth instar A. honmai larvae inoculated with AdhoNPV or AcMNPV plus 1% Tinopal were not significantly different from that of larvae inoculated with virus alone. The LD50 values of AdhoNPV and AcMNPV against the A. honmai larvae were approximately 500 and 50000 OBs/larva, respectively in either presence or absence of the brightener. To elucidate the mechanism of different viral-enhancing effect of the brightener between A. honmai and S. litura larvae, we compared the larval PM structure and early infection process of AcMNPV to midgut of these larvae inoculated with the brightener. Forth instar A. honmai and S. Litura larvae were inoculated with OBs (107 OBs/larva) of AcMNPV in either presence or absence of 1% Tinopal, and occlusion-derived virus (ODV) of AcMNPV was localized by immunohistochemistry with the anti-ODV serum. The amount of viral DNA entered into the midgut epithelial was analyzed by DNA dot-blot hybridization. Immunolocalization analysis showed that the access of ODVs to midgut epithelial of S. litura was prevented by peritrophic membrane (PM), whereas ODVs reached to midgut epithelial when the brightener disrupted PM. In contrast, ODVs reached to midgut epithelial of A. honmai, the PM was disrupted by the brightener or even not. DNA dot-blot hybridization analysis suggested that the addition of brightener to viral suspension increased the amount of AcMNPV entered into the midgut cells in S. litura, but did not in A. honmai. Our results suggest that the efficacy of PM for prevent virus infection differed between the species. PM disruption by the brightener facilitates virus infection, but its effect depends on insect species.

V-11 BLOCKAGE OF ADOXOPHYES HONMAI NUCLEOPOLYHEDROVIRUS INFECTION IN THE MIDGUT OF A NON-PERMISSIVE INSECT, HOMONA MAGNANIMA

Ayako Hirao, Shohei Okuno, Jun Takatsuka, Takayoshi Ishii, Madoka Nakai, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

Abstract: Blockage of Adoxophyes honmai nucleopolyhedrovirus infection

in the midgut of a non-permissive insect, Homona magnanima
Ayako Hirao, Shohei Okuno, Jun Takatsuka, Takayoshi Ishii, Madoka Nakai, and Yasuhisa Kunimi Tokyo University of Agriculture and Technology, Fuchu-shi, Tokyo 183-8509, Japan
Abstract Adoxophyes honmai nucleopolyhedrovirus (AdhoNPV) is a host-specific baculovirus whose host range is limited to the genus Adoxophyes (Lepidoptera: Tortricidae); Homona magnanima, another tortricid species, is non-permissive to AdhoNPV. Little is known about the mechanisms governing whether a baculovirus can or cannot infect a particular insect species, and in this study we set out to determine where and how AdhoNPV infection is blocked in a non-permissive insect in vivo. We have focused on primary infection in the midgut: viral gene transcription and DNA replication. Fifth-instar of permissive (A. honmai) and fourth-instar of non-permissive (H. magnanima) insects were inoculated per os with 150 and 15000 AdhoNPV occlusion bodies/larva, respectively. DNA and RNA were extracted from midgut cells of these larvae at 0, 3, 6, 12, 24, 48 and 72 hour post inoculation (hpi). Transcription was detected by RT-PCR, and viral DNAreplication by quantitative PCR. In the non-permissive H. magnanima, transcription of the early genes ie-1 and lef-8 was detected, but expression of the late genes vp39 and polh was undetectable. All four genes were transcribed in the permissive A. honmai. Viral DNA did not increase in midgut cells of non-permissive H. magnanima, but did in the permissive A. honmai at 12 hpi. This suggests that DNA replication was not occurred in the non-permissive H. magnanima. Similar studies were done for secondary infection, as budded viruses were injected into hemocoel of these larvae. Our results indicate that AdhoNPV does not infect H. magnanima larvae because virus replication in the midgut fails to occur after early gene expression.

V-12 DIVERSITY OF ADOXOPHYES HONMAI ENTOMOPOXVIRUS FIELD ISOLATES FROM JAPAN

Jun Takatsuka, Shohei Okuno, Takayoshi Ishii, Yuko Takahashi, Madoka Nakai, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

Abstract: Adoxophyes honmai and Homona magnanima (Lepidoptera: Tortricidae) are economically important pests of tea plants in Japan. An entomopoxvirus (EPV) isolated from A. honmai in Tokyo infects six species of tea and orchard pests, including H. magnanima, and is a candidate biological control agent for these pests. To examine genetic relationships between A. honmai entomopoxvirus (AhEPV) and other invertebrate EPVs, the AhEPV spheroidin and fusolin genes were sequenced. The spheroidin gene has an open reading frame (ORF) of 3039 bp, encoding a predicted 116-kDa polypeptide which shows high amino acid sequence similarity to the spheroidins of other lepidopteran EPVs (e.g. 73% identity with Amsacta moorei EPV spheroidin). The 1056-bp fusolin gene ORF is predicted to encode a 40-kDa protein whose amino acid sequence is also highly similar (greater than 60% identity) to those of other lepidopteran EPV fusolins. From a survey of several tea fields in different regions of Japan (Tokyo, Ibaraki, Shikoku, Kyushu and Hiroshima), we found diverse AhEPV genotypes among the restriction endonuclease profiles of DNA extracted from individual infected insects. Isolates from Tokyo, Ibaraki and Kyushu were chosen for further phenotypic studies. Neonate larval bioassays showed that all three isolates were similarly virulent to A. orana larvae, but that the Kyushu isolate was less virulent than the other two isolates to H. magnanima larvae.

V-13 ENHANCEMENT OF NUCLEOPOLYHEDROVIRUS INFECTIVITY AGAINST MAMESTRA BRASSICAE (LEPIDOPTERA: NOCTUIDAE) BY GRANULOVIRUS PROTEINS AND A FLUORESCENT BRIGHTENER

Chie Goto, Shigeyuki Mukawa, *National Agricultural Research Center, JAPAN*

Abstract: We evaluated the enhancing effects of proteins derived from Xestia c-nigrum granulovirus capsules (GV proteins) and a fluorescent brightener, Tinopal UNPA-GX, on the infectivity of Mamestra brassicae nucleopolyhedrovirus (MabrNPV) against its original host. Both GV proteins and Tinopal UNPA-GX are known as enhancers of several entomopathogenic viruses. The addition of GV proteins (1 mg/ml) or Tinopal UNPA-GX (1%) to MabrNPV polyhedra reduced the LD50 by 170- to 300-fold and 2,000- to 50,000-fold, respectively, against fifth-instar larvae using the droplet feeding method. These additives caused a significant extension of the lethal time, in fifth-instar larvae of M. brassicae inoculated with MabrNPV at an LD95 equivalent dose (i.e., 7.6 days at 106 PIBs/larva without an additive, 8.4 days at 104 PIBs/larva with GV proteins, and 8.7 days at 101.5 PIBs/larva with Tinopal UNPA-GX). In order to evaluate the MabrNPV titer at these equivalent doses, we measured the concentration of MabrNPV viral DNAs in the host hemocoel by real-time quantitative PCR. The concentration of viral DNAs in larvae inoculated with MabrNPV alone, increased and reached a plateau earlier than in larvae inoculated with MabrNPV and an additive. These findings suggested that the early death of larvae inoculated at an LD95 equivalent dose without additives, resulted from high virus load during the primary infection. GV proteins and Tinopal UNPA-GX are beneficial for in vivo mass production of MabrNPV because these additives allow efficient low dose infection and higher virus yields. These additives also reduced the LC50 of MabrNPV against second-instar larvae that were inoculated by the diet contamination method, and slightly accelerated the lethal time at LC50 or LC75 equivalent doses. Our findings indicate that the enhancement of infectivity is important not only for field application but also for mass production of virus insecticides.

V-14 GROWTH AND SURVIVAL OF METEORUS PULCHRICORNIS IN MYTHIMNA SEPARATA INFECTED WITH ENTOMOPOXVIRUS

Aki Fujimoto, *Tokyo Univercity of Agriculture and Technology, JAPAN*; Shohei Okuno, Takayoshi Ishii, Jun Takatsuka, Kazuko Nakanishi, Madoka Nakai, Yasuhisa Kunimi, *Univercity of Agriculture and Technology, JAPAN*

Abstract: Growth and survival of Meteorus pulchricornis in Mythimna separata infected with entomopoxvirus
Aki FujimotoShohei OkunoTakayoshi IshiiJun TakatsukaKazuko NakanishiMadoka NakaiYasuhisa Kunimi
Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan
When the gregarious endoparasitoid Cotesia kariyai (Hymenoptera: Braconidae) parasitizes Mythimna separata (Lepidoptera: Noctuidae) larvae that are infected with an entomopoxvirus (MyseEPV), the embryos and@larvae of the parasitoid die inside the host before emergence. Previous studies showed that a toxic factor, lethal to Cotesia kariyai, was present in virion-free plasma (VFP) from M. separata infected with MyseEPV. A 28-KDa polypeptide was purified and named Protein Lethal to C. kariyai (PLCK). It is not known whether PLCK affects parasitoids other than C. kariyai, however, and in this study we examined the effect of PLCK on Meteorus pulchricornis (Hymenoptera: Braconidae). Like C. kariyai, M. pulchricornis is a braconid endoparasitoid; however, it is a generalist, parasitizing not only M. separata, whereas C. kariyai is a specialist. We observed larval growth and survival of these two parasitoids after exposure to PLCK, both in vivo and in vitro. Percentages of emergence, pupation and adult eclosion of M. pulchricornis larvae developing in MyseEPV-infected or VFP-injected hosts were not significantly different from those in non-infected or in hosts injected with hemolymph from healthy insects, whereas no C. kariyai larvae emerged from M. separata infected with MyseEPV. Similarly, M. pulchricornis larvae survived for seven days in IPL-41 medium containing PLCK, but C. kariyai died within two days in the same medium, showing pathognomonic symptom such as the cuticle detached from the somatic tissues. We also examined immunohistological localization of PLCK within parasitoid larvae developing in medium containing PLCK, to determine whether PLCK attaches to particular tissues in C. kariyai but not in M. pulchricornis. In summary, PLCK has no effect on the development and survival of M. pulchricornis, but is lethal to C. kariyai.

V-15 PRIMARY INFECTION IN ADOXOPHYES HONMAI LARVAE THAT ARE RESISTANT TO A NUCLEOPOLYHEDROVIRUS

Hirokazu Shikata, Madoka Nakai, Jun Takatsuka, Shohei Okuno, Takayoshi Ishii, Yasuhisa Kunimi, *Tokyo University of Agriculture and Technology, JAPAN*

Abstract: We have established a colony of Adoxophyes honmai (Lepidoptera, Tortricidae) that is resistant to a nucleopolyhedrovirus (AdhoNPV: Baculoviridae). We selected resistant A. honmai by feeding neonate larvae in successive generations with the LC70 (70% lethal concentration) or LC60 of AdhoNPV. Based on the ratio of the LC50 values in neonates from selected (S) and unselected (U) strains, the highest degree of acquired resistance in neonate larvae ranged from approximately 1,000- to 50,000-fold after the 25th generation. We have examined the mechanism of A. honmai larval resistance to AdhoNPV. Baculoviruses infect the host insect per os (oral infection). The infection can be divided into two phases: primary infection of midgut cells, and secondary infection of tissues in the hemocoel (e.g. fat body). In 5th-instar larvae, the degree of acquired resistance of S-strain larvae to virus administered per os was about 1,000,000-fold. The ratio of the LC50 values for S- and U-strain larvae was about 200-fold when budded virus was injected into the hemocoel of 5th-instar larvae. This result suggests that the mechanism of resistance is associated with both primary and secondary infections. Physical conditions in the larval midgut lumen (e.g. pH; polyhedron-digesting ability and protein composition of the midgut juice; permeability of the peritrophic matrix) were the same in both strains, and there were no significant differences in several factors related to insect immunity (e.g. the number of hemocytes and their ability to encapsulate beads; phenoloxidase activity in the hemolymph). Fifth-instar S- and U-strain larvae were inoculated per os with 105 occlusion bodies of AdhoNPV, and viral gene expression in the midgut was monitored by RT-PCR in a time course experiment. At this dose, more than 90% of U-strain A. honmai larvae, but less than 10% of S-strain larvae, become infected with AdhoNPV. Transcription of ie-1 and

polh was first detected at 6 and 24 hours post-inoculation (hpi), respectively, in the midgut of U-strain larvae. However, transcription of neither of these genes was detected in S-strain larval midgut during observations until 72 hpi. The absence of viral gene expression in midgut cells indicates that AdhoNPV infection of S-strain larvae is blocked predominantly in the midgut.

V-16 CHARACTERIZATION OF THE FP25K GENE OF HELICOVERPA ARMEGIRA SINGLE-NUCLEOCAPSID NUCLEOPOLYHEDROVIRUS

Dong Wu, Fei Deng, Xiulian Sun, Li Yuan, *Joint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, CHINA*; Just M. Vlak, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Zhihong Hu, *Joint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, CHINA*

Abstract: It was reported before that the fp25k gene of baculovirus is responsible for the generation of few polyhedra (FP) phenotype during baculovirus infection. Here we characterized the fp25k gene of Helicoverpa armegira single-nucleocapsid nuclopolyhedrovirus (HaSNPV). 3-RACE analysis showed the Ha-fp25k transcription could be detected from 18 hours post infection. The FP25K protein was also detected from 18 hours post infection by Western-blot using specific antiserum. A recombinant HaSNPV bacmid with fp25k gene deleted was constructed by homologous recombination in E. coli and was transfected into HzAM1 cell line. One-step growth curve result showed that the fp25k deleted virus had 5-10 folders higher yield of budded virus production. Fp25 gene rescue assay indicated that the higher yield of BV was caused by fp25k deletion. Transmission electron microscopy results showed few ODV localized in the nucleus infected with fp25k deleted virus. These results suggested that Ha-fp25k gene involved in the BV and ODV synthesis, as well as the polyhedra maturation.

V-17 HAS ACMNPV PIF THE SAME ROLE AS SPLINPV PIF?

Serafín Gutiérrez, Miguel López-Ferber, *Laboratoire de Pathologie Comparée INRA/CNRS/Universtié de Montpellier II, FRANCE*

Abstract: The pif gene of Spodoptera littoralis nucleopolyhedrovirus (SpliNPV) is essential for oral infection of Spodoptera littoralis larvae. This gene is conserved among the sequenced baculovirus. Thus, the pif role may be also conserved among baculoviruses. The SpliNPV PIF protein has been only localised in the envelope of the occlusion derived virions (ODV), which suggests that PIF is required for ODV infectivity or formation. The pif gene of Autographa californica NPV (AcMNPV) is also essential for oral infection in Trichoplusia ni larvae (R.D. Possee, personnal communication). However, a recent work carried out to identify the protein composition of the AcMNPV ODV has not detected PIF among the ODV structural proteins (Braunagel et al, 2003; PNAS, 100, 9797-802). This result raises the question on whether PIF has the same function in SpliNPV and in AcMNPV. We aimed to determine if AcMNPV pif has the same function as SpliNPV pif. First, we constructed an AcMNPV deletion mutant (AcMNPV-Delta119) lacking the ORF 119 (homolog to SpliNPV pif). We compared the pathogenicity of this mutant and a wild-type clone (AcMNPV-1.2) both by oral inoculation and by injection into the hemocoel of S. littoralis and Spodoptera frugiperda larvae. We followed the fate of the oral inoculations using PCR. Finally, we fed S. frugiperda larvae with a mixture of viral occlusion bodies of AcMNPV-1.2 and AcMNPV-Delta119 and checked the resulting virus offspring for the presence of AcMNPV-Delta119 DNA. No mortality due to AcMNPV-Delta119 infection was observed in any orally-inoculated larvae of both hosts. AcMNPV-Delta119 DNA was not detected in larvae at different times after oral inoculation. On the other hand, AcMNPV-Delta119 was roughly as pathogenic as AcMNPV-1.2 when administered by injection. No AcMNPV-Delta119 DNA was detected in the offspring resulting from the oral infections with a mixture of viruses. The presented results show that the deletion of AcMNPV pif probably blocks oral infection at an early stage. The absence of oral infectivity due to pif deletion could not be overcome by co-inoculation with occlusion bodies of a wild-type clone. Therefore, it is not likely that AcMNPV PIF is contained as a free protein in the matrix of the occlusion bodies. These data suggest that AcMNPV PIF acts during ODV infection or formation, as it has been suggested for SpliNPV PIF.

V-18 ORF107 OF HASNPV ENCODES A STRUCTURE PROTEIN OF BOTH BV AND ODV

Xiaoyu Pan, Gang Long, Zhihong Hu, *Joint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*

Abstract: In this report, a unique gene Ha107of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HaSNPV) was investigated. By computer analysis, HA107 was found to contain ten predicted transmembrane helixes and two predicted hydrophilic loops (aa 136 C aa 190 and aa 214 C aa 304). It was predicted as a putative polytopic membrane protein with a molecular weight of 51.2 kDa. By using 3RACE analysis, we found Ha107 was transcribed as a polyadenylated transcript and was detected from 24 h post-infection. The transcripts polyadenylation was initiated from 38nt downstream of the putative translation stop codon. By western blot using a polyclonal antibody against HA107 (aa136-aa304), a 52 kDa protein was recognized in HaSNPV infected cells, which is in harmony with the theoretical size. HA107 protein was found in the nucleocapsids of both budded virions (BV) and occlusion derived virions (ODV). To understand HA107 function in detail, experiments on protein-protein interaction and topology determination will be established.
This research is supported by a 973 grant (2003CB114202) and NSFC grants (030210 and 30025003). E-mail: ganglong58@hotmail.com

V-19 TRANSLATION ARREST MECHANISM IN ACMNPV-INFECTED LD652Y CELLS

Christy Mecey, Wade Williams, *Michigan State University, USA*; Monique M. Van Oers, *Wageningen University and Research Centre, THE NETHERLANDS*; Suzanne Thiem, *Michigan State University, USA*

Abstract: Global translation arrest is observed in Autographa californica M nucleopolyhdrovirus (AcMNPV)-infected Ld652Y by 16 h.p.i., resulting in a nonproductive infection. However all temporal classes of viral mRNA are transcribed and are translatable in vitro. Expression of a novel protein, host range factor 1 (hrf-1), isolated from LdMNPV prevents translation arrest. Recombinant AcMNPV expressing hrf-1 (vAchrf-1) replicates normally in Ld652Y cells. Polysome profiles of AcMNPV-infected Ld652Y cells showed a monosome peak at 12 h.p.i. in AcMNPV-infected cells indicating a block in translation initiation. In this study we investigated two well-characterized molecular mechanisms that control translation initiation as possible mechanisms for translation arrest in AcMNPV-infected Ld652Y cells. We first investigated a potential block in the binding of eIF4E to capped mRNA. We investigated 4E cap-binding by comparing the binding of 4E to 5'-methyl-guanosine agarose among Ld652Y cell lysates from mock-, AcMNPV-, and vAchrf-1-infected cells. There was no detectable variation in the relative amounts of cap-bound eIF4E suggesting that translation arrest is not due to impaired cap binding. We next investigated the phosphorylation state of eIF2a. Phosphorylated eIF2a binds the nucleotide exchange factor, eIF2B, inhibiting its activity and preventing translation initiation. This is a major control point for translation initiation that is controlled by a family of stress-induced kinases, which includes the dsRNA induced kinase, PKR, which is commonly activated by virus infection in vertebrates. Western blot analysis of the eIF2a phosphorylation state in mock-, AcMNPV- and Achrf-1-infected Ld652Y cells at 12 h p.i. showed an increase in phosphorylated eIF2a in AcMNPV-infected cells relative to mock-infected cells suggesting that translation arrest in AcMNPV-infected Ld652Y cells is due to eIF2-a phosphorylation. Although the AcMNPV pk2 protein, an inhibitor of eIF2a kinases, was expressed it did not prevent phosphorylation of eIF2a. These data indicate that translation arrest in AcMNPV-infected Ld652Y cells involves phosphorylation of eIF2a by a kinase that is not inhibited by pk2.

V-20 PRELIMINARY CHARACTERIZATION OF GENOME ORGANIZATION FOR TNSNPV ISOLATES FROM GREENHOUSE POPULATIONS OF TRICHOPLUSIA NI

Martin Erlandson, Amanda Neudorf, *1Agriculture and Agri-Food Canada, Saskatoon Research Centre, CANADA*; David Theilmann, *Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, CANADA*

Abstract: Cabbage looper, Trichoplusia ni, populations in greenhouses from the Fraser Valley (FV) of British Columbia were sampled and screened for the presence of baculoviruses. Both single nucleopolyhedrovirus (SNPV) and multiple nucleopolyhedrovirus (MNPV) baculoviruses were detected in these populations and multi-plex species-specific PCR analysis showed that Autographa californica MNPV (AcMNPV) and Trichoplusia ni SNPV (TnSNPV)-like isolates were present. TnSNPV isolates were the most prevalent, representing greater than 95% of NPV isolates collected in eight greenhouses sampled over two seasons. Restriction endonuclease analysis of genomic DNA from each isolate indicated that the TnSNPV isolates all had identical REN profiles which were distinct from a TnSNPV isolate (TnSNPV-RJ) previously collected from cabbage looper populations from New York state (R. Jaques, AFFC, London, Ontario). Dose response bioassays were conducted in 2nd, 4th, and 5th instar T. ni larvae to compare the infectivity and virulence of selected TnSNPV field isolates and TnSNPV-RJ. There were no significant differences in LD50 values for any of the isolates although one isolate FV#34 was somewhat more virulent. We have previously reported on the complete genome sequence of

the TnSNPV isolate RJ from New York state. To determine the genetic relatedness of the new TnSNPV isolates from western North America, we cloned REN fragments from two field isolates, TnSNPV-FV#34 and #5, and undertook a "sniff sequencing" strategy from both ends of the clones. These sequences were aligned and compared to the complete TnSNPV-RJ sequence. The results to date indicate that the genome sequence of the two Fraser Valley isolates have 99% identity to TnSNVP-RJ and the vast majority of differences consist of single nucleotide changes, largely transitions. A few three to six nucleotide insertion/deletions also were noted. Those differences in REN profiles of TnSNPV-RJ compared to the FV isolates where sequence information is available all result from single nucleotide substitutions and there appears to be no major genomic re-arrangements present in the various isolates. The TnSNPV populations isolated from greenhouse cabbage looper populations appear to be very homogeneous in comparison to other baculovirus systems.

V-21 **LOW VARIATION IN SUSCEPTIBILITY OF SPODOPTERA LITTORALIS STRAINS TO SLNPV AND IN VIRULENCE VARIABILITY OF FOUR EGYPTIAN S. LITTORALIS NUCLEOPOLYHEDROVIRUS ISOLATES**

Luc-Olivier Brun, *Virology Center, IRD- Faculty of Agriculture, Cairo University, EGYPT*; Said El-Salamouny, *Department of Economic Entomology and pesticides, Faculty of Agriculture, Cairo University, EGYPT*; Wael Kamal, *Virology Center, IRD- Faculty of Agriculture, Cairo University, EGYPT*

Abstract: The virulence of four Spodoptera littoralis (Boisd.) nucleopolyhedrovirus isolates collected from three regions: Giza (two isolates 1&2), Alexandria and Fayoum, Egypt, were tested against S. littoralis neonate larvae. The source of S. littoralis colony was collected from Giza region and maintained on continuous rearing on artificial media. The bioassay data showed no big variations in the obtained median lethal concentration (LC50) with a value of 1.6x105, 5.6x10 4, 2.5x104 and 1.7x104 PIB's/ ml diet, for the Giza 1, Giza 2, Alexandria and Fayoum viral isolates, respectively. At the same time, the Fayoum viral isolate was used to compare the larval susceptibility of three S. littoralis strains collected from Baharia Oasis, Fayoum Oasis and Giza regions. öThe LC50 values were 1.6x105, 5.4x104 and 7.3x104 PIB's/ ml diet for the above mentioned three strains, respectively. The observed low variability of the LC50 among virus strains or insect strains was in the normal variability range under our bioassay condition. The genomic variability between the four viral isolates is under investigation.
Keywords: Bioassay, nucleopolyhedrovirus, Spodoptera littoralis, virulence.

V-22 **HOST SPECIFICITY OF SPODOPTERA SPP. NUCLEOPOLYHEDROVIRUSES IS NOT DETERMINED BY VIRUS ENTRY OR THE PRIMARY INFECTION CYCLE**

Oihane Simón, Trevor Williams, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Miguel López-Ferber, *2Laboratoire de Patologie Comparée, UMR 5087, INRA-CNRS-Université de Montpellier II, FRANCE*; Primitivo Caballero, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

Abstract: The multicapsid nucleopolyhedroviruses (NPVs) of Spodoptera exigua (SeMNPV), S. frugiperda (SfMNPV), and S. littoralis (SpliNPV) are genetically similar (78% homology) but differ in their degree of host specificity. Infection by each of the three NPVs in these three Spodoptera host species was determined by oral inoculation of larvae with occlusion bodies (OBs) or intrahaemocelic injection with occlusion derived virions (ODVs). RT-PCR analysis on total RNA from inoculated insects, targeted at immediate early (ie-0), early (egt, DNA polymerase), late (chitinase) and very late genes (polyhedrin), indicated that each of the NPVs initiated an infection in all three host species tested. SpliMNPV produced a fatal NPV disease in both heterologous hosts, S. frugiperda and S. exigua, by oral inoculation or injection. SfNIC is lethal to heterologous hosts, S. exigua and S. littoralis, but infected larvae do not melt and disintegrate and progeny OBs were not observed. SeMNPV is able to replicate in heterologous hosts and all genes required for replication are present in the genome as the virus primary infection cycle was observed. However, gene expression is significantly lower in heterologous hosts. SeMNPV pathogenesis in S. frugiperda and S. littoralis was blocked at the haemocoel transmission stage and finally cleared. SeMNPV mixtures with SpliMNPV or SfMNPV did not extend the host range of SeMNPV; in all cases only the homologous virus was observed to proliferate. We conclude that entry and the primary virus infection cycle are not determinant factors for SeMNPV infection of heterologous Spodoptera species.

V-23 **QUANTIFYING THE GENETIC DIVERSITY OF SPODOPTERA EXIGUA MNPV POPULATIONS IN SOIL RESERVOIRS IN SOUTHERN SPAIN**

Rosa Murillo, Delia Muñoz, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Carmen Ruíz-Portero, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, SPAIN*; M. Dolores Alcázar, *3Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; José E. Belda, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; Trevor Williams, Primitivo Caballero, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

Abstract: The beet armyworm, Spodoptera exigua (Lepidoptera: Noctuidae) is the main lepidopterous pest of vegetables in greenhouse crops of Southern Spain. A strain of the multicapsid nucleopolyhedrovirus of S. exigua (SeMNPV) with demonstrated efficiency against this pest, was isolated in this area as a mixture of virus-killed larvae in 1990, and named SeMNPV-SP2A. Previous studies indicated a high prevalence of genotypic heterogeneity in SeMNPV-killed insects. We extended this work by isolating SeMNPV from the soil, a natural reservoir of virus occlusion bodies (OBs). Soil samples, randomly collected over an 18 month period in an area of 500 km2 with 40,000 ha of greenhouses, were incorporated into a semi-synthetic diet and bioassayed against first instar S. exigua. The presence of SeMNPV OBs was detected in up to 33% of soil samples over the whole area studied, with no significant differences between samples from different crops or from locations with distinct agricultural practices. Seasonally, the Spring and Summer soil samples had significantly greater OB titres, with median mortality of 10,5%, and those from Autumn and Winter, with median mortalities of 4.2% and 2.1%, respectively. Genotypic variability of virus isolates, as analyzed by restriction fragment length polymorphism with BglII, revealed at least nine different isolates. Six of them with a single dominant genotype and three others with mixtures of at least two genotypes. Some of these isolates showed identical profiles to some of the genotypes purified from SeMNPV-SP2, whereas others displayed new profiles. Phenotypic characteristics of the most abundant isolates are currently being determined.

V-24 **NUCLEOPOLYHEDROVIRUS (SEMNPV) AND OPTICAL BRIGHTENER FORMULATIONS FOR CONTROL OF SPODOPTERA EXIGUA IN GREENHOUSES IN SOUTHERN SPAIN.**

Rodrigo Lasa, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*; Carmen Ruíz-Portero, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, SPAIN*; M. Dolores Alcázar, *Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; José E. Belda, *Departamento de Biología Aplicada, Cite-IIB Universidad de Almería, Unidad de Entomología, Laboratorio de Sanidad Vegetal, SPAIN*; Primitivo Caballero, Trevor Williams, *Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN*

Abstract: Beet armyworm, Spodoptera exigua (Hübner), causes serious damage in the greenhouse crops of Almería, in Southern Spain. This pest has developed resistance to most chemical insecticides. The use of a multicapsid nuclearpolyhedrovirus (SeMNPV) as a biological insecticide has proved to be effective in glasshouses in northern Europe. The incorporation of stilbene optical brighteners into baculovirus formulations can significantly improve ultraviolet protection and viral pathogenicity. We evaluated the efficacy of a Spanish isolate (SP2) of SeMNPV formulated with and without the optical brightener Leucophor AP in a pepper crop planted in a 800m2 greenhouse in Almería. The experiment involved four treatments: (i) control without virus, (ii) 108 OB/m2 SeMNPV, (iii) 108 OB/m2 SeMNPV + 1% Leucophor AP, (iv) 0.05% Lufenuron (IGR, chemical control). All applications included a commercial wetter-sticker. Mortality observed in larvae collected at 2, 5 and 8 days after treatment and reared in the laboratory until death were 61, 90 and 90%, respectively, when treated with virus alone and 91, 91 and 92%, respectively, when treated with virus + optical brightener. The mortality observed, was higher that observed with the chemical treatment (39, 25 and 10%, respectively). A diet incorporation bioassay of leaves collected at each timepoint revealed excellent persistence of OBs on leaf surfaces. Persistence was significantly greater on the leaves of the lower crop canopy compared to leaves on the upper part of the plant. OB persistence was not significantly improved when formulated with the optical brightener.

V-25 **A NEW ASCOVIRUS (SEAV6A) ISOLATED FROM SPODOPTERA EXIGUA LARVAE IN CALIFORNIA**

Yeping Tan, Francis Tan, Dennis K. Bideshi, *Department of Entomology, University of California, UNITED STATES*; Yves Bigot, *Unit of Insect Parasite Genetics, University of Tours, FRANCE*; Brian A. Federici, *Department of Entomology, University of California, UNITED STATES*

Abstract: A new ascovirus (tentatively, SeAV6a) has been isolated from larvae of Spodoptera exigua collected from the San Joaquin Valley, California. To determine its relationship to several other ascoviruses, specifically, Spodoptera frugiperda AV (SfAV1a), Trichoplusia ni AV (TnAV2a), and Heliothis virescens AV (HvAV3a), comparative analyses based on virion proteins, genome organization, Southern hybridization and nucleic acid sequences were performed. SDS-PAGE analysis of virion protein profiles revealed that SeAV6a most closely resembled SfAV1a, although distinct SfAV1a peptide bands migrating at about 100-kDA and 30-kDA were absent in SeAV6a virions. Comparisons of HinDIII and EcoRI restriction fragments showed that SeAV6a and SfAV1a were more similar to each other than to HvAV or TAV. In addition, most of the SfAV1a restriction fragments hybridized with a SeAV6a genomic probe, whereas only a few HvAV3a and TnAV2a bands hybridized, even at 420C. To determine the phylogenetic relationship of SeAV6a to the other ascoviruses, nucleotide sequences encoding the major capsid protein (mcp) and DNA polymerase (dnapol) were compared. Results showed that the SeAV6a and SfAV1a mcp genes were 92% identical, which was significantly higher then when the genes of this new isolate were compared with the corresponding genes of HvAV3a or TnAV2a (63%). Similar results were observed with the dnapol gene, for which SeAV6a and SfAV1a were 91% identical, compared to 75% for SeAV6a when compared to either HvAV3a or TnAV2a. These results provide evidence that SeAV6a is a major variant of SfAV1a, and possibly a new species of ascovirus.

V-26 **IDENTIFICATION OF A NOVEL SHRIMP PROTEIN PHOSPHATASE AS THE INTERACTING PARTNER FOR LATENCEY-ASSOCIATED PROTEIN ORF427 OF WHITE SPOT SYNDROME VIRUS**

Liqun Lu, Jimmy Kwang, *Temasek Life Sciences Laboratory, SINGAPORE*

Abstract: White spot syndrome virus (WSSV) is a major pathogen in shrimp that causes high mortality and huge economic losses in shrimp aquaculture. Previously, WSSV has been suggested to be a latent virus in normal shrimps and several viral transcripts (including orf 427, orf 151, and orf 366) has been identified in SPF shrimps through a WSSV-specific DNA microarray study [Khadijah et al., J Virol. 2003 Sep;77(18):10162-7]. In order to characterize the role of latency-associated protein ORF427 involved in the viral infection cycle, a shrimp cDNA library was constructed to screen interacting proteins of ORF427. Employing the yeast two-hybrid system, a novel shrimp protein phosphatase (named PPs), sharing 93% homologue with human protein phosphatase 1, has been identified able to bind ORF427 in vivo in Yeast. The interaction was further confirmed by in vitro co-immunoprecipitation experiments. Interestingly, this novel shrimp protein phosphatase consists of only 199 aa and appears to be the smallest protein phosphatase known so far. Analysis of the sequence of this protein phosphatase showed that it contains almost all the functional catalytic domain of Human protein phosphatase, while lacks the corresponding C-terminal non-catalytic sequence. To characterize its function, the shrimp protein phosphatase was highly expressed in bacterial and the purified protein showed phosphatase activity when tested against pNPP in a standard phosphatase assay. Furthermore, the mRNA and its translated products could be detected in normal shrimp cells; localization studies employing the EYFP-PPS fusion constructs showed that the PPs exists mainly in the lysosome of uninfected shrimp PMO cells. Our results suggested that the identified protein phosphatase, PPs, may represent a novel member of protein phosphatase family and might involve in the regulation of WSSVs life cycle through interaction with ORF427 of WSSV.

V-27 **SUPPRESSION OF FIELD POPULATIONS OF BALSAM FIR SAWFLY WITH ITS NUCLEOPOLYHEDROVIRUS**

G. Moreau, E.G. Kettela, G.S. Thurston, S. Holmes, C. Weaver, B. Morin, *Canadian Forest Service, CANADA*; D.B. Levin, *Department of Biology, University of Victoria, CANADA*; C.J. Lucarotti, *Canadian Forest Service, CANADA*

Abstract: Recent increases in the area devoted to intensive forest management in North America have been accompanied by changes in the levels of defoliation attributable to some insects previously viewed as secondary pests. For example, the intensity and duration of balsam fir sawfly (Neodiprion abietis [Harris]) outbreaks have recently increased in managed balsam fir (Abies balsamea [L.] Mill) forests in western Newfoundland. A nucleopolyhedrovirus (NeabNPV) is responsible for the collapse of balsam fir sawfly populations in natural conditions and may provide an efficacious yet environmentally-friendly tactic to suppress epidemic populations of this insect. This study examines the effects of aerial applications of NeabNPV on increasing, peaking and declining populations of its host. Results indicate that balsam fir sawfly densities were distinctly lower in the generation following an aerial application of NeabNPV, but only when treatments were directed against increasing or peaking populations. When directed against declining populations, NeabNPV applications apparently did not influence the natural collapse of outbreaks. Although the artificial introduction of NeabNPV did not consistently affect densities of the treated generation, it had an effect on host biology in the weeks following the treatment as levels of NeabNPV infection increased and frass production (concomitantly larval feeding) decreased in treated areas. Thus, this study suggests that increasing or peaking popul! ation outbreaks of the balsam fir sawfly may be successfully suppresse d by aerial applications of its nucleopolyhedrovirus at rates as low as 1 109 polyhedral inclusion bodies per hectare.

V-28 **EUROPEAN LEUCOMA SALICIS MNPV IS CLOSELY RELATED TO ORGYIA PSEUDOTSUGATA MNPV OF NORTH AMERICA**

Agata Jakubowska, Monique M. Van Oers, *Laboratory of Virology Wageningen University, NETHERLANDS*; Jadwiga Ziemnicka, *Department of Biocontrol and Quarantine Institute of Plant Protection, POLAND*; Just M. Vlak, *Laboratory of Virology Wageningen University, NETHERLANDS*

Abstract: The satin moth Leucoma salicis L. (Lepidoptera, Lymantriidae) is a serious defoliator of poplar trees (Populus spp.) in Europe and Asia (China, Japan). In the beginning of the last century it was introduced to North America and now occurs locally in southwestern British Columbia and New England. We have characterized a multicapsid nucleopolyhedrovirus isolated in Poland from L. salicis larvae (LsMNPV). Three baculoviral conserved genes, polh, lef-8 and ac22 (pif-2), were amplified in a polymerase chain reaction using gene-specific degenerate primer sets. Sequences analysis revealed a very high homology of these LsMNPV genes with a MNPV from the Douglas-fir tussock moth Orgyia pseudotsugata (Lepidoptera, Lymantriidae). The latter insect is found only in North America. Restriction enzyme analysis of both viral genomes confirmed that LsMNPV and OpMNPV are closely related baculoviruses. Nevertheless a small number of restriction fragment length polymorphisms was observed. Two regions were chosen as putative molecular determinants of the respective viruses: ctl-2 encoding a conotoxin- like protein (disulfide-rich ion channel antagonist) and present only in a few baculoviral genomes, and lef-7 encoding late expression factor 7. These genes are thought to undergo most frequently positive selection in alternate hosts and may modulate the ability of NPVs to replicate in cell cultures of different hosts. Both genes appeared suitable for unequivocal identification of either virus. The genetic relationships between LsMNPV and OpMNPV and the consequences for their taxonomy and nomenclature will be discussed. Finally, we will address the issue of how theses viruses evolved over time into diverse ecological niches.
AJ is a recipient of a Marie Curie Training fellowship of the European Union (Functional Biodiversity and Crop Protection), contract n HPMT-CT-2000-00199. E-mail: monique.vanoers@wur.nl.

MC-1 **RESISTANCE MANAGEMENT FOR BACILLUS THURINGIENSIS SPRAYS AND TOXINS; IS IT COMPATIBLE WITH THE USE OF BACULOVIRUSES AS ADDITIONAL BIOCONTROL AGENTS?**

Ben Raymond, Ali Sayyed, *Imperial College London, UK*; Denis Wright, *Imperial College London, UK*

Abstract: The use of Bacillus thuringiensis in insect control, either as a spray or as toxins within transgenic crops, can increase the potential for the use of additional biological control agents. However, what are the implications of the use of additional biological control agents for the resistance management for Bt? Selection for resistance to Cry1Ac in two strains of diamondback moth, Plutella xylostella, did not produce any change in resistance to two baculoviruses, PxGV and AcalNPV. Coinfection bioassays using AcalNPV and Cry1Ac revealed that low doses of NPV and toxin inhibited each other as mortality agents for Cry1Ac susceptible insects, while no such inhibition was found for Cry1Ac resistant insects. Although the strength of this interaction and the dosages at which inhibition was found indicate that this interaction is unlikely to have a strong effect on insects in the field, the predicted direction of such an inhibition would be to maintain an increased fitness of Cry1Ac susceptible relative to resistant insects in the presence of virus. Subsequent experiments using insects maintained on whole plants in the laboratory have confirmed that Cry1Ac susceptible insects are significantly more fit than Cry1Ac resistant insects in the presence and absence of virus. Most significantly of all, in the presence of low doses of toxin and high doses of NPV the fitness advantage of Cry1Ac sus-

ceptible insects is maintained relative to Cry1Ac resistant insects. While furthers experiment to explore the causes behind this result are ongoing all our available data indicate that the use of NPVs may, in the worst case scenario, have no effect on the evolution of Cry1Ac resistance, and at best, may have benefits in slowing the evolution of resistance.

MC-2 **A NOVEL MECHANISM FOR BACILLUS THURINGIENSIS CRY1AC RESISTANCE IN A FIELD-DERIVED POPULATION OF THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA**

Ali Sayyed, *Imperial College London, UK*; Graham Moores, *Rothamsted Research, UK*; Fred Kemp, *University of Reading, UK*; Robin Gunning, *NSW Ag, AUSTRALIA*; Denis Wright, *Imperial College London, UK*

Abstract: A number of insects have been shown to develop target-site (loss of binding) resistance to specific Bt Cry toxins under laboratory selection and one species, Plutella xylostella, has developed widespread resistance to Bt products under the intensive field selection commonly found in crucifer crops. There is evidence that additional mechanisms of resistance may be present in some insect populations and a proteinase-based mechanism has been described in Plodia interpunctella. Recent work on Helicoverpa armigera in Australia by R.V. Gunning and colleagues has provided evidence for a novel, metabolic resistance mechanism, which may confer broad-spectrum resistance across Bt toxins. Evidence for the same mechanism is now reported in a Cry1Ac-resistant laboratory re-selected strain of P. xylostella. The mechanism is synergisable by piperonyl butoxide (which suggests that it is either mixed function oxidase or esterase-based) and an analogue, which specifically inhibits esterases. Initial studies using surface plasmon resonance indicate that enhanced esterases found in this P. xylostella strain have a strong affinity for the Bt toxin and that sequestration of the toxin is the mechanism by which resistance is occurring.

MC-3 **DANISH CENTRE FOR BIOLOGICAL CONTROL**

Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Annie Enkegaard, *The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, 4600 Slagelse, DENMARK*; Niels Bohse Hendriksen, *The National Environmental Research Institute, Department of Environmental Chemistry and Microbiology, Frederiksborgvej 399, 4000 Roskilde, DENMARK*; Dan Funck Jensen, *Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Jørgen Brøchner Jespersen, *The Danish Institute of Agricultural Sciences, Danish Pest Infestation Laboratory, Skovbrynet 14, 2800 Lyngby, DENMARK*; John Larsen, *The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, 4600 Slagelse, DENMARK*; Anne Mette Madsen, *The National Institute of Environmental Health, Department of Chemical Working Environments, Lersø Parkallé 105, 2100 Kbh. ., DENMARK*; Hans-Peter Ravn, *Forest and Landscape, Department of Applied Ecology, Hørsholm Kongevej 11, 2970 Hørsholm, DENMARK*

Abstract: Danish Centre for Biological Control was established 2003 and aims to support the development and use of biological control in Denmark and internationally based on principles of ecological sustainability. Focus is on:

Biological research and development of products for release Biological research to enhance natural regulation Risk assessment Teaching and other dissemination

The centre will consider biological control of all groups of damaging organisms: pest insect mites and slugs; plant diseases; mammals; weeds and endoparasites.

The centre consists of the core groups in Denmark who have a broad established net-work co-operation with other national and international research groups companies and authorities.

In the period 2003-2005 the centre will organise international workshops and conferences on different aspects of biological control. The centre is in this period funded by FELFO. The first conference was held nov 27, 2003, with focus on occupational health in biocontrol. Abstracts can be found on our web-page. In 2004, a workshop will be held November with specific attention to effects of biocontrol on human health and environment.

http://www.Centre-biological-control.dk (English version)
http://www.Center-biologisk-bekaempelse.dk (Danish version)

MC-4 **QUALIFICATION AND QUANTIFICATION OF CULTURABLE MICROORGANISMS IN MARKETED MICROBIAL PEST CONTROL AGENTS**

Anne Winding, Bjarne Munk Hansen, *Dept. Env. Chem. Microbiol., NERI, DENMARK*; Anita Fjelsted, *Danish EPA, DENMARK*

Abstract: In Denmark twelve products of microbial pest control agents are on the market. These include the following bacteria: Bacillus thuringiensis subsp. kurstaki, B. thuringiensis subsp. israelensis, and Streptomyces griseoviridis and the following fungi: Tricoderma harzianum, T. polysporum, Verticillium lecanii, Phlebiopsis gigantea and Beauveria bassiana. Important parts of the evaluation and authorisation are assurance of the right microorganism in the product, information on the concentration, and microbiological purity of the product. The EU Member states are obliged to carry out analytical control of pesticides. In Denmark this control has until now only been done for chemical pesticides. With the present study this control was extended to include a control of the microbial pesticides that presently are on the Danish market. In the present investigation the products were collected at the suppliers in duplicates representing two different production batches. The microorganisms were separated, diluted and cultured on solid media as described by the manufacturer. Physical and chemical separation of the Bacillus thuringiensis-spores was optimised. The number of colony forming units was counted after appropriate incubation time and the purity of the products visually evaluated. Representative isolates were restreaked on agar plates. The identity of B. thuringiensis in three products was confirmed by 16S-23S ribosomal spacer DNA analysis and formation of toxin crystals visualised by phase contrast microscopy. The initial identification of the fungi (five species) and Streptomyces griseoviridis was determined by traditional techniques and confirmed by identification at DSMZ. The number of microorganisms is not included in the product information of B. thuringiensis based products, however, the content is defined in International Units. In products based on fungi the number of culturable units were generally within or below the range provided in the product information and the number of contaminating microorganisms was low. In a few cases, however, the presence of other microorganisms than the active microorganism amounted to approx. 10%.

MC-5 **EFFECTS OF SEVERAL ABIOTIC FACTORS ON THE VIRAL ENHANCING ABILITY OF THE SPINDLE OF ANOMALA CUPREA ENTOMOPOXVIRUS**

Wataru Mitsuhashi, Ritsuko Murakami, Kazuhisa Miyamoto, *National Institute of Agrobiological Sciences, JAPAN*

Abstract: Proteinaceous structure called the spindle of an entomopoxvirus (EPV) from Anomala cuprea (Coleoptera: Scarabaedae) is known to enhance the infectivity of some nucleopolyhedroviruses (NPVs) and the Anomala cuprea EPV (AcEPV) itself. The enhancing degree was high; especially in the case of Bombyx mori NPV in B. mori larvae, about 200,000-fold enhancement was observed. Thus, this proteinaceous body seems to be potential synergist of bio-control agents such as NPVs or EPVs. The high stability of the enhancing activity of the spindle in field is required when it is used as a synergist. However, little is known on the effect of environmental factors, e. g. high temperature and UV ray, on the enhancing activity of the spindle. Further it is preferable to know the stability of its activity in several chemicals, because these properties would be important in the preparation of the synergist of microbial insecticides from A. cuprea larvae. In the present study, we examined effects of several abiotic factors on the viral enhancing ability of the AcEPV spindle. Spindles heated at 75, 85 or 95C for 30 min retained their high ability to enhance the infectivity of BmNPV in B. mori larvae. Also, they were stable in 0.2 % Benzalkonium Chloride solution for 45 min, which is the condition that this chemical acts as a strong germicide. These results suggest that the spindle is a relatively stable material. In addition, we present effects of UV ray, formaldehyde, ethyl alcohol, etc. on the ability of the spindle.

MC-6 **EFFECT OF BEAUVERIA BASSIANA, VERTICILLIUM LECANII, BACILLUS THURINGIENSIS SUBSP. TENEBRIONIS AND AZADIRACTIN COMPOUNDS ON SITOPHILUS ORYZAE (L.) AND TRIBOLIUM CONFUSUM DU VAL IN STORED RYE**

Dimitris Kontodimas, Nickolas Kavallieratos, Spyridon Mantzoukas, *Benaki Phytopathological Institute, GREECE*; Christos Athanassiou, *Agricultural University of Athens, GREECE*; Maria Anagnou-Veroniki, *Benaki Phytopathological Institute, GREECE*

Abstract: The insecticidal effect of Naturalis [Beauveria bassiana (Balsamo) Vuillemin at 7.16% w/v, i.e 2.3x107 conidia/ml], Mycotal [Verticilium lecanii (Zimmerman) Viegas at 16.1% w/w, i.e. 8.44x106 spores/gr], Novodor (Bacillus thuringiensis subsp. tenebrionis at 3% w/v) and

NeemAzal T/S (azadirachtin A at 1% w/v) was evaluated against the rice weevil Sitophilus oryzae (L.) (Coleoptera: Curculionidae) and the flour beetle Tribolium confusum Du Val (Coleoptera: Tenebrionidae) on stored rye. Three solutions of each compound were used and 100ml of each solution were sprayed in one Kg of rye, for each case. Naturalis was applied at 2500, 5000 and 10000ppm of B. bassiana, Mycotal at 2500, 5000 and 10000ppm of V. lecanii, Novodor at 750, 1500 and 3000ppm of B. t. tenebrionis and NeemAzal at 500, 1000 and 2000ppm of azadirachtin. Then, 30 adults of each species were exposed to the treated substrate, separately for each case. The mortality of S. oryzae was estimated after one, two, seven and 14 days of exposure. In the 14-day count, at the highest application rates of Naturalis, NeemAzal, Mycotal and Novodor the mortalities were 100, 100, 71 and 66% respectively. The mortality of T. confusum was estimated after one, two, seven, 14 and 21 days of exposure. In the 21-day count, at the highest application rates of Naturalis, NeemAzal, Mycotal and Novodor the mortalities were 100, 78, 33 and 31% respectively. In addition, the application of the four formulations tested significantly reduced progeny production in the treated substrate, in comparison with the untreated rye. Hence, offspring production was suppressed at least 58% in S. oryzae treatments and at least 25% in T. confusum treatments in comparison with the untreated rye. The potential of using some of these formulation/dose rate combinations, as reliable alternatives to conventional pesticides is also discussed.

MC-7 **ARE NOMURAEA RILEYI EPIZOOTICS TRIGGERED BY THE MICROENVIRONMENT OF SOYBEAN PLANT AREA OR FAVORED BY SELECTIVE FUNGICIDES?**

Daniel R. Sosa-Gómez, Jose J. Da Silva, *Embrapa Soja, BRAZIL*; Francislene Angelotti, *Universidade Estadual de Maringá, BRAZIL*; Ivan T.V. Licursi, *Fundação Dalmo Giacometti, BRAZIL*; Eduardo Polloto, *Universidade Estadual de Maringá, BRAZIL*

Abstract: Nomuraea rileyi is one of the most important natural controlling factors of the velvetbean caterpillar (VBC), Anticarsia gemmatalis, and soybean loopers (Pseudoplusia includens and Rachiplusia nu) populations. In Brazil, their epizootics, usually initiate at the end of December, declining in March. Epizootic initiation and intensity can be delayed if deleterious fungicides are applied on soybean fields, mainly if application is done early, at the beginning of the epizootic phase. In order to know if the epizootic process is triggered by the microenvironmental conditions favored by soybean canopy, and if selective fungicides favored it, we monitored the VBC population density and quantified the soybean plant area (leaves, petiole, and stems) through six growing seasons. The same soybean plots were used during the experiment; soybean was sowed with 45 cm between rows. Sampling was performed once or twice a week, using the ground cloth method for VBC population and the area of 10-15 plants was determined in a leaf area meter. The maximum mortality by N. rileyi occurred in 1997/1998, 1999/00 and 2003/2004 reaching ca. 19 larvae/ 10 m of row. Number of cadavers per meter row was lower (maximum of 7.0 larvae in 10 meter row) in March 3, 2001. In four seasons epizootics (1997/98, 1998/99, 2000/01 and 2003/04) initiated when the soybean plant reached an area between 1,000 and 1,500 cm2. The rain regime was lower in the season 99/00, and the synchronism of the first disease case with the canopy was not maintained. In this year, the first case of the N. rileyi was detected when the soybean area was around 2,400 cm2. Germination bioassays and mycelial growth with agrochemicals (procloraz, tebuconazole, benomyl, sulphur, fluquiconazole, triticonazole, fosethyl, difeconazole, carbendazim, tetraconazole, azoxistrobin, propiconazol, flutriafol, triflumurom, lorsban, lufenurom, neem, spinosad, cyflutrin, propamocarb-hydrochloride) demonstrated that the fungicide propamocarb-hydrochloride was stimulant to germ tube growth. The fungicide flutriafol (Impactâ), the insecticides diflubenzuron (Dimilinâ) and spinosad (Tracerâ) did not affect N. rileyi growth. The remaining formulations reduced N. rileyi growth. Attempts to favor epizootics with two field applications of the fungicide Previcurâ (propamocarb-hydrochloride, at 1,083 g a.i. Ha-1 each application), which stimulate N. rileyi growth, failed. No increases in the prevalence of N. rileyi diseases were observed.

MC-8 **TRANSGENIC RISK ASSESSMENT: POTENTIAL EFFECTS OF TRANSGENIC CHITINASE AND 1,3-GLUCANASE EXPRESSION ON GRAPE VINE ARTHROPODS**

Hugo M. Arends, Claudia Vogel, Johannes A. Jehle, *Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate, GERMANY*

Abstract: The transgenic expression of enzymes that inhibit the growth of fungi is a novel genetic approach in plant breeding to enhance fungal resistance. A strategy to obtain improved resistance against fungal diseases is the combinative 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 possible effects of transgenically expressed CHI and GLU on non target organisms were investigated by feeding the enzymes to the beneficial predatory mite Typhlodromus pyri and the cabbage moth Mamestra brassicae which occasionally feeds on grape vine. The effect of the enzymes on these arthropods was tested using CHI and GLU expressed in the baculovirus expression system. After isolation, the enzymes were biochemically characterised (pH optimum, temperature optimum) and used in bioassays. Additionally, the expression level of CHI was quantified in different tissues of transgenic grape vine using Western blotting. No direct effect of CHI and GLU on the mortality or the development of T. pyri and M. brassicae could be observed. Beside the direct effects, it is investigated whether the expression of CHI and GLU has a possible synergistic effect on the activity of M. brassicae nucleopolyhedrovirus and the Bacillus thuringiensis preparation Xentari on larvae of M. brassisae. The results will be presented and discussed.

MC-9 **ISOLATION OF ENTOMOPATHOGENS FROM SOUTH AFRICAN SOILS USING THE GALLERIA MELLONELLA-BAIT TECHNIQUE**

Justin Hatting, *ARC-Small Grain Institute, SOUTH AFRICA*; Selcuk Hazir, *Hacettepe University, TURKEY*; Gloria Macucwa, Hannetjie Jooste, Astrid Jankielsohn, *ARC-Small Grain Institute, SOUTH AFRICA*

Abstract: During 2002, a three-year government-funded project entitled Bio-insecticides: a biorational approach to insect pest management was launched by a multi-disciplinary consortium comprising the South African Agricultural Research Council's Small Grain Institute (ARC-SGI) and Plant Protection Research Institute, the University of KwaZulu-Natal, Pietermaritzburg, and a private company, Plant Health Products (Pty) Ltd. In addition, entomologists from two private sector institutions and three ARC institutes are acting as field collaborators within this project. A 'Central Service Laboratory' (CSL) was established at ARC-SGI in Bethlehem, Free State Province, with quarantine facility to accommodate the safe-handling of entomopathogens isolated directly from insect cadavers as well as from soil samples collected throughout South Africa. Following countrywide surveys a total of 1506 soil samples were processed at the CSL employing the Galleria mellonella-bait technique. These samples yielded 441 isolates of entomopathogenic fungi (87% Beauveria bassiana versus 13% Metarhizium anisopliae)and 76 isolates of entomopathogenic nematodes (provisional identifications: 61% Steinernematidae versus 39% Heterorhabditidae). An entomopathogenic strain of the bacterium Serratia marcescens was also isolated. Following this exercise, the consortium on this project currently holds the largest collection of both indigenous entomopathogenic fungi and nematodes in South Africa. Laboratory bioassays are now being conducted at the CSL for quantification of virulence (LC50) and to establish the host range of these microbes. Immediate target pests within the scope of this project include American bollworm (Helicoverpa armigera), whitefly (Bemisia tabaci), Russian wheat aphid (Diuraphis noxia), false codling moth (Cryptophlebia leucotreta), black vine weevil (Otiorhynchus sulcatus), and Western flower thrips (Frankliniella occidentalis).

MC-10 **ENTOMOPATHOGENIC FUNGI FOR WHITE GRUB CONTROL IN NEPAL**

Yubak Dhoj Gc, *Institute of Agriculture and Animal Sciences, Rampur, Chitwan,, NEPAL*; Siegfried Keller, *Swiss Federal Research Station for Agroecology and Agriculture, SWITZERLAND*

Abstract: With an objective to explore the possibility of biocontrol of white grubs using entomopathogenic fungi, an exploratory study was conducted in the Syangja and Parbat districts in Nepal in the winter of 2001/2002. In order to explore the occurrence of indigenous fungal pathogens of white grubs, field and laboratory experiments were carried out and informations were collected from all available sources. White grubs collected in fields with arable crop and kept in the laboratory were found to be attacked by the entomopathogenic fungus Metarhizium anisopliae Disease prevalence was between 0 and 2% depending on host origin and species. Bioassays revealed that the Nepalese isolates of this fungus species were as pathogenic as a Swiss isolate used for comparison purposes. Therefore, future work will be done exclusively with Nepalese isolates. Analysis of soils from three different regions showed that M. anisopliae is common and was present in about 50% of the samples irrespective of their origin. However, the fungus densities were low. Another entomopathogenic fungus, Beauveria bassiana, was isolated as well from a few soil samples. Based on these first results, the possibilities to develop mycoinsecticides and to integrate them into existing pest management systems are considered as very promising.

MC-11 **LABORATORY BIOASSAYS OF PAECILOMYCES FUMOSOROSEUS ON COPTOTERMES FORMOSANUS: THE EFFECTS OF TERMITE SEPARATION AND SPORE CONCENTRATIONS ON TERMITE SURVIVAL**

William Meikle, Guy Mercadier, Alan Kirk, Franck Derouane, *European Biological Control Laboratory, FRANCE*; Rebecca Rosengaus, *Northeastern University, USA*; Yurong He, *Laboratory of Insect Ecology, CHINA*; Chuck Quimby, *European Biological Control Laboratory, FRANCE*

Abstract: As part of a larger USDA-ARS project on the management of Formosan subterranean termites, Coptotermes formosanus, exploration for natural enemies of termites, either parasitoids or pathogens, was conducted in six countries. Several fungal strains were isolated from termites collected in China, the area of origin for C. formosanus by a team from the European Biological Control Laboratory, and one isolate of Paecilomyces fumosoroseus was demonstrated to be virulent and selected for further laboratory studies. Using the Chinese C. formosanus, experiments were conducted to 1) quantify the density of cuticular microbes on the termites and the number of spores recovered from a termite after treatment in a Potter tower using suspensions of different P. fumosoroseus conidial densities; and 2) examine the effects of separation and conidia concentration on termite survivorship. We found natural natural microbial loads of about 60 bacterial spores and about 30 fungal spores per termite, after one washing, and although these loads were variable, they were broadly similar to those observed for dampwood termites in the US. The median survival time for termites kept in petri dishes was a function of both the concentration of the spore suspension with which the termites were treated, and whether the termites were kept separately or together after treatment. No significant difference was observed in median survival time between control (no spores) termites kept separately after treatment and those kept as a group after treatment. However, grouped termites lived significantly longer than isolated termites after exposure to either of two concentrations of conidial suspensions.

MC-12 **VIRULENCE OF NEW STRAINS OF ENTOMOPATHOGENIC HYPHOMYCETES (DEUTEROMYCOTA, HYPHOMYCETES) TO ORTHOPTERAN INSECTS**

Yuriy Tokarev, Maxim Levchenko, Anton Naumov, George Lednev, *All-Russian Insitute for Plant Protection, RUSSIA*

Abstract: Development of biological control measures of locusts and grasshoppers is one of most priority and fast developing directions in modern IPM programs. The most important from this point of view are entomopathogenic hyphomycetes (Deuteromycota, Hyphomycetes). Due to a high level of reproduction of Calliptamus italicus and Locusta migratoria on the south of Russia, we studied activities of fungi against locusts under lab and field conditions. The research was conducted during five years in three south-eastern regions: Altaiskiy, Primorskiy and Novosibirskiy regions, and resulted in isolation of five new strains - one of Metarhizium anisopliae and four of Beauveria bassiana, including two from C. italicus. Under lab conditions, all studied fungal strains were found virulent for crickets Gryllus bimaculatus and locusts L. m. migratorioides. All larval stages tested were susceptible when treated with suspension of 1-5x107 conidia per ml. Mortality of III-IV instar larvae of insects at 17th day post infection (p.i.) was 65-100%. The highest activity was attributed to M. anisopliae isolated from C. italicus, which caused 85% (107)-100%(5x107) mortality of IV instar locust larvae at 13th day p.i. When III instar larvae were treated with 5x107 suspension, 100% mortality was observed at 7th day p.i. At lower concentrations (1x106 - 1x107) mortality reached 70-85% at 14th day p.i. The most virulent strain of B. bassiana was also isolated from C. italicus population in Novosibirskiy region. At 15th day p.i. mortality of III instar larvae of locusts treated with 107 and 5x107 suspensions, reached 65 and 95%, respectively. For IV instar larvae, these values reached 60 and 70%, respectively. Laboratory experiments were conducted at humidity lower 80%, that is close to that in the field. Thus the tested strains are quite xerophilic, that favors their field application. In field trials, fungi were tested on IV-V instar larvae of L. m. migratoria in Astrakhanskiy region. At concentrations of 107 and 5x107 conidia per ml, at 17th day p.i. B. bassiana strain from Novosibirskiy region caused 47.1 and 55.9% mortality, and M. anisopliae - 76.5 and 82.3%, respectively. Although M. anisopliae used in our studies was found the most virulent and its in vitro growth was the fastest, conidia hatching was slower comparing to Beaveria strains and started only 14 hrs after moisturing. As a result, in a trial when a shower rain occured 12 hrs p.i., M. anisopliae efficacy was reduced two fold comparing to rainless trial, while activity of Beaveria strains was not decreased.

MC-13 **CONJUGATIVE TRANSFER, STABILITY AND EXPRESSION OF A PLASMID ENCODING A CRY1AC GENE IN BACILLUS CEREUS GROUP STRAINS**

Xiaomin Hu, *Wuhan Institute of Virology, CHINA*; Bjarne Munk Hansen, *National Environmental Research Institute, DENMARK*; Jørgen Eilenberg, *Agricultural University, DENMARK*; Niels Bohse Hendriksen, *National Environmental Research Institute, DENMARK*; Lasse Smidt, *National Institute of Occupational Health, DENMARK*; Zhiming Yuan, *Wuhan Institute of Virology, CHINA*; Gert Bolander Jensen, *National Institute of Occupational Health, DENMARK*

Abstract: Bacillus thuringiensis is a member of the B. cereus group, which also contains the closely related species B. cereus, B. mycoides, B. pseudomycoides, B. anthracis and B. weihenstephanensis. The taxonomy of the group is controversial and it has been suggested that these species should all be grouped as members of the species B. cereus. B. thuringiensis, including strains used commercially, have enterotoxin encoding genes. Some B. thuringiensis strains show in vitro cytotoxicity levels similar to those of B. cereus strains suggesting a potential to cause diarrhoea in humans. It is therefore important to evaluate the potential risk of individual B. thuringiensis strains used as insecticides and to look for some means of constructing or selecting B. thuringiensis strains for biological control with low levels of expression of B. cereus-like enterotoxic traits. However, at least some of the enterotoxic traits are expected to play a role in the insecticidal activity. One way to investigate the role of the enterotoxins in the insecticidal process is to transfer Cry toxin encoding plasmids by conjugation to a number of B. cereus group bacteria, including bacteria known to have low enterotoxic activity. However, only limited work has been done to determine recipient host range and plasmid stability of transconjugants of B. thuringiensis. Here we describe conjugation of pHT73, containing cry1Ac and tagged with an erythromycin resistance gene, from B. thuringiensis subsp. kurstaki KT0 to several Bacillus cereus group strains, including recipients with a known low enterotoxic activity. The study demonstrated that pHT73 can be transferred to B. thuringiensis subsp. kurstaki, several Bacillus cereus strains and one Bacillus mycoides. Under non-selective conditions, the stability of the pHT73 plasmid in the transconjugants was found to be 58.2%-100% after 100 generations and 4.096.0% after 200 generations.

MC-14 **OCCURRENCE OF BACILLUS CEREUS AND B. THURINGIENSIS IN FIELD PLOTS WITH CURLY KALE (BRASSICA OLEARACEA ACEPHALA)**

Niels Bohse Hendriksen, Bjarne Munk Hansen, *National Environmental Research Institute, DENMARK*

Abstract: The natural occurrence of Bacillus cereus and B. thuringiensis on Curly kale (Brassica olearacea acephala), was investigated in 17 plots in Denmark, as a background for the assessment of the use of B. thuringiensis as a microbial pest control agent. During September the number of Bacillus cereus and B. thuringiensis spores occurring in the soil, on lower and on upper leaves were quantified by plate counting. From all samples a total of 524 colonies were isolated and subcultured. PCR analysis of the 16S-23S rDNA spacer region revealed that 98% of the isolates was correctly identified by colony morphology as belonging to the B. cereus group. B. thuringiensis isolates constituted 1.3% of these on the basis of crystal formation. The highest number was found in the soil (mean 9.0 x 104 cfu/g), while 1.4 x103 cfu/g were found on the lower leaves and as few as 15 cfu/g were found on the leaves from the top of the plants. The number of spores found in soil and on leaves is log-normal distributed, with the spores more contagiously distributed on the upper leaves as compared to the lower, which again are more contagiously distributed than in the soil. The variation between leaves within the same plot and type is often similar to the variation between plots. The number of spores on lower leaves is partly explained by the amount of soil adhering to the leaves. Growth on the leaves seem also to be of importance, as analysis of isolates by a molecular typing method (RAPD) reveal that clones of B. cereus group cells are found on leaves with a high number of spores.

MC-15 **BIOASSAY WITH MOSQUITOS FOR EVALUATION OF TRANSCONJUGANT BACILLUS SPP. CONTAINING THE PBTOXIS PLASMID**

Jens Efsen Johansen, *The Agricultural University, DENMARK*; Xiaomin Hu, *Wuhan Institute of Virology, CHINA*; Bjarne Munk Hansen, *National Environmental Research Institute, DENMARK*; Zhiming Yuan, *Wuhan Institute of Virology, CHINA*; Jørgen Eilenberg, *The Agricultural University, DENMARK*

Abstract: Due to the genetic similarities of B. thuringiensis and B. cereus, B. thuringiensis has the potential to cause B. cereus-like symptoms in hu-

mans. At least some of the traits involved in human pathogenesis are expected also to play a role in insect pathogenesis. In the search for insecticidal Bacillus spp. with low or no effects on humans we constructed several strains containing the B. thuringiensis subsp. israelensis plasmid pBtoxis (tagged with antibiotic resistance) encoding toxins active against mosquito's (Diptera /Culex quinquefasciatus). Strains were constructed by conjugation and one of the transconjugants (DH05) was able to harbour, maintain and divide with plasmids coding for toxins against Diptera (pBtoxis) and Lepidoptera (pHT73). Bioassays were performed according to standard methods recommended by WHO with three target colonies of mosquito's (two colonies, which showed different resistances to Bacillus sphaericus strains and one susceptible laboratory colony). The donor strains had LC50 toxicities to mosquitos in the range of 0.00446-0.03450 mg spore-crystal/l and transconjugants had a LC50 in the range of 0.003-2.74 mg spore-crystal/l). The positive control B. thuringiensis subsp. israelensis 4Q5 containing pBtoxis had a LC50 of 0.00394 mg spore-crystal/l. We did not find any differences between toxicity effects of the Bacillus strains against the different mosquito colonies that had similar LC50 values for the individual transconjugants. Currently we study the most promising candidates of transconjugants with high mosquito toxicity in order to evaluate the B. cereus-like toxicity.

MC-16 **FURTHER DEVELOPMENTS IN THE COMMERCIAL LABORATORY PRODUCTION OF THE NUCLEOPOLYHEDROVIRUS OF ANTICARSIA GEMMATALIS IN BRAZIL**

Flavio Moscardi, *Embrapa Soja, BRAZIL*; Braulio Santos, *Universidade Federal do Paraná, BRAZIL*

Abstract: The nucleopolyhedrovirus of the velbetbean caterpillar, Anticarsia gemmatalis Hübner (AgMNPV) is being employed annually on nearly 2.0 million ha of soybean in Brazil. Up to the 2003 season all virus production was made in the field for further processing and formulation by the companies involved with its commercialization. However, this type of production reached a plateau and the companies could not cope with the increasing demand for the biological insecticide in the last four seasons. Furthermore, quality of field-collected material started to decrease in the last years making it difficult to adjust the final formulated product to the established quality standards. Improvements on the procedures for the laboratory production of the AgMNPV, as presented in the SIP 2003, resulted in a final product of much better quality and at a competitive cost with those of chemical insecticides. The procedures were further adjusted through the implementation in one of the companies (COODETEC) of a Pilot Laboratory for AgMNPV production. Initially, in this laboratory (ca. 100 m2), 25,000 to 30,000 A. gemmatalis larvae were inoculated per day. During four months of production, estimated cost of the final product to treat one hectare of soybean was ca. 0.32 US$ and could be sold to the farmers at the same cost (ca. 1.00 US$) as the product originated from field production. In the sequence, COODETEC expanded the pilot facilities to over 200 m2 and currently is inoculating around 120,000 to 150,000 larvae per day, achieving the same cost of the final product to treat one hectare. The production has been so successful that this company has planned to build a 1,200 m2 laboratory facility to produce around 20 to 22 metric tons of dead larvae per year, which would result in product enough to treat 1.2 to 1.3 million hectares/year. This is twice the amount of AgMNPV that this company usually produces under field conditions. The laboratory production procedures of the AgMNPV are to be adopted by other private companies in the near future (2004/2005), increasing the availability of the biological insecticide to the soybean growers as well as improving the quality of the final product.

MC-17 **SELECTION OF ENTOMOPATHOGENIC FUNGI FOR MICROBIAL CONTROL OF APHID PESTS IN US GREENHOUSES**

Melanie Filotas, *Department of Entomology, Cornell University, U.S.A.*; Stephen P. Wraight, *USDA Agriculture Research Service, US Plant, Soil and Nutrition Laboratory, U.S.A.*; John Sanderson, *Department of Entomology, Cornell University, U.S.A.*

Abstract: Although numerous studies have screened fungal isolates against aphid pests, most have targeted the adult stage of a single pest species. We have found that adults are a poor target for screening assays because, although adult aphids are highly susceptible to most fungal strains, their high rates of reproduction are not sufficiently reduced prior to death to effectively control pest populations. Screening assays against aphids may thus be better aimed at identifying isolates effective at killing nymphal stages before they are capable of population increase. We conducted a series of laboratory bioassays assessing the efficacy of isolates of entomopathogenic fungi against the green peach aphid, Myzus persicae, and the melon aphid, Aphis gossypii, the two most common aphid pests of US greenhouse crops. As an initial screen, one-day-old first instar M. persicae and A. gossypii were exposed to spray applications of a single dose (ca. 1000 spores/mm2) of 48 isolates of three Hyphomycete fungi (20 B. bassiana, 19 Metarhizium anisopliae and nine Paecilomyces fumosoroseus). Resulting mortalities ranged from $0.4 + 0.2$ to $61.6 + 6.3$ % for M. persicae and $1.3 + 1.2$ to $56.9 + 10.3$ % for A. gossypii. While both species of aphids were equally susceptible to B. bassiana ($P > 0.5$), A. gossypii tended to be more susceptible than M. persicae to isolates of M. anisopliae and P. fumosoroseus ($P < 0.05$). However, nymphal stages were generally not highly susceptible to most isolates, with less than 25% mortality of test insects observed for 38 of 48 isolates. This was likely due to loss of fungal spores via frequent molting of these rapidly developing insects. Nevertheless, we were able to identify some isolates which were effective against both aphid species. Five of these (two B. bassiana, two M. anisopliae and one P. fumosoroseus), as well as the Beauveria products Naturalis and BotaniGard and the Metarhizium product F52, were selected and are currently being subjected to more detailed multiple dose bioassays.

MC-18 **SCREENING OF SHUFFLED ALPHA-AMYLASE INHIBITORS TO COTTON BOLL WEEVIL ALPHA-AMYLASES**

Maria F. Grossi de Sa, Maria Cristina Mattar da Silva, Rafael Perseghini Del Sarto, Marise Ventura Coutinho, Edson Luiz Zangrando Figueira, *Embrapa Recursos Genéticos e Biotecnologia. Parque Estacão Biológica, BRAZIL*

Abstract: The cotton boll weevil A. grandis (Coleoptera: Curculionidae) is an important pest of Brazilian cotton crop. As well as other insect-pests, the cotton boll weevil secretes a high level of alpha-amylases and proteinases in its midgut lumen in order to digest the diet's starch and proteins as carbohydrates and amino acids source, respectively. The development of this pest in target plants takes place mainly in fruits and floral buds, in which the bromatological characterization has shown a high starch level. On the other hand, its control is very hard and expensive due to its endophytic larval development. So, biotechnology approaches applying proteins involved in plant defense such as the alpha-amylase inhibitors (alpha-AIs) are a powerful tool for the control of this insect-pest. However, there is not any described alpha-AIs genes effective to the cotton boll weevil alpha-amylases, and, in this context, this research focused on the screening of alpha-AIs using phage display methodology from a previously generated alpha-AIs shuffled library. This library was produced by homologous gene recombination (DNA shuffling) of alpha-AI1 and alpha-AI2 from common and wild bean respectively, resulting in 106 recombined genes. For that purpose, the recombinant alphaAI genes were carried out on 4 rounds of phage display selection against the A. grandis alpha-amylases. The selected genes on the fourth round were expressed into E. coli TOP10F' cells and the AI activity was determined against the A. grandis alpha-amylases. The wild type inhibitor genes used to create the shuffled library did not inhibit the assayed alpha-amylases; nevertheless, 95% of the selected recombinant proteins evaluated have presented inhibitory activity from 3 to 100 AIU against the cotton boll weevil alpha-amylases. The selected recombined genes are being characterized and overexpressed to proceed with the bioassays against A. grandis. The advantage of using DNA shuffling and phage display will be discussed as strategy to create high number of mutants by in vitro homologous gene recombination and select mutant inhibitors for important agricultural pests. Supported by Embrapa, Facual, Fialgo and CNPq.

MC-19 **TARGETED DISSEMINATION OF BIOCONTROL AGENTS BY USING THE HONEY BEE:**

Heikki M. T. Hokkanen, *Applied Zoology, University of Helsinki, FINLAND*; Ingeborg Menzler-Hokkanen, *Rural Research and Training Centre, University of Helsinki, FINLAND*

Abstract: Honeybees are famous as good pollinators of plants, i.e. in carrying passively small living particles on their body from one place to another. They are also very precise in visiting diligently specific flowers, such as apple blossoms or oilseed rape flowers. Due to specific signalling systems used by flowers dependent on insect pollination, bees do not miss a single blossom advertising its abundant mead yield. It is therefore amazing that only during little over a decade attention has been paid to utilising bees to transport and precision-deliver beneficial microbes to crop plants. Encouraging results have been obtained in research on bee-mediated delivery of biocontrol agents to crops such as apples, oilseed rape, strawberries, alfalfa, and others. We assessed the potential applications of this strategy in the Nordic countries in the biological control of pests and plant diseases. There are many important crop plants grown in the Nordic countries, frequently visited by the honeybees. Most of these crops have key pests or diseases attacking the flowers, thus forming a potential target for precision application of biocontrol agents via honeybees. Most important of these crops in area and in importance to the honeybee is oilseed rape (or turnip rape). Both the key insect pest and the most important disease could be targeted with this strategy. In Finland the blossom beetle Meligethes needs control over the whole growing area (65 000 ha) almost every year, and Sclerotinium requires control on one-third of the area every few years. Our list of potential targets includes many species, which have not yet been considered from this pont of view. These include Butyrus

and Botrytis on raspberry, Zophodia and Pachynematus on currants, Depressaria daucella on caraway, Frankliniella on greenhouse cucumber, and Apion on clover seed cultivation. We thus feel that there is much scope for applying this multidisciplinary precision strategy in pest management in the Nordic countries.

MC-20 **THE INTERACTION BETWEEN ROOT HERBIVOURUS LARVAE AND BENIFICIAL SOIL ORGANISMS IN NURSERY PEAT VS. FOREST SOIL - A POT EXPERIMENT.**

Edda Sigurdís Oddsdóttir, *Icelandic Forestry Research, ICELAND*; Jørgen Eilenberg, *The Royal Veterinary and Agricultural University, DENMARK*; Robin Sen, *Department of Biosciences, University of Helsinki, FINLAND*; Gudmundur Halldórsson, *Icelandic Forestry Research, ICELAND*

Abstract: Since the settlement of Iceland in 874, the country has suffered from severe ecosystem degradation and large-scale soil erosion. Birch (Betula pubescens Ehrh.), which is the only native forest-forming tree, is believed to have covered at least 25% of land area at the time of settlement, but now occupies only 1%. It is presently the most common species used in afforestation and is extensively used in land reclamation, mostly on degraded land devoid of the soil biota normally associated with birch. Furthermore, it is often planted in hostile environments where it can be expected to be especially vulnerable to pest problems. Otiorhyncus spp. larvae are a serious problem in afforestation, killing 10-20% of newly planted seedlings. The aim of this project is to study the mechanisms of the multitrophic interactions between Otiorhyncus larvae, birch seedlings and beneficial soil organisms in pots. Birch seeds were sown in a) FinnPeat, b) FinnPeat inoculated with mycorrhizal fungi and c) Icelandic birch soil from Hafnarskógur. After 4 months in pots, Otiorhynchus sulcatus larvae were introduced and plants inoculated with Metarhizium anisopliae. Plants and larvae were harvested 2 weeks after introduction of larvae. A difference could be seen in larvae/pupae mortality between plants inoculated with ectomycorrhiza vs nontreated plants and plants inoculated with insect pathocenic fungi vs noninoculated plants. Synergetic effects were detected in treatments were both mycorrhizal and insect pathogenic fungi were inoculated. These results indicate that inoculation with mycorrhiza and insect pathogenic fungi may increase larvae mortality in the rhizhosphere and therefore decrease plant mortality due to larvea feeding on roots.

MC-21 **NATURALLY OCCURRING INSECT PATHOGENIC FUNGI ON KEY COFFEE PESTS, AND THE INFLUENCE OF MANAGEMENT PRACTICES**

Arnulfo Monzòn, *UNA, NICARAGUA*; Ingeborg Klingen, *Planteforsk, NORWAY*; Falguni Guharay, *CATIE, NICARAGUA*

Abstract: In coffee systems, shade management and pests control practices are related to the occurrence of arthropods and micro-organisms, because such practices alter the environmental conditions in the system. The insect pathogenic fungi Beauveria bassiana is used as a microbial control agent against coffee berry borer Hypothenemus hampei, in several countries in America, and it has also been reported to infect the coffee leaf miner Leucoptera coffeella. Only few studies have, however, focused on the natural infection level and the effect of shade and pest management on the dynamic of B. bassiana or other insect pathogenic fungi on key coffee pests. Understanding the dynamic of B. bassiana in the field and its genetic diversity is very important not only to favour its natural occurrence and enhance the natural control, but also to select strains with a good field performance and virulence. The main objectives of this PhD study conducted in Nicaragua are therefore to: a) determine the natural occurrence and infection level of B. bassiana on H. hampei and L. coffeella in unsprayed coffee plantations throughout two successive seasons; b) clarify whether B. bassiana is present as an endophyte in coffee trees, and determine whether B. bassiana injected into coffee plants can establish and persist; c) reveal the effect of shade and use of pesticides on insect pathogenic fungi on H. hampei and in the soil, and d) compare B. bassiana isolates obtained from L. coffeella, H. hampei and the soil at the same and different locations by the use of PCR techniques. The research is being carried out over a period of two years, 2004 and 2005. The natural occurrence and infection level of B. bassiana and the study on endophytic B. bassiana will be studied in unsprayed coffee plantations in the north and the pacific zone of Nicaragua. The experiment on the effect of shade and management practices will be carried out in plots with different pest management practices already established by CATIE in Masatepe, Nicaragua.

MC-22 **BRASSICA HOST PLANT AND FERTILIZER IMPACTS ON STEINERNEMA FELTIAE EFFICIENCY**

Melita Zec-Vojinovic, Heikki M. T. Hokkanen, *Laboratory of Applied Zoology, FINLAND*

Abstract: The impact of Steinernema feltiae on pollen beetle and flea beetles in oilseed rape has been shown to be excellent when applied in high dose with watering can at the right time, but application as slow-release nematode delivery system ('NemaBagTM', more suitable for farmers) yielded disappointing control results in first field trials. To understand and to overcome the problems in nematode efficiency when applied via NemaBags we conducted greenhouse experiments. Aim of this study was to show the possible impact of the rapeseed plant, organic fertilizer, and synthetic fertilizer on Steinernema feltiae efficiency. The experiment consisted of 7 treatments and 6 repetitions. Variables were plant (four leaf stage Brassica rapa), organic fertilizer, and synthetic fertilizer, used for treatments in combinations as follows: plant and organic fertilizer, plant and synthetic fertilizer, only plant, only organic fertilizer and only synthetic fertilizer. In all of these treatments NemaBag (15 kIJ/m2) and T. molitor larvae were added for efficacy testing so that the nematodes had to cross the rhizosphere and/or fertilizer placement zone in order to get to the bait larva, placed at 6 cm distance. In two control treatments bait larva only, and larva plus NemaBag were used. Overall, high percentage of bait larvae in the nematode treatments (84.37%) was dead after four days. Results showed that the test parameters did influence nematode efficiency, as well as nematode replication in dead cadavers. Plants alone lowered the nematode efficiency by about 43%, and fertilizers alone by 34%, while the combination plant+fertilizer lowered nematode efficiency by about 46%. When the test plant was used together with a fertilizer, efficiency was 6% higher in the combination plant+organic fertilizer, compared with synthetic fertilizer. Nematode replication was influenced by plant and fertilizer as well. Nematode replication was higher, when plant was combined with a fertilizer (plant+synthetic fertilizer combination) than with plant only. If we consider fertilizers only, higher nematode replication was with organic fertilizer. From these results it is possible to conclude that if nematodes are applied at early plant stage, plant and fertilizers lower the nematode efficiency. If nematodes are used as bio-insecticides it will be better, according to our results, to use organic fertilizer than synthetic fertilizer. Further studies will show the impact of the same parameters on nematodes when they are applied at the flowering stage.

N-1 **ECOLOGICAL CHARACTERIZATION OF HETERORHABDITIS SP. (CABORCA STRAIN) (NEMATODA: HETERORHABDITIDAE), A NATURAL PATHOGEN OF DICEROPROCTA ORNEA (HOMOPTERA: CICADIDAE) FROM SONORA, MEXICO**

Benjamin Rivera-Orduño, *División de Ciencias Administrativas, Contables y Agrarias, MEXICO*; S. Patricia Stock, *Department of Plant Sciences, University of Arizona, Tucson AZ 85721-0036, USA*

Abstract: Several species of cicadas are represented in the Sonoran Desert, with one of the most common species at lower elevations being Diceroprocta ornea (Walker) (Homoptera: Cicadidae). Over the past ten years, this insect has become a major pest of asparagus (Asparagus officinalis) in the region of Caborca, (Sonora State, Mexico). Like most cicadas its life cycle is long, usually involving multiple years spent underground as nymphs (2 17 years), followed by a brief (roughly 2-6 weeks) adult life above ground. Nymphs and adults, feed on the xylem fluid of asparagus plants (at the crown level) using piercing and sucking mouthparts. Adults mate on the asparagus stems, and females lay eggs between the shoots and the stem. The eggs hatch later in the season and the new nymphs drop down and burrow underground and begin feeding on roots. Asparagus yield and crop quality is severely affected by the cicadas. The state of Sonora is the major asparagus production area of Mexico, and a major exporter of this crop to USA and Canada. The increasing demand for organic asparagus from these countries has put pressure for non-chemical control alternatives for this crop. To promote this effort, a survey for natural pathogens on native insect pests, including cicadas, was initiated in the spring of 2003. As a result, an entomopathogenic nematode, Heterorhabditis sp. (Caborca strain) was recovered from infested fourth-instar nymphal stages. The nematode was established in the laboratory and is currently being characterized using molecular and morphological diagnostic tools. To effectively implement this nematode for the management of the cicadas or other desert insect pests, some basic data on the ecological characterization of this nematode is needed. In this study we provide data on the laboratory host range, temperature and moisture requirements on infectivity, development and reproduction of this entomopathogenic nematode. This information is currently non-existent, yet fundamental for the evaluation of the efficacy of this native entomopathogenic nematode, and critical for subsequent experimental and management activities.

N-2 **EVALUATING EFFICACY OF APPLICATION OF ENTOMOPATHOGENIC NEMATODES VIA A DRIP LINE IRRIGATION SYSTEM**

Andrew Brown, *Imperial College London, UK*; Simon Piggott, Jeremy Pearce, *Becker Underwood, UK*; Denis Wright, *Imperial College London, UK*

Abstract: One of the major constraints for the use of entomopathogenic nematodes (EPN) as biological control agents in agricultural systems is their uneven distribution during application. For example, improvements in application methods to give more even emission of EPN along drip irrigation lines could lead to more efficient pest control and improve the market potential for these biopesticides. One of the most important factors influencing uneven distribution during application is settling of EPN, this is especially the case in slow release methods such as drip line irrigation. Using an 18 m T-tape laboratory test irrigation rig to sample water and EPN emission along its length we have modelled the nematode output data to enable it to be applied to larger, commercial irrigation systems. We have shown that although a constant release of water is given with increased distance, EPN emission decreases exponentially with increased distance from the point of introduction for over 80% of its length. With the aim of improving EPN distribution along the irrigation line by reducing settling the effects of various modifications were tested, including the addition of polymers to the nematode solution to increase viscosity, mechanical agitation to reduce settling, and altering pressure and flow rate along the irrigation line by increasing the input pressure or increasing the overall length of the line. Glasshouse trials will be carried out to investigate the applicability of the most effective modifications identified with the laboratory irrigation rig. Observations on commercial drip line-applications of Phasmarhabditis hermaphrodita to combat slugs on lettuce will also be discussed.

N-3 **NON-TARGET EFFECTS OF ENTOMOPATHOGENIC NEMATODES ON SOIL MICROBIAL COMMUNITY AND NUTRIENT CYCLING PROCESSES: A MICROCOSM STUDY**

E. A. B. De Nardo, *Department of Entomology, Ohio State University, EMBRAPA Meio Ambiente, U.S.A.*; Parwinder S. Grewal, D. McCartney, B. R. Stinner, *Department of Entomology, Ohio State University, U.S.A.*

Abstract: In this study we evaluated changes in the soil microbial biomass, respiration rates, and nitrogen pools as indicators of potential non-target effects of entomopathogenic nematodes on soil ecosystems for the first time. Two microcosm tests were conducted using soil collected from the field with no history of entomopathogenic nematode applications. Treatments consisted of applications of Steinernema carpocapsae All strain in the presence or absence of the wax moth Galleria mellonella larva as a target insect, compared with the untreated control (only soil). In the second experiment an insecticide treatment (trichlorfon) was added. Soil microbial respiration, microbial biomass (total nitrogen), and mineral nitrogen (NH4-N, NO3 -N) were measured using standard methods up to 32 to 64 days. No negative effect was detected in the soil microbial biomass, respiration and nitrogen pools after application of S. carpocapsae. However, a significant increase of ammonium level was measured during almost the entire period of the test in the nematode plus larva treatment. This high levels of ammonium may help to clarify several findings associated with the suppression of plant parasitic nematodes by the application of entomopathogenic nematodes. In contrast, the application of the insecticide trichlorfon significantly suppressed the microbial biomass and the nitrification process. Although our results did not show any negative impact of the use of inundative application of S. carpocapsae on important soil parameters, the potential effects of compounds released by infected hosts on other non-target organisms should be explore in future studies.

M-1 **COMPARATIVE ULTRASTRUCTURAL ANALYSIS OF THREE SPECIES OF THE GENUS PARANOSEMA FROM ORTHOPTERA AND COLEOPTERA**

Yulia Sokolova, *Instirute of Cytology Russian Academy of Sciences, St.Petersburg, RUSSIA*; Irma Issi, Yuriy Tokarev, *Institute for Plant Protection, St.Petersburg, RUSSIA*; Elena Morzhina, *Instirute of Cytology Russian Academy of Sciences, St.Petersburg, RUSSIA*; Carlos Lange, *Center for Parasitological Studies, La Plata National University, ARGENTINA*

Abstract: Recently by means of comparative phylogenetic analysis of SSUrDNA it has been shown that Nosema grylli (N.g.), a microsporidian parasite of the cricket Gryllus bimaculatus is closely related to N. locustae (N.l.), and to N. whitei (N.w.) from the confused flour beetle, Tribolium confusum. The sequence divergence and morphological traits clearly separate this group of Nosema parasites from the true Nosema clade containing N. bombycis. The generic name of N. grylli and its close relative N. locustae, has been therefore changed to Paranosema n. comb. N. whitei was left in the former status due to lack of data on fine morphology (Sokolova and Lange, Acta Protozool. 41(2002): 229-237; Sokolova et al. J.Invertebr.Pathol.84 (2003):159-172). Here we present more evidences on structural similarity of three species. In addition to similar spore morphology and sporogony type, all three species possess a characteristic stage in their life cycle, which we call meront/sporont transitional stage. The most striking feature of this phase is that the nucleus is filled with numerous electron-dense bodies, which do not react with antibodies against DNA (tested on P.g.). The question of whether the nucleus maintains its diplokaryotic arrangement at this stage or whether it becomes uninucleate is still unclear. Cells with one, two, or four unpaired nuclei were occasionally observed both on Giemsa-stained smears and on ultrathin sections from all three species. The observed stage with unpaired and comparatively small nuclei might belong to early merogony as well. E. Canning observed the similar stages in N.l. (Parasitology 43(1953): 287-290) and included presence of a uninucleate stage in the diagnosis of the species. Another typical feature of the species is an exospore with electron dense protrusions, which in P.g. and P.l. are seen only in sporoblasts and early spores, but in N.w. - in mature spores also. All three microsporidia develop in fat body cells and produce characteristic tubular structures during sporogony. Being so similar morphologically as well as genetically, N.w., thus, should be eventually transferred to the genus Paranosema. Supported by Russian Foundation for Basic research: grant #03-04-49629.

M-2 **HYPERTROPHY OF SPODOPTERA FRUGIPERDA CELLS INDUCED BY MICROSPORIDIAN INFECTION**

Hidetoshi Iwano, Hideki Tanaka, Tetsufumi Yazu, Kouji Iyama, Tosihiko Hukuhara, *Nihon University, JAPAN*

Abstract: An entomogenous microsporidium, Microsporidium sp. Sd-NU-IW9069, which was isolated from the lawn grass cutworm, Spodoptera depravata, caused systemic infection in larvae of the silkworm, Bombyx mori. Spores purified from the larvae were primed with 0.2M KCl for hatching and combined with Spodoptera frugiperda (IPLB-Sf21AEU) cells that were cultured at 27 in IPL-41 insect medium enriched with 10% FBS. Sporoplasms and meronts were observed in the cultured cells within the first 48 hr post-inoculation (pi). Sporogonial stages were seen at 60 hr pi. Many-coiled type spores developed in infected cells at 120 hr pi. The change in the morphology of infected cells became conspicuous after 24 hr pi. Their cytoplasm was hypertrophied up to three times in diameter and their nuclei increased in number up to 10 per cell. Ultrathin sections revealed that the multinucleate cells were not syncytia but giant monocellular cells. Non-infected cells did not exhibit any of these changes. The proportion of infected cells did not change during 48-168 hr pi. but decreased thereafter due to the disintegration of the cytoplasm of infected cells. The results indicate that the microsporidian infection induces pronounced hypertrophy and extra karyokinesis in Spodoptera frugiperda cells.

CA-1 **TEMPERATURE AND THE NORTHERN RANGE OF PLASMODIUM VIVAX IN EUROPE**

Lena Huldén, *University of Helsinki, FINLAND*

Abstract: Endemic malaria (mostly Plasmodium vivax) was common in north Europe during the 19th century and earlier research has considered the summer-isoterm 16C as the north border for the disease. This study tests the correlation between temperature and endemic malaria in areas, where the summer-temperature was considerably lower and malaria a common disease. The historical material and the rare use of quinine made Finland 1800-1880 suitable for an analyze. Anopheles messae and A. beklemischevi are still common species in the whole country.
There are two sources for collecting the malaria data: the reports by district doctors and the records with the causes of deaths by the local ministers. Combined together they can be used for a statistical analyze of the local malaria epidemics. The oldest Finnish temperature series from Helsinki started in 1829. The temperature data used for the east of Finland is a series from St. Petersburg and for the west of Finland the temperature data from the means of Stockholm and Uppsala is used.
The historical data exhibited a distinct yearly cyclicity that was syncronous in the whole country. But no correlation between temperature and malaria cases was found. The temperature only occasionally rose to 16C during summer and then the anophelines only exist as larvae. The adult anophelines appear later, mate and then seek shelter in sheds and houses for hibernating. Plasmodium vivax was in northern conditions dependent of a vector that hibernated indoors.
Malaria conquered new areas during 1800-1880. It spread rapidly to the north and in Finland the east lakedistrict got infected, when it became economically important and the population there grew. The mechanism for the spreading of Plasmodium vivax was a complicated pattern of human and anophelian behavior. Big working sites brought together seasonal workers. Some of them had the disease and infected the anophelines during early autumn. The hibernating mosquitoes then infected the local house-

holds during the winter. Infected anophelines spread malaria in spring by biting uninfected day laborer, who would move on for work opportunities. Domestic anophelines in Finland still hibernate indoors in sheds. This study shows a further need for risk studies for airports, railwaystations and shopping centers. The combination of crowds together with hibernating anophelines and imported malaria can create risksituations and conditions parallell to the 19th century. The spreading of malaria in north Europe, where the vector is common, depended on the number of mosquitobites on man, not on the outside temperature.

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 10

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 2

Chair: R. de Maagd; D. Pauron

16:00 **STU**

PHAGE-DISPLAY PEPTIDES THAT BIND TO THE CRY11A TOXIN OR TO THE RECEPTOR, REVEALED AN IMPORTANT ROLE OF DOMAIN II REGIONS IN RECEPTOR INTERACTION AND TOXICITY TO AE. AEGYPTI

Luisa Elena Fernández-Altuna, *Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO*; Lorenzo Segovia, *Cellular Biology and Biocatalisis Department of the Instituto de Biotecnología, UNAM, MEXICO*; Oswaldo Lopez, *Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO*; Sarjeet Gill, *Cell Biology & Neuroscience of University of California-Riverside, USA*; Alejandra Bravo, Mario Soberón, *Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO*

Abstract: The mosquitocidal bacterium Bacillus thuringiensis subsp. israelensis (Bti) produces four major endotoxins proteins: Cry11Aa, Cry4A, Cry4B and Cyt1A; that show toxicity in the range of many synthetic chemical insecticides. Due to the emerge of insect-resistance to chemical insecticides Bti formulations are being used worldwide for mosquito control. Bti produce endotoxins as crystaliferous inclusion bodies during the sporulation. Once in the insect midgut occur the solubilization and the protoxins are activated through of intestinal proteases. The activated toxins bind to a receptor in the midgut epitelium and the conformational change in the toxin molecules triggers the insertion of their pore-forming domain into the membrane. The insertion in the membrane leads to osmotic unbalance and larval death. High specificity of the Bt toxins is determinate by the interaction between toxins and specific receptors on midgut epithelial cells of the target insects. For Cry1A lepidopteran toxins, two receptors have been characterized, a GPI-anchored aminopeptidase (APN) and a cadherin-like protein (Bt-R1). In the case of the mosquiticidal toxins Cry11A and Cry4A, the identity of the receptor molecules remains unknowns. Cry11A toxin is the most active Bti toxin against Ae. aegypti and the regions of the toxin involved in the interaction with the receptor are still unknown. Previous works in our laboratory demonstrated that phage display could be a powerful methodology to identify receptor binding epitopes of Cry toxins. In this work, we isolated random phage displaying peptides select that inhibited the interaction of the toxin with the receptor on midgut membrane vesicles (BBMV). Prediction of exposed amino acid regions on domain II revealed six putative exposed regions. One phage peptide that interfere the interaction of the toxin to the receptor bound to an exposed loop region in domain II. Heterologous competition experiments with the binding of Cry11A to BBMV using synthetic peptides corresponding to the exposed loop regions confirmed the role of this loop region in receptor interaction. Single point mutation of this region revealed important residues involved in receptor interaction. We identified a putative epitope in the domain II involved in the interaction between Cry11A and its receptor. Finally, one peptide-phage selected against Ae. aegypti BBMV and that interfere toxin receptor interaction was identified. This peptides-phage will be useful for identification of Cry11A receptor molecules. Progress on receptor interaction will be reported.

16:15 **STU**

ANALYSIS OF THE INTERACTION BETWEEN CRY11A AND CYT1A OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS: BIOLOGICAL ROLE IN SYNERGISM

Claudia Pérez, Luisa Fernández, *IBT-UNAM, MEXICO*; Sarjeet Gill, *University of Riverside, California, UNITED STATES*; Mario Soberón, Alejandra Bravo, *IBT-UNAM, MEXICO*

Abstract: The synergism between the Bti toxins is one of the most interesting molecular events in the study of the mode of action of Cry and Cyt proteins. Different groups have probed by bioassays against Culex spp., Aedes spp. and Anopheles spp. larvae, that these toxins increased their biological effect when they work together. Actually, the construction of recombinant bacteria and algae that expressed different Bti proteins has been increased in the last years. Moreover, the fact that mosquito resistance to Cry toxins is overcome by the presence of Cyt, gives a new and interesting vision about the potenciality of synergism for copping with insect resistance. However, there are no reports about the molecular mechanism of the synergism between Cry and Cyt toxins. The aim of this work is to study at the molecular level, the specific interaction between Cry and Cyt toxins. If we understand this, we could increased the effectiveness of Bti for the mosquito control and even used this information to make recombinant of Cyt that can help Cry toxins active against lepidopteran and coleopteran insects to increased their insecticidal capacity. In this work we demonstrate the interaction between Cry11A and Cyt1A toxins with different methods. We used ligand blot assays, binding assays of Cry and Cyt to BBMVs of A. aegypti in homologous and heterologous competitions, co-immunoprecipitation assays with specific antibodies and ELISA. We developed a Two-Hybrid System in yeast (S. cerevisiae) expressing different regions of Cry11A and Cyt1A proteins to identify the regions of interaction. Whit these results, we demonstrated that these toxins interact in their native and desnaturalized form. Finally, the interaction of specific fragments of Cyt1A toxin with domains II and III of Cry11A toxin observed in the two hybrid system, was confirmed by analyzing the binding of Cry11A toxin to peptide arrays of Cyt1A in nitrocellulose membranes. Our results represent the first study that analyze the molecular basis of synergism between Cry and Cyt toxins. We propose that Cry and Cyt toxins interact between them in a specific way, and the Cyt toxin could function as a Cry receptor in the mosquito midgut. These ideas could be important guidelines to establish a more strict and useful conditions to make recombinant strains of Bti that can be used in biological control with high success.

16:30

CHARACTERIZATION OF THE CELLULAR MODE OF ACTION OF THE BACILLUS SPHAERICUS BINARY TOXIN IN AN EPITHELIAL CELL LINE.

Yannick Pauchet, *INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux , FRANCE*; Frédéric Luton, *IPMC, CNRS-UMR 6097, FRANCE*; Claude Castella, *INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux , FRANCE*; Jean-François Charles, *Institut Pasteur, Unité de génétique des génomes bactériens, FRANCE*; David Pauron, *INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux , FRANCE*

Abstract: The Bacillus sphaericus binary toxin (Bin) is made of two subunits BinA and BinB and is a larvicidal agent used to control mosquito populations. The toxicity of Bin is linked to the presence of a receptor, named Cpm1, on the apical side of the midgut epithelial cells of Culex pipiens larvae. Cpm1 is anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) and displays an alpha-glucosidase activity (Darboux et al., 2002). To get clues to the mode of action of Bin, we have transfected the mammalian cell line MDCK with the Cpm1 cDNA. We isolated clonal cell lines which can reconstitute an epithelium in vitro when cultured in adequate conditions. Though, by expressing Cpm1 in those cells, we aimed to mimic the interaction between the toxin and the mosquito larval midgut epithelium. Immunoblot analysis performed with an anti-Cpm1 polyclonal antibody showed that Cpm1 expressed in MDCK cells has a molecular weight of about 66 kDa. When transfected cells were treated with phosphatidylinositol-specific phospholipase C, the recombinant Cpm1 was released in the culture medium which indicated its anchorage by a GPI. By immunofluorescence, Cpm1 was detected on the apical side of polarized MDCK cells. Binding experiments performed with [125I]-Bin showed that membranes prepared from MDCK cells expressing Cpm1 bound the toxin with a Kd value similar to the value reported for the native receptor expressed in C. pipiens midgut brush border membranes or expressed in lepidopteran Sf9 cells. These data demonstrate that Cpm1 expressed in MDCK cells is functionally active. Large vacuoles appeared in Cpm1-MDCK cells treated with 50 nM of trypsin-activated Bin but no cell lysis was observed which is consistent with previous observations reported on the midgut cells of Culex pipiens larvae fed with Bin (Charles et al, 1987). Confocal microscopy experiments done with fluorescently-labelled derivatives of BinA and BinB showed that, after binding to the apical side of polarized Cpm1-MDCK cells, both subunits entered the cells and aggregated in specific compartments which are currently under investigation.

16:45 **UNFOLDING EVENTS IN THE MONOMERIC CRY1AB TOXIN DURING TRANSITION TO MEMBRANE INSERTED OLIGOMERIC PORE: DOMAIN I IS THE ONLY INTEGRAL MEMBRANE DOMAIN**

Carolina Rausell, *Instituto de Biotecnología. Universidad Nacional Autónoma de Mexico, MEXICO*; Jorge Sanchez, Carlos Munoz-Garay, Claudia Morera, Mario Soberón, Alejandra Bravo, *Instituto de Biotecnología. Universidad Nacional Autónoma de Mexico., MEXICO*

Abstract: The primary action of Cry toxins is to lyse midgut epithelial cells in the target insect by forming lytic pores on the apical membrane. In order to exert their toxic effect, it is required a transition from crystal inclusion protoxins to membrane inserted oligomeric channels. In the case of Cry1A toxins we have shown that oligomerization occurs after BT-R1 receptor binding and before membrane insertion. Upon oligomerization, Cry1Ab toxin undergoes conformational changes that leads to rearrangement of Trp side chains reducing the accessibility of Trp residues to soluble quenchers. Upon membrane penetration a second conformational change occurs favouring that Trp residues come in close contact with the membrane and anchoring the pre-pore to the lipid bilayer. Insertion into the membrane is the limiting step in other pore-forming toxins. It has been proposed that proteins must partially unfold to facilitate membrane insertion and channel formation. In most cases, unfolding is triggered by acidic pH. In this work, we analyzed the stability of the monomeric Cry1Ab toxin, the oligomeric pre-pore in solution and the membrane inserted pore at different pHs. Equilibrium unfolding was induced by urea and monitored by measuring the intrinsic tryptophan fluorescence of the protein. At pH 11 all toxin structures became less stable than at acidic pH, showing high denaturation at lower urea concentrations. These data could be correlated with the conditions that Cry1 toxins would encounter in vivo inside the midgut lumen of the larvae which in the case of Lepidopteran insects is highly alkaline Our data show that the pre-pore complex and membrane inserted pore has different conformations since they displayed different unfolding patterns by changes in pH. We also analyzed the thermal denaturation of the monomeric and oligomeric structures of Cry1Ab by monitoring ANS binding to hydrophobic regions exposed in partially unfolded proteins and by analysing the energy transfer of Trp residues to ANS bound to the unfolded protein. Our data show that in the membrane inserted pore the domains II and III are highly sensitive to heat denaturation in contrast to domain I that remains folded, suggesting that only domain I is protected by the membrane. x

17:00 **CLONING AND EXPRESSION ANALYSIS OF GENES INVOLVED IN INSECT RESPONSE TO BACILLUS THURINGIENSIS TOXINS**

Salvador Herrero, *Laboratory of Virology, Wageningen University and Plant Research International, Wageningen, THE NETHERLANDS*; Tsanko Gechev, Petra L. Bakker, *Plant Research International, Wageningen, THE NETHERLANDS*; William Moar, *Department of Entomology and Plant Pathology, Auburn University, USA*; Just M. Vlak, Monique M. Van Oers, *2Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Ruud De Maagd, *Plant Research International, Wageningen, THE NETHERLANDS*

Abstract: The response of insects to a sublethal dose of Bacillus thuringiensis (Bt) toxin involves a change in the expression of a large number of genes. Understanding the reaction of the insect to pathogen or toxin attack and knowing which of the changes can be responsible for the development of resistance, might improve the efficacy of Bt-based products. Additionally, knowing the genes responsible for resistance could make it possible to detect resistance in a population at an early stage before it becomes wide-spread and may implement tactics to delay its development. Suppression Subtractive Hybridization (SSH) was used to make cDNA libraries of genes that are up- or down-regulated (as compared to controls) in the midgut of last instar larvae of the beet armyworm, Spodoptera exigua, when exposed to Bacillus thuringiensis Cry1Ca toxin. A similar approach was employed to compare gene expression between toxin susceptible and resistant insects. Several clones of these libraries were selected by their homology to genes that could be involved in the mode of action of Cry1Ca or in the insect response to this toxin. These clones included fragments from four different midgut aminopeptidases and several members of the serine protease family. Full-length sequences of the selected clones were obtained using RACE-PCR and hypothetical protein sequences were compared with members of the same family in other lepidoptera. Northern blot analyses were performed to study and compare the expression level of these genes in different developmental stages and tissues. Expression levels of these genes were also compared among different populations of S. exigua, one of them selected for resistance to Cry1C toxin and another selected for resistance to a Bt-based product such as XenTari.

17:15 **GALLERIA MELLONELLA LARVAE AS A MODEL FOR INTESTINAL INFECTIONS: BACTERIAL LOCALIZATION AND VIRULENCE GENE EXPRESSION**

Christina Nielsen-LeRoux, *Unité Génétique Mirobienne et Environnement, INRA, la Minière, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*; Myriam Hajaij, Christophe Buisson, Patricia Nel, Elisabeth Guillemet, *Unité Génétique Mirobienne et Environnement, INRA, la Minière, FRANCE*; Laurence Fiette, *Unité d'Histotechnologie et Pathologie, Institut Pasteur, FRANCE*; Didier Lereclus, *Unité Génétique Mirobienne et Environnement, INRA, la Minière, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE*

Abstract: Even though Cry toxins are the major insecticidal factors of Bacillus thuringiensis (Bt), by oral infection, other compounds are also important. Galleria mellonella larvae is used as a model to study the effect of Bt virulence factors different from Cry toxins. The importance of the Bt pleiotropic PlcR regulator was demonstrated by reduced mortality in Galleria mellonella of spores from a Bt 407 cry- plcR mutant, infected orally by force-feeding 5 instars with spores or vegetative cells combined with a sub lethal dose of Cry1C toxin. (Salamitou et al., 2000). PlcR governs many putative virulence factors (phospholipases, entero toxins, hemolysins, proteases etc.) Recently a putative zinc protease InhA2 was discovered to be important for pathogenesis via the oral route, probably interfering with peritrophic membrane and /or intestinal midgut cells (Fedhila et 2003 and abstract by Hajaij M. this volume). Among other possible PlcR regulated genes and with putative impact on these intestinal barriers, we investigated a new gene (mpbe, metalloprotease bacillus enhancin) encoding a protein with homology to Enhancin of T.ni GV baculovirus, having a peritrophic membrane protein as target. Results from molecular characterization, in vitro and in vivo gene expression and gene interruptions will be shown. In order to investigate on the localization of Bt cells during the various steps conducting to septicemia and to identify at which levels attenuated mutants are blocked. We have visualized the infection process of Bt wild type and different mutants. Plasmid born transcriptional fusion was made between the gfp (green fluorescence protein) gene and promoters from either various Bt genes (plcA, mpbE and cry1) or the constitutive expressed Apha3 promoter. Bt 407 cry- wild type, PlcR, InhA2 and the flagella (FlhA) mutants are transformed with these plasmids and spores or vegetative cells are used for force-feeding. Infection kinetics and gene expression are recorded by fluorescence microscopy and by histopathological observations. For instance, Bt 407 cry- wild type colonized mainly posterior midgut cells, necrosis is observed and hemolymphe infection is probably processed from that site. PlcR and FlhA mutants were found to develop in the intestinal lumen, but did not cross the midgut cells, while no clear differences were found for the InhA2 and the MbpE mutants compared to the wild type. Salamitou S. et al., 2000 The regulon PlcR is involved in the opportunistic properties of Bacillus thuringiensis and Bacillus cereus in mice and insects. Microbiology 146 :2825-2832 Fedhila, S., Gohar, M., Slamti, L., Nel, P., Lereclus,D., 2003. The Bacillus thuringiensis PlcR-regulated gene inhA2 is necessary, but not sufficient, for virulence. J. Bacteriol. 185 :2820-2825.

17:30 **IN VIVO INDUCTION OF APOPTOSIS BY XENORHABDUS NEMATOPHILA IN INSECT PHAGOCYTES**

Fabienne Vigneux, Carlos Ribeiro, *Laboratoire d'Ecologie Microbienne des Insectes Interactions Hôtes-Pathogènes UMR 1133 INRA-Université de Montpellier II, 34090 Montpellier, FRANCE*; Stephen Baghdiguian, *Institut des Sciences de l'Evolution, UMR 5554, Université de Montpellier II, 34090 Montpellier, FRANCE*; Michel Brehélin, *Laboratoire d'Ecologie Microbienne des Insectes Interactions Hôtes-Pathogènes UMR 1133 INRA-Université de Montpellier II, 34090 Montpellier, FRANCE*

Abstract: Xenorhabdus nematophila is a Gram negative bacterium belonging to the family of Enterobacteriaceae, symbiotically associated with the nematode Steinernema carpocapsae (Steinernematidae). This symbiotic bacterial helminthic couple is pathogenic for numerous insect species. In experimental infestations X. nematophila by itself is also highly pathogenic for numerous insects. It is able to escape defence reactions, especially phagocytosis, to kill insect and to colonize all the insect tissues. Therefore, bacterial virulence factors and their action modalities are studied in different laboratories. The alpha-Xenorhabdolysin (alpha-X), a toxin with cytolytic activity on insect hemocytes (=immunocytes), has been purified from X. nematophila 24h-old nutritive broth-growth. The alpha-X is under the control of flhDC, the flagellar master operon of X. nematophila. Insertional inactivation of this gene leads to loss the alpha-X activity to a very attenuated virulence phenotype. Granulocytes, which are the functional equivalent of vertebrate macrophages, were the most sensitive hemocytes to alpha-X. The first target of the toxin is the plasma membrane where channels selective for small cations are induced. Then en-

doplasmic reticulum vacuolation and cell swelling occur before cell death. The size of channels built in macrophage plasma membrane, increases with toxin concentration which leads to a rapid cell lysis. When tested at doses which do not trigger cell necrosis, we have observed that alpha-X seems to elicit apoptosis. So, different biochemical, histological and cytological approaches were used to characterize this apoptosis in vitro after incubation of hemocyte monolayers with purified alpha-X . We also show that after larval infestation with X. nematophila, apoptosis of granulocytes was also elicited in vivo. This work gives new insights in the understanding of the toxic activity of alpha-X and moreover in the study of the bacteria-host relationships.

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Contributed Papers (Division of Fungi)

fungi / contributed paper session 3

Chair: Judith Pell; Ingeborg Klingen

16:00 **THE ABILITY OF FUNGAL INFECTED APHIDS TO PRODUCE AND RESPOND TO ALARM PHEROMONE**

Helen Roy, *Anglia Polytechnic University, UK*; Jason Baverstock, Keith Chamberlain, Judith K. Pell, *Rothamsted Research, UK*

Abstract: The effect of infection by an aphid specific (Pandora neoaphidis) and a generalist (Beauveria bassiana) fungal pathogen on the alarm response of aphids was investigated. The response of pea aphids, Acyrthosiphon pisum, to the aphid alarm pheromone (E)-Beta-farnesene (EBF) was not modified by infection with B. bassiana. Approximately 50 % of aphids responded to synthetic alarm pheromone. These results were further supported by observations of the response of settled uninfected and B. bassiana-infected aphids to the simulated attack (aphid squeezed until death) on an adjacent uninfected aphid. Air entrainments of both uninfected aphids and aphids at different stages of B. bassiana infection demonstrated that B. bassiana infected aphids produced less alarm pheromone than uninfected aphids and that this reduction was apparent at an early stage of infection. This finding was supported by subsequent behavioural experiments involving the response of uninfected aphids to the simulated attack of B. bassiana-infected aphids. In contrast, it was apparent from air entrainment that pea aphids infected with P. neoaphidis showed an increase in production of alarm pheromone supporting previous behavioural observations. Both B. bassiana and P. neoaphidis-infected pea aphids showed a dramatic increase in alarm production just prior to conidiation. These results are discussed with particular emphasis on the different life history strategies of these two pathogens. We hypothesise that the obligate, specialist pathogen, P. neoaphidis, is under greater selection pressure resulting in modified host behaviour to increase pathogen transmission and survival, than the generalist pathogen, B. bassiana.

16:15 **PREVALENCE OF INSECT PATHOGENIC FUNGI AND PARASITOIDS ON THE BLACK CHERRY APHID, MYZUS CERASI**

Ingeborg Klingen, Karin Westrum, *The Norwegian Crop Research Institute, Plant Protection Centre, NORWAY*; Gunnhild Jaastad, *The Norwegian Crop Research Institute, Ullensvang Research Centre, NORWAY*

Abstract: Both insect pathogenic fungi and parasitoids are important for the regulation of insect pests in organic and integrated fruit production. In perennial crop systems, where the pest spends significant periods of time in permanent habitats, biological control is often successful. This is in part due to the stable and robust perennial ecosystem that acts as a reservoir for insect pathogens, parasitoids and other natural enemies of pests. Black cherry aphid (Myzus cerasi) is one of the most important pests on cherries all over the world. However, there are few studies on M. cerasi and insect pathogenic fungi. In this study the occurrence and importance of insect pathogenic fungi and parasitoids as natural enemies of the black cherry aphid was investigated throughout two successive seasons in Norway. Results show that in the first part of the season, from the last part of May to mid July, mostly parasitoids were found in dead aphids. Parasitoids of the following species were found: Ephedrus plagiator and E. persicae. The hyperparasitoids Alloxystra victrix and Dendrocerus sp. were also found in this part of the season, and a few individuals infected with fungi in the order Hyphomycetes (Verticillium lecanii). From the middle of July, fungi in the order Entomophthorales (Entomphthora planchoniana, Erynia neoaphidis and Conidiobolus obscurus) were found in dead aphids. Number of aphids killed by fungi increased towards the end of July. There was, however, a big variation in infection level between trees.

16:30 **STU** **INTRAGUILD INTERACTIONS BETWEEN THE APHID PATHOGEN PANDORA NEOAPHIDIS AND THE PARASITOID APHIDIUS ERVI: IMPLICATIONS FOR MULTI-SPECIES BIOCONTROL**

Jason Baverstock, *Plant and Invertebrate Ecology Division, Rothamsted Research, Division of Agricultural Sciences, The University of Nottingham, UNITED KINGDOM*; P. G. Alderson, *Division of Agricultural Sciences, The University of Nottingham, UNITED KINGDOM*; Judith K. Pell, *Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM*

Abstract: Intraguild interactions occur between species that occupy the same trophic level and compete for similar prey/ hosts. These interactions may have positive or negative effects on the fitness of the species involved and need to be assessed when designing multi-species biocontrol programmes. The aphid-specific pathogenic fungus Pandora neoaphidis and the parasitoid Aphidius ervi are both natural enemies of the aphid Acyrthosiphon pisum, requiring successful parasitisation of an aphid-host to complete their life-cycle. The success rate of parasitoid introductions for the biological control of herbivorous pests is low, and this is thought to be due to an over-estimation in the top-down pressure applied by parasitoids. Greater control may be achieved using multiple natural enemy species. Therefore, for P. neoaphidis and A. ervi to be effective multi-species biocontrol agents they must co-exist spatially. However, coexistence may result in intraguild competition and therefore a net-decrease in the ability of the natural enemy species to control the herbivorous pest. Here we investigated whether A. ervi enters and forages in aphid colonies containing P. neoaphidis and indirectly assessed the effect of competition for hosts on the population sizes of both the parasitoid and the fungus. Laboratory based experiments indicated that the presence of P. neoaphidis-sporulating cadavers did not affect the entry rate of A. ervi into aphid colonies nor did they affect the foraging behaviour of the parasitoid once the aphid colony had been entered. Aphidius ervi attempted to oviposit in P. neoaphidis-infected aphids but was unable to out-compete the fungus. Subsequent polytunnel experiments confirmed the findings of the laboratory based experiments at a larger spatial scale, and indicated that over a single generation of the parasitoid, P. neoaphidis and A. ervi could co-exist. These results suggest that P. neoaphidis and A. ervi may be effective multi-species biocontrol agents. Experiments designed to assess the outcome of the P. neoaphidis-A. ervi interactions on the population size of both the natural enemies and the pest population over several generations of the parasitoid and fungus are currently in progress.

16:45 **THE RELATIONSHIP OF NUMBER OF CONIDIA, MOLTING AND INSECT DEVELOPMENTAL STAGE TO SUSCEPTIBILITY OF COTTON APHID, APHIS GOSSYPII, TO THE FUNGUS VERTICILLIUM LECANII**

Jeong Jun Kim, Dae Joon Im, *Division of Entomology, NIAST, RDA, KOREA*; Kyu Chin Kim, *Dept. Agrobiology, Chonnam National University, KOREA*; Dong Ro Choi, *Division of Entomology, NIAST, RDA, KOREA*; Donald Roberts, *Dept. Biology, Utah State University, USA*

Abstract: Aphids are some of the most serious pests of greenhouse vegetables in the world. Verticillium lecanii has high virulence to aphids and whiteflies and is under consideration as a microbial control agent. An isolate from Korea, V. lecanii CS625, is being evaluated as a mycopesticide for control cotton aphid, A. gossypii, in Korean greenhouses. A study was conducted to understand the influence of molting on mortality of aphids at various developmental stages. Mortality of cotton aphid inoculated with V. lecanii CS625 varied with the developmental stage of the host. LT50 in 3rd instar nymphs and adults was shorter than in 1st instar nymphs. The number of spores attached to the surface of 1st instar nymphs was approximately one half of that on 3rd instar nymphs and adults. Also, rates of spore germination on the surface of 1st instar nymphs were lower than on the surface of other stages of the aphid. The difference in spore germination on the different developmental stages may be related to differences in cuticle composition, especially lipids, of each stage (no data). Molting removed conidia from the host body. After molting, the numbers of conidia attached to insect cuticles and to exuviae, respectively, were significantly different. The results suggest that early nymphal stages of aphids may escape fungal disease by molting quickly and the ecdyses remove conidia from the body before they can penetrate the host. Accordingly, low mortality in 1st instar nymphs was due to three factors: low numbers of attached conidia, low germination rates and rapid ecdysis. In addition, spore sprays influenced the biology of cotton aphid. Both fecundity and longevity were significantly reduced following treatment with conidial suspensions. Also, nymphal development of cotton aphid was slightly slower after spraying with low concentrations of conidia.

17:00 **RECENT RESEARCH ON FUNGOUS PATHOGENS OF MITES (ACARI) IN POLAND**

Cezary Tkaczuk, Ryszard Miêtkiewski, *University of Podlasie, POLAND*; Stanislaw Balazy, *Research Centre for Agricultural and Forest Environment, POLAND*

Abstract: First cases of mite mycoses caused by Hirsutella modulosa and Verticillium eriophytis on Dendrolaelaps and uropodid species in Poland were recorded in 1970s. A considerable number of mycosed individuals from numerous samples of Agricultural University and Research Centre for Agricultural and Forest Environment (RCAFE) in Poznañ, collected in different habitats, were investigated in 1980s and about 40 species of fungi showing signs of pathogenic character were encountered. Apart from 12 species belonging to commonly known entomopathogenic taxa (the genera Beauveria, Hirsutella, Paecilomyces, prostrate forms of Verticillium sensu W. Gams, Conidiobolus and Pandora), about 20 different resting spore forms were found and identified or described under the generic-form name Tarichium, counted into the order Entomophthorales. More extensive studies were undertaken in 1990s by the University of Podlasie in co-operation with RCAFE on mycoses of phytophagous mites of the families Eriophyidae Tarsonemidae and Tetranychidae. Among the mycopathogens of Abacarus hystrix on grasses Hirsutella thompsonii and H. kirchneri dominated with the accompanying H. gregis, H. necatrix and one yet unidentified species. The mean host mortality caused by them in late summer and autumn reach 25-30%, with not rare local cases to overstep 50%. H. thompsonii var. synnematosa occurs regularly in Eriophyes piri on pear leaves and H. nodulosa together with H. kirchneri on Phytonemus pallidus ssp. fragariae in strawberry plantations. High autumnal mortality of Tetranychus urticae, caused by Neozygites floridiana occurs almost every year in gardens and this pathogen also infects Bryobia species. A new entomophthoralean pathogen of eriophyids A. hystrix named Neozygites abacaridis was described and hibernating phases of aforementioned pathogens were recognised. Continued researches on predacious or saprophagous mites accompanying subcortical insects, decayed wood and forest litter allow to suppose that the species Hirsutella brownorum, H. rostrata, H. haptospora, Lecanicillium-like forms, Neozygites species and Conidiobolus coronatus are the most common pathogens of mites, but dead individuals filled with unidentified Chytridiomycota-like structures were found,too.

17:15 **NEOZYGITES FLORIDANA KILLING TETRANYCHUS URTICAE IN STRAWBERRIES AND THE INFLUENCE OF MANAGEMENT SYSTEM**

Ingeborg Klingen, Nina Trandem, *The Norwegian Crop Research Institute, Plant Protection Centre, NORWAY*

Abstract: Neozygites floridana is a fungus in the order Entomophthorales that infects and kills the two-spotted spider mite, Tetranychus urticae. N. floridana may be responsible for abrupt population declines frequently observed in T. urticae populations on maize and soybean in the mid-western and south-eastern USA where farmers are encouraged to adapt their fungicide spray programmes to avoid suppressing the fungus. To our knowledge few systematic studies have been conducted on N. floridana as a mortality factor of T. urticae in strawberry fields. Some preliminary studies have, however, been conducted in Poland which indicate that N. floridana might be important for the regulation of T. urticae in strawberry. A cropping system that enhances the prevalence of N. floridana might be important for the reduction of T. urticae in strawberry. In our studies we therefore aim to clarify the effect of different strawberry growing systems and pesticides on the infection level and killing capacity of N. floridana. In one study, the occurrence of N. floridana in T. urticae in organic and conventional strawberry fields was compared in the summer 2002 and 2003. Strawberry leaves were collected from two fields, one organic and one conventional, from each of six different locations in Norway. Leaf samples were collected twice: First sampling was in June/July and the second in July/August. Preliminary results show that N. floridana infected and killed T. urticae in all strawberry fields studied. Infections from 0 to 19% were registered, and the highest infection rates were observed in the second sampling. A preliminary analysis do not show any relationship between occurrence of N. floridana and strawberry cropping systems. In a second study the aim was to clarify how commonly used pesticides in strawberries affect the killing capacity of N. floridana to T. urticae. A pilot laboratory study was conducted with three fungicides; Euparen (tolylfluanid), Teldor (fenhexamide), Switch (cyprodinil +fludioksinil) and one acaricide: Mesurol (mercaptodimethur/methiocarb). The results indicated that both Teldor and Mesurol did affect the N. floridana killing capacity, while Euparen and Switch did not have such an effect.

17:30 **USE OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA FOR BIOCONTROL OF IXODIDAE TICK SPECIES**

Greg Westwood, Brett Kirkland, Eun-Min Cho, Nemat Keyhani, *University of Florida, U.S.*

Abstract: Nymph and adult ticks from five different tick species; Dermacentor variabilis, Ixodes scapularis, Rhipicephalus sanguineus, Amblyomma maculatum, and A. americanum were treated with conidia and blastospores of the entomopathogenic fungus Beauveria bassiana (Bals.) Vuill. Dose response experiments indicated that a critical concentration of fungal spores was required for infection and mortality. Over a 28 d time course, fungal suspensions of either B. bassiana conidia or blastospores (108 conidia/ml) resulted in 50-70% mortality in adult I. scapularis and R. sanguineus, greater than 90% mortality in A. maculatum, but less than 20% mortality in D. variabilis and A. americanum ticks. A. maculatum and R. sanguineus nymphs were highly susceptible to B. bassiana, displaying greater than 60% mortality within 14 d post-infection and >90% mortality within 21-28 d post-infection. D. variabilis and A. americanum nymphs were also more susceptible than their corresponding adults, displaying mortalities ranging from 20-40% 28 d post-infection. I. scapularis nymphs, on the other hand, appeared to be slightly less susceptible than adults (45% mortality, 28 d post-infection). The addition of nutrients to fungal cell suspensions did not have any noticeable effects on mortality towards any of the tick species tested. Significant mortality against D. variabilis and A. americanum adults (60-80%) was noted only when B. bassiana fungal cells with growth media carryover were used as the inoculum against the ticks. The entomopathogenic fungus B. bassiana may have the potential for controlling populations of I. scapularis, R. sanguineus, and A. maculatum, and under certain conditions D. variabilis and A. americanum.

17:45 **INVESTIGATIONS OF COLORADO POTATO BEETLE MORTALITY FOLLOWING FOLIAR**

Stephen P. Wraight, Mark E. Ramos, *USDA-ARS, U.S. Plant, Soil and Nutrition Laboratory, U.S.A.*

Abstract: Beauveria bassiana (Bb) has the potential to kill Colorado potato beetle (CPB) larvae within a few days after infection. However, under suboptimal conditions, (e.g., during hot weather), pathogenesis is slowed, allowing the beetles to complete larval development and enter the soil to pupate. Little is known about the fate of these larvae. A field population of CPB was exposed to sprays of Bb strain GHA (all sprays applied at the rate of 2.5 x 1013 conidia/ha). Treatments (5 replicates in an RCB design) included a series of 4 sprays at 3-4 day intervals targeting early instars (4X treatments), a single spray targeting large larvae (1X treatments), and a carrier control. Larval populations were sampled until development was completed; mature larvae were collected immediately as they dropped to the ground and placed in soil cages in the various treatment plots (20/cage). Cages consisted of plastic pots (25 cm diam. x 24 cm deep) with screened bottoms. These were embedded in the soil of each plot and filled to field level with soil prior to initiation of the sprays; they were left uncovered during all treatments. Results revealed 1) 22% mortality among larvae taken from control plots and placed in soil cages in control plots; 2) 1617% mortality among larvae taken from control plots and placed in soil in 1X or 4X treatment plots; 3) 3738% mortality among larvae taken from either the 1X or 4X plots and placed in control soil; 4) 50% mortality among larvae taken from 1X plots and placed in 1X-treated soil; and 5) 51% mortality among larvae taken from 4X plots and placed in 4X soil. Counts of larvae prior to drop indicated 0% and 31% reductions in larval numbers relative to controls in the 1X and 4X plots, respectively. Counts of emerged adults feeding in the plots ultimately indicated 50% and 70% reductions in numbers of 1X and 4X treated beetles, respectively. These results showed that mortality in the soil was due primarily to infections acquired from the foliar sprays, but that significant additional infection occurred when previously-treated larvae were exposed to treated soil, and that sprays against large larvae were more efficient than sprays against small larvae. Implications of the results with respect to CPB management will be discussed.

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Contributed Papers (Division of Nematodes)

nematodes / contributed paper session 1

Chair: Holger Philipsen; Rob van Tol

16:00 **GENETIC IMPROVEMENT FOR PREVENTION OF BENEFICIAL TRAIT DETERIORATION IN HETERORHABDITIS BACTERIOPHORA THROUGH CREATION OF INBRED LINES**

Cheng Bai, David I. Shapiro-Ilan, *USDA-ARS, SAA, U.S.A.*; Randy Gaugler, *Dept. Entomology, Rutgers University, U.S.A.*; Keith R. Hopper, *USDA-ARS, U.S.A.*

Abstract: Continuous subculturing of organisms used for biological pest suppression can lead to detrimental genetic changes and loss of utility. In this study, we demonstrated that genetically homozygous inbred lines deter beneficial trait loss in the entomopathogenic nematode, Heterorhabditis bacteriophora. We created 22 inbred lines from a genetically diverse foundation strain. Three inbred lines and the foundation strain were repeatedly subcultured in the insect host, Galleria mellonella. Trait stability was evaluated after 6, 11, and 16 in vivo passages by comparing subcultured populations and non-subcultured (or minimally subcultured) populations. Subculturing of the foundation strain resulted in more than a 30% loss in traits deemed beneficial for biological pest suppression i.e., virulence to an insect host (Diaprepes abbreviatus), reproductive capacity, heat tolerance (at 38oC), and host seeking ability. In contrast the three inbred lines were impervious to detectable changes in all beneficial traits. Creation of inbred lines maybe a useful tool in maintaining beneficial traits of other biological pest control agents, and other organisms that are routinely subcultured.

16:15 **PLANTS PROTECT THEIR ROOTS BY ALERTING THE ENEMIES OF GRUBS**

Rob Van Tol, Marleen Riemens, Frans Zoon, *Plant Research International, P.O. Box 16, 6700 AA Wageningen, NETHERLANDS*

Abstract: Although many ecologists are aware of the presence and importance of natural enemies in the soil that protect the plants from herbivores, the existence and nature of tritrophic interactions are poorly understood. So far, attention has focused on how plants protect their above-ground parts against herbivorous arthropods, either directly or indirectly (i.e. by getting help from the herbivore's enemies). Recently we provided the first evidence that indirect plant defences also operate underground. Chemicals released from the roots of Thuja occidentalis and Taxus baccata when attacked by weevil larvae Otiorhynchus sulcatus attracted entomopathogenic nematodes Heterorhabditis megidis.

The transport of these chemicals from the producer to the receiver in soil is a highly complicated process depending on many, often interdependent, biotic and abiotic factors. Transport of chemicals through soil may take place through diffusion and convection in the gaseous and water phases. This transport will be influenced by the partitioning of the chemicals between air, water, organic matter and other phases and also by adsorption to surfaces such as the mineral surface. Our recent results suggest that the attractive plant odour probably reach the nematode's sensory organs by other routes than air.

In addition, the chemicals may be transformed through abiotic and biotic processes. The transformations may lower the activity of the compounds but may also result in more potent structures, or in higher compound mobilities. All of these processes depend on factors such as acidity, presence of organic and inorganic molecules (nutrients), temperature and so on, all of which may have a spatial and temporal variation.

Next to identification our research focuses on the environmental chemistry of these signalling root exudates in order to understand their routes and fate in the soil between emitter and receiver. Concepts from environmental chemistry are introduced in chemical ecology, thereby expanding and renewing our understanding of the role of infochemicals in natural ecosystems.

16:30 **CROP INFLUENCE ON THE ABUNDANCE OF STEINERNEMA FELTIAE**

Holger Philipsen, *Department of Ecology, The Roytal Veterinary and Agricultural University, DENMARK*; Otto Nielsen, *Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK*

Abstract: The influence crops on the abundance of Steinernema feltiae Filipjev (Rhabditida: Steinernematidae) was studied on an experimental site at the university. Crops included were barley, oil seed rape, pea, red clover, white clover, ryegrass and chicory. Over a period of three years the chosen crops were grown in plots with a size of 100 m2 or more as main crop, cover crop or catch crop. During growing seasons soil samples were collected at different times to obtain information on incidence of Steinernema feltiae using bait techniques. Occurrence of insects in the crops were monitored during growing seasons, either by collecting insects falling from the plants or by taking soil samples from crops with populations of soil dwelling insect species. It was found that the different crops had different effects on populations of S. feltiae. A positive effect was observed after having grown oil seed rape, pea and clover. That effect can probably be explained by the relative higher numbers of insect host availability in those crops.

16:45 **ESTABLISHMENT AND PERSISTENCE OF ENTOMOPATHOGENIC NEMATODES IN CONVENTIONAL AND ORGANIC AGRICULTURE**

Alper Susurluk, Ralf-Udo Ehlers, *Institute for Phytopathology, Department of Biotechnology and Biological Control, Christian-Albrechts-University Kiel, GERMANY*

Abstract: Entomopathogenic nematodes (EPNs) (Heterorhabditis bacteriophora and Steinernema feltiae) were applied at a dose of 0.5 million/m with a sprayer on different crops in fields of a conventional and organic farm. All environmental data related with application areas were documented at that time of the application. Before the application of EPNs, the soil samples were collected from the target fields to check for natural occurrence of EPNs. After application, soil samples of 2 cm diameter and 10 cm depth were taken every month. From each field, about 50 soil samples/month were collected. EPN were trapped with 2 Galleria mellonella larvae at 25 degrees Celcius for 3 days. This procedure was repeated twice. During the application petri dishes were put on the soil (5 meter intervals) and the quality of EPNs was tested immediately after spraying. It was detected that the plant cover had an effect on establishment and persistence of EPNs in the soil. H. bacteriophora was established successfully on lupine (Lupinus luteus) and on pea (Pisum sativum) in organic fields, but was not recovered from treated potato (Solanum tuberosum) fields a month after application. In conventional fields the rate of positive soil samples with H. bacteriophora decreased from 70% to 10% in clover (Trifolium campestre) during the first month. In wheat (Aestivum sativum) and oil seed rape (Brassica napus) between 20 and 30% of the samples were positive 3 months after application. Any kind of tillage reduced the occurrence of EPNs. The impact of potential host insects on the persitence is discussed. In organic field, the presence of bean weevils (Sitona lineatus) probably enhanced establishment and persistence of H. bacteriophora. Establishment of H. bacteriophora had no negative effect on endemic populations of S. feltiae.

17:00 **INTERACTIONS BETWEEN FUSARIUM OXYSPORUM F. SP. ASPARAGI (ASCOMYCOTA: PYRENOMYCETES) AND HETERORHABDITIS CABORCA STRAIN (HETERORHABDITIDAE) IN GALLERIA MELLONELLA LARVAE**

Jennifer Bauman, *Department of Plant Sciences. University of Arizona. Tucson AZ 85721-0036, USA, USA*; Benjamin Rivera-Orduño, *División de Ciencias Administrativas, Contables y Agrarias, Universidad de Sonora, Santa Ana, Sonora, MEXICO*; S. Patricia Stock, *Department of Plant Sciences. University of Arizona. Tucson AZ 85721-0036, USA, USA*

Abstract: The entomopathogenic nematode Heterorhabditis sp (Caborca strain) is a native pathogen of nymphs of Diceroprocta ornea (Homoptera: Cicadidae) in the state of Sonora Mexico. Cicadas have become a major pest of asparagus in this region and the isolation of natural occurring entomopathogenic nematodes offers great potential for controlling these insect pests. An augmentative biological control approach, consisting of increasing natural population levels of this nematode could be an effective management strategy. However, approximately 70% of the cicadas collected from these fields have been found to be infested with Fusarium oxyxporum f.sp. asparagi, a soilborne fungus highly pathogenic to asparagus. We hypothesize that F. oxysporum might affect the success of Heterorhabditis in controlling the cicada populations in this system. To address this question, laboratory experiments were conducted to evaluate the infectivity and reproductive success of Heterorhabditis sp (Caborca strain) in the presence of F. oxysporum, using G. mellonella larvae as a host. Treatments considered two F. oxysporum inocula (100 and 1,000 spores/insect) and three F. oxysporum + Heterorhabditis application times: simultaneous, prior to, and after nematode inoculation. Preliminary data indicated that nematode infectivity (IJ penetration and establishment) and reproduction were reduced by the presence of F. oxyxporum, with prior and simultaneous fungus exposure treatments having significant effects on both nematode infectivity and reproduction. These results suggest that the presence of F. oxyxporum in the soil affects the success of the native Heterorhabditis to naturally control the cicada population. However other biotic and abiotic factors might also influence the dispersal and success of this nematode in the field. Further studies are currently being conducted to evaluate these parameters.

17:15 **CONTROL OF PLUTELLA XYLOSTELLA USING NOVEL FORMULATION TECHNIQUES TO IMPROVE PERFORMANCE OF ENTOMOPATHOGENIC NEMATODES ON THE FOLIAGE**

Sibylle Schroer, Ralf-Udo Ehlers, *Institute for Phytopathology, Dept. Biotechnology & Biol. Control, Christian-Albrechts-University Kiel, GERMANY*

Abstract: In the past decades the Diamondback moth (DBM), Plutella xylostella, developed resistance against every insecticide applied on Brassica crops world-wide. In 2001 an EU funded project (DIABOLO) started with the objective to manage resistance in DBM populations and to support natural antagonists. Novel integrative biological strategies are tested in China and Indonesia. One particular subject of the project is the substitution of chemical insecticides with entomopathogenic nematodes (EPN). The natural habitat of EPN is the soil environment. To achieve satisfying control results on the foliage, survival and infectivity of EPN requires innovative formulation technology. Screenings among different EPN species confirmed the highest pathogenicity of Steinernema carpocapsae against 3rd instar DBM. Major port of entry into DBM larvae is by active penetration through the anus. The formulation with 0.3% surfactant and 0.3% polymer hinder larval movement and breathing, while environmental conditions support EPN movement and penetration activity. The use of the formulation decreased the infective time (IT)50 from 50 to 18 hours using 10 EPN/larva. The infective dose (ID)50 is lowered from 12 to 1 EPN/larva. Without formulation adjuvants 70% of the EPN applied to cabbage foliage run of the leaves. The ability to prolong EPN persistence on the leaf was evaluated using the polymer-surfactant-formulation enriched with 0.25% cross-linked polyacrylamide (PA), silica fumed (SF) or 0.25% alginate gel. Ten hours after application the decrease of EPN infectivity is 50% using the polymer-surfactant-formulation, with or without PA or SF. However, using the alginate gel, infectivity was decreased by only 10%.

17:30 **CONTROLLING THE QUALITY OF ENTOMOPATHOGENIC NEMATODE PRODUCTS**

Arne Peters, *E-nema GmbH, GERMANY*; Ursula Koelzer, *GAB Biotechnologie GmbH, GERMANY*; Klaus Iwahn, *Öre Bio-Protect GmbH, GERMANY*; Frank Stepper, *Sautter & Stepper GmbH, GERMANY*

Abstract: In todays commercial world controlling product quality is of ever growing importance. Due to their inherently large variation, biological products, like entomopathogenic nematodes, pose specific challenges for quality control. The association of retailers and distributors of beneficials in Germany (Verein der Nützlingsanbieter Deutschlands) has finalised a project on standardising methods for assessing the quality of entomopathogenic nematode products and for maintaining it within the shipment line to the end user. A labour- and budget-efficient method for counting and infectivity-assessment was designed and tested for its resolution between different production batches and for reproducibility in various laboratories. All partners did instantly get reproducible results in counting nematodes, while only 2 out of 3 got reproducible results with the infectivity assay. The counting method was shown to detect deviations of approx. 10% from the label-specified amount in the packages. Based on LC50-calculations, thresholds for rejecting nematode products for bad quality are defined. The temperature which nematode products are exposed to during shipment was determined and the effect of temperature on nematode survival was assessed. Based on these observations, a threshold temperature and time together with standardised packaging and shipment times was defined which distributors are committed to follow. These measures gives the end-user the security to obtain good quality products and should in turn extend the use of beneficial nematodes.

Wednesday, August 4^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 6

Symposium (Division of Viruses)

Role of native immune systems/molecular host response

Chair: Diana Cox-Foster; John Burand

16:00 **IMMUNE SYSTEMS (D. HULTMARK)**

D. Hultmark, -, -

Abstract: Abstract is not available at the time of printing.

16:25 **IMMUNE SYSTEMS (I. FAYE)**

I. Faye, -, -

Abstract: Abstract is not available at the time of printing.

16:50 STU **LECTIN-INDUCED HEMOCYTE INACTIVATION: A PARADIGM FOR PARASITOPID-MEDIATED IMMUNE-SUPPRESSION?**

Richard Glatz, *University of Adelaide, AUSTRALIA*; Sassan Asgari, *University of Queensland, AUSTRALIA*; Otto Schmidt, *University of Adelaide, AUSTRALIA*

Abstract: Many insect parasitoids that deposit their eggs inside immature stages of other insect species inactivate the cellular host defence to protect the growing embryo from encapsulation. Suppression of encapsulation by polydnaviruses-encoded immune-suppressors is correlated with specific alterations in hemocytes that include cytoskeletal rearrangements, and the loss of ability to spread on foreign surfaces. We have previously shown that the Cotesia rubecula polydnavirus gene product CrV1 is sufficient to cause immune suppression when injected into the host hemocoel, where CrV1 is taken up by hemocytes. Uptake is dependent on dimer formation, which is also a prerequisite for lipophorin binding, an indication that CrV1-lipophorin complexes are involved in hemocyte uptake. Since CrV1 resembles oligomeric lectins regarding interaction with lipophorin and uptake in resting cells, we examined the cytoskeleton during lectin-mediated uptake reactions and observed F-actin depolymerization resembling cytochalasin D inactivation. These observations suggest that some oligomeric adhesion molecules, which may include immune-suppressors, internalise receptors from the cell surface and in the process depolymerize actin-cytoskeleton. Although other more complicated mechanisms are possible, cellular immune inactivation in insect parasitoids may involve massive uptake reactions driven by lipoprotein complexes, which destabilize actin-cytoskeleton. Since recycling of membrane-vesicle to the periphery requires actin-cables, consecutive uptake reactions deplete receptors from the cell surface.

17:15 **A RECIPE FOR DEATH: THE INTERPLAY BETWEEN HONEYBEE IMMUNITY, IMMUNOSUPPRESSION BY MITES, AND PICORNA-LIKE VIRUSES**

Diana Cox-Foster, Xiaolong Yang, Miaoqing Shen, Liwang Cui, Nancy Ostiguy, *Penn State University, USA*

Abstract: A major question underlying host/pathogen interactions is how a host can defend itself against pathogens and parasites and how the pathogens or parasites overcome these defenses. The honey bee is an excellent model system for this question, given the new bee genomic data, bee genetics and life history of this insect. Parasitic Varroa mites are a major contributing factor in loss of honey bee colonies and have been previously suggested to kill bees by activating bee pathogens such as viruses. We hypothesized that mites feeding upon bees immunosuppress the bee via salivary protein secretions, in a similar manner as ticks feeding upon mammalian hosts. We tested this hypothesis by multiple methods: examining both the survivorship of bees following a challenge with a non-pathogenic bacteria, evaluation of immune defenses (cellular immune responses and antimicrobial production), and comparison of the gene expression levels of antimicrobial peptides and immunity-related enzymes. Our data indicate that mites are immunosuppressing the honey bees in a number of ways. To ask if mites are activating bee viruses, we examined five picorna-like viruses and the natural pathogenesis/association of the viruses with bees. Bees were found to contain detectable viral genomes for two picorna-like viruses (Sacbrood virus (SBV) and deformed wing virus (DWV)) and one dicistroviridae virus (Kashmir bee virus (KBV)) by RT-PCR, with many bees have co-infections of more than one virus. These viruses were found in colonies lacking any mite-infestation and the levels of viral RNA were significantly higher in individual bees when parasitized by mites. For the KBV virus, many bees lacked any detectable capsid proteins, indicating that these viruses were truly persistent or latent. In multiple bee colonies, all three viruses have been detected in adult bees (workers, drones, queens), eggs, larvae, pupae, and all food stores. These data indicate that these viruses can be vertically transmitted and suggest that there is excellent potential for horizontal transmission via the worker secretions into honey, brood food, and pollen stores. In addition, experimental data suggest that the presence of other microbes may trigger an amplification of DWV to extremely high levels. The relationship of the DWV virus with bees is intriguing given recent reports of a very similar virus (99% homology) being associated naturally with bees and found in the brains of aggressive guard bees.

Wednesday, August 4^{th}, 2004
Time: 20:00 - 22:00, Lecture Rooms 1, 12

Division meetings: MC, F

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 12

Symposium (Division of Bacteria)

New advances in research and development of insecticidal proteins

Chair: James Baum; Trevor Jackson

08:30 **CRY TOXIN DISPLAY: ITS JUST A PHAGE WE'RE GOING THROUGH**

Susana Vilchez, Craig Pigott, *Department of Biochemistry, Cambridge University, UNITED KINGDOM*; Juliette Jacoby, *Dept. of Medicine, Cambridge University, UNITED KINGDOM*; David Ellar, *Department of Biochemistry, Cambridge University, UNITED KINGDOM*

Abstract: The successful use of Bacillus thuringiensis (Bt) insecticidal toxins to control agricultural pests could be undermined by the evolution of insect resistance. Under selection pressure in the laboratory a number of insects have gained resistance to the toxins and one case of field resistance has been recorded. The use of protein engineering to develop novel toxins active against resistant insects could offer a solution to this problem. The display of proteins on the surface of phages has been shown to be a powerful technology to search for proteins with new characteristics from combinatorial libraries. However this potential of phage display to develop Cry toxins with new binding properties and new target specificities has hitherto not been realised because of the failure of displayed Cry toxins to bind their natural receptors. In this work we describe the construction of a display system in which a Cry toxin is fused to the amino terminus of a bacteriophage capsid protein. The resultant phage is viable, infectious, and the displayed toxin successfully interacts with a natural receptor. Using a second cry toxin we are developing this method to replace the exposed hypervariable surface loops in domain II of the Cry toxin with antibody CDRs from an antibody phage library containing 109 unique antibodies. In this way it is hoped to create toxins with entirely novel specificities. Against a background of 200 or so known Cry toxins this strategy has the potential to generate a portfolio of novel insecticidal proteins that is larger by several orders of magnitude.

09:00 **GENESIS OF MON 863, A TRANSGENIC CORN HYBRID RESISTANT TO CORN ROOTWORM FEEDING DAMAGE**

Ty Vaughn, James Baum, *Monsanto, USA*

Abstract: Corn rootworms (Diabrotica spp.) are widely distributed throughout the corn growing regions of the US and are also present in Canada, Mexico, and Brazil. Diabrotica species have also been found in Europe, with the 1992 discovery of the insect in Yugoslavia. In little more than a decade, corn rootworms have spread to ten European countries, including Hungary, Bulgaria, Romania, Slovakia, Italy, Switzerland, Ukraine, Austria, France, and the Czech Republic. This presentation describes the genesis of improved corn hybrids that are protected from damage due to feeding by CRW larvae. Using modern molecular techniques, Monsanto Company has engineered a variant of the wild type cry3Bb1 gene from B. thuringiensis that encodes a protein with enhanced insecticidal activity against corn rootworms and exhibits high levels of expression in transgenic corn plants. The resulting Cry3Bb1 variant is approximately eight times more lethal to corn rootworm larvae than the wild type protein. A DNA vector containing the variant cry3Bb1 gene was linked to a constitutive plant promoter and was introduced into corn cells. Corn event MON 863 was selected from hundreds of transformation events produced and developed for commercialization as YieldGard Rootworm Corn. This presentation reviews the development of the Cry3Bb variant protein, the nature of the target CRW pest and control of CRW by resistant hybrids.

09:30 **INSECTICIDAL PROTEINS FROM PAENIBACILLUS STR IDAS1529**

Scott Bintrim, Scott Bevan, Baolong Zhu, Weiting Ni, Don Merlo, Ernie Schnepf, *Dow AgroSciences LLC, USA*

Abstract: Paenibacillus spp. are Gram positive spore-forming bacteria that are found in many natural environments. Several species have been found to be pathogenic to scarab beetle grubs (P. popilliae and P. lentimorbus) or honeybees (P. larvae) while one species is nonpathogenic but is insect associated (P. apiarius). Recently, novel Cry18 and Cry43 proteins have been identified from P. popilliae and P. lentimorbus, respectively,

that are orally toxic to coleopteran larvae. We have isolated a strain of Paenibacillus, designated as strain IDAS1529, that produces proteins which are orally toxic to lepidopteran larvae. Phylogenetic analysis of the 16S rDNA sequence from Paenibacillus str. IDAS1529 showed close affiliation to the P. popilliae-P. lentimorbus-P. thiaminolyticus group within the genus Paenibacillus. A cosmid library made from total DNA isolated from Paenibacillus str. IDAS1529 was used to isolate recombinant cosmids that were orally toxic to lepidopteran larvae. Sequence analysis of one of these cosmids identified six open reading frames arranged in a putative operon that had 38-48% deduced amino acid sequence identity to the insecticidal toxin complex genes tcaA, tcaB, tcaC, and tccC identified in the entomopathogenic bacterium Photorhabdus luminescens. Downstream of this putative operon was another open reading frame that had 40% amino acid sequence identity to Cry1Ac. Further analysis of this Cry sequence, designated as Cry1529, showed that this protein is distantly related to the Cry proteins obtained from other Paenibacillus species and is more closely related to a group of Cry proteins identified in Bacillus thuringiensis that are Lepidoptera (Cry1, Cry9), Coleoptera (Cry3, Cry7 ,Cry 8), and Diptera (Cry4) toxins. The presence of the toxin complex genes in Paenibacillus str IDAS1529 is the first known occurrence of these genes in a Gram positive organism. A molecular survey of other Paenibacillus species identified toxin complex genes in a strain of P. apiarius and indicates that these genes are not unique to Paenibacillus str DAS1529.

10:30 **PHOTORHABDUS: A NATURAL BORN KILLER.**

Nick Waterfield, Andrea Dowling, Michelle Hares, Phil Daborn, Richard ffrench-Constant, *Biology and Biochemistry, University of Bath, UNITED KINGDOM*

Abstract: The genus Photorhabdus contains insect pathogenic Gram-negative bacteria that are carried within the guts of entomopathogenic nematodes in a symbiotic relationship. Upon entry into suitable insect prey, the heterorhabditid nematodes regurgitate the Photorhabdus which then rapidly set up a lethal septicaemia, killing the insect and converting the tissues into more bacteria which serve as a food source for the partner nematodes. When the insect resources are exhausted, infective juvenile worms re-associate with the bacteria before leaving the cadaver in search of new prey. Genomic analysis has revealed that Photorhabdus encodes an astonishing array of virulence factors suggesting a high degree of functional redundancy or overkill. We are currently studying in detail two families of novel insecticidal toxins used in this infection process. I will present our recent findings on the structure and function of members of two novel toxin families, the injectably toxic Makes Caterpillars Floppy (mcf) protein and the orally toxic Toxin Complex (tc) proteins. The Mcf toxins are large single polypeptide molecules of approximately 3000 amino acids (aa), which show a domain structure similar to the large Clostridial toxins. Mcf kills target cells through the induction of the apoptosis pathway and we are currently investigating exactly how this is achieved. In contrast, the Toxin Complex requires 3 polypeptides for full toxicity, exemplified by TcdA (2517aa), TcdB (1477aa) and TccC (1044aa). Heterologous expression studies in both Escherichia coli and in mammalian cells have allowed us to ascribe functions to the different proteins and individual domains of this highly complex toxin system.

11:00 **NOVEL SERRATIA ENTOMOPHILA ANTI-FEEDING GENES CONTAIN A PUTATIVE DEFECTIVE PROPHAGE ACTIVE AGAINST THE GRASS GRUB COSTELYTRA ZEALANDICA**

Mark Hurst, Trevor Jackson, Travis Glare, *AgResearch, NEW ZEALAND*

Abstract: Strains of Serratia entomophila and S. proteamaculans (Enterobacteriaceae) cause amber disease in the grass grub Costelytra zealandica (Coleoptera: Scarabaeidae), an important pasture pest in New Zealand. Larval disease symptoms include cessation of feeding, clearance of the gut, amber coloration, and eventual death. A 155-kb plasmid, termed pADAP for amber disease associated plasmid is essential for amber disease symptoms. pADAP has been fully sequence and found to encode two virulence associated regions; i) the previously documented sep virulence encoding region that exhibits strong similarity to the tc-type toxins of Photorhabdus luminescens and Xenorhabdus nematophilus; and ii) a novel anti-feeding gene cluster. The presence of anti-feeding gene/s beyond the sep region was indicated by maintenance of an anti-feeding component after transposon insertions in any of the sep genes. Based on deletion analysis of pADAP and subsequent sequence data, a 47-kb clone was constructed, which when placed in either an Escherichia coli or a Serratia background exerted strong anti-feeding activity and often led to rapid death of the infected grass grub larvae. Sequence analysis showed that the anti-feeding clone was comprised of 18 ORFs, several of which, contain virus associated domains. These ORFs have accordingly been termed afp for anti-feeding prophage. As with the sep virulence associated region, homologues to the afp cluster are also found as six separate entities in the genome of P. luminescens subsp. laumondii TTO1. Of specific interest, at the 3' distal end of the anti feeding operon and its P. luminescens homologues, are two ORFs encoding putative toxins. To date, the function of the prophage type molecule is unclear, but it can be speculated that it forms a novel toxin delivery system resembling in structure phage tail-like bacterocins, such as enterocoliticin of Y. enterocolitica, or xenorhabdicin from X. nematophilus.

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 1

Workshop (Cross-Divisional)

Risk assessment

Chair: Tariq Butt; Ralf Ehlers

08:45 **RISK ASSESSMENT AND REGISTRATION (G. STERK AND W. RAVENSBERG)**

G. Sterk, -, *BELGIUM*; W. Ravensberg, -, *NETHERLANDS*

Abstract: Abstract is not available at the time of printing.

09:15 **REGISTRATION OF MICROBIAL PLANT PROTECTION PRODUCTS AND ACTIVE MICRO-ORGANISMS IN EU**

Anita Fjelsted, *Ministry of Environment, Danish Environmental Protection Agency, DENMARK*

Abstract: Since July 1993 the microbial plant protection products and the active micro-organisms in these products have in EU been regulated according to EU Directive 91/414/EEC. This Directive has 6 Annexes. Two of these contain data requirements for the active micro-organisms and the formulated products. Annex I of the Directive is a table with all microbial strains (as well as chemical active substances) that have gone through the EU evaluation process and subsequently have been accepted - and therefore obtained an "Annex I inclusion". In the EU regulation system, the microbial plant protection products can be separated in two categories. Those that were placed at the EU market before July 1993 (containing existing/old micro-organisms) and those that were not (containing new micro-organisms). Until today 9 applications for Annex I inclusion for new micro-organisms have been submitted and EU evaluations of theses have been initiated. At present only three of these micro-organisms have been included in Annex I. The EU evaluation process for these micro-organisms have lasted for several years. The existing/old micro-organisms have until now only been evaluated at national level. However, also these micro-organisms will in the future have to go through the EU evaluation process. Applications for Annex I inclusion for these approximately 50 microbial strains need to be submitted in autumn 2005.
With this presentation I will: h explain the EU regulation and the EU evaluation process, h describe some of the most important data requirements, h describe the possibility of submitting identical applications to all OECD member countries, h give advise to researchers and industry h describe initiatives taken for the improvement of the EU evaluation system as well as initiatives taken to help industry

10:15 **NONTARGET EFFECTS OF ENTOMOPATHOGENIC FUNGI: ARE WE FINALLY ABLE TO GENERALIZE?**

Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Siegfried Keller, *Federal Research Station for Agroecology and Agriculture, 8046 Zürich, SWITZERLAND*; John D. Vandenberg, *USDA Agricultural Research Service, U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA*

Abstract: Many species of entomopathogenic fungi have been released for insect control. Most inundative and inoculative releases of fungi have included one of only a few species of Beauveria, Metarhizium and Paecilomyces. Our presentation will draw from a wealth of information about the nontarget effects of these releases. The following elements will be considered:
Ecological versus physiological host range of a fungus Other nontarget effects against invertebrates Potential replacement of, or recombination with, naturally occurring fungal strains Nontarget effects over time including dissemination and persistence The impact of inundative versus inoculative releases Comparisons among releases in different crops Comparisons among studies in different climates or different parts of the world
Our presentation will mainly be based on data from:
1) Studies performed in the EU funded project 'BIPESCO'. Here, data on non- target effects of Metarhizium anisopliae and Beauveria brongniar-

tii were obtained 2) Studies performed in North America using Beauveria bassiana and Paecilomyces fumoso-roseus
We aim to find common themes among past and current projects. We wish to stimulate workshop discussion of the status of knowledge of nontarget impacts of these fungi that can elucidate the lessons learned and our future needs. In particular, we hope to contribute ideas pertinent to registration of fungi for use in insect pest management.

10:45 **DO COMMERCIALISED FUNGAL BIOCONTROL AGENTS PRODUCE RELEVANT METABOLITES WHICH HARM HUMANS AND THE ENVIRONMENT?**

Hermann Strasser, *Institute of Microbiology, Leopold-Franzens University Innsbruck, AUSTRIA*; Claudio Altomare, *Institute of Sciences of Food Productions, Bari, ITALY*; Tariq Butt, *School of Biological Sciences, University of Wales Swansea, WALES*

Abstract: Do fungal biocontrol agents (BCAs), more specifically their metabolites, pose a risk to human health? This is a question of paramount importance which is being addressed by the EU-funded consortium RAFBCA (QLK1-CT2001-01391, http://www.rafbca.com). Some of the findings of this project are discussed with particular attention being focussed on: (1) The range of compounds produced by fungal BCAs, (2) whether they pose a risk to producers and applicators, (3) if the metabolites enter the food chain, and (4) strategies to simplify risk assessment of metabolites. Realistic risk assessment strategies are important to ensure public safety and to provide a clear, cost effective registration procedure which enables industry to accelerate registration of useful agents.

Thursday, August 5^{th}, 2004
Time: 08:30 - 12:00, Lecture Room 6

Contributed Papers (Division of Viruses)

virus / contributed paper session 3

Chair: P. J. Krell; M. M. van Oers

08:30 **CHARACTERIZATION OF HEPTAD REPEATS OF THE F PROTEIN OF HASNPV: SIMILARITY VERSUS NOVELTY**

Gang Long, Xiaoyu Pan, *The Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*; Zihe Rao, *Laboratory of Structure Biology and MOE Laboratory of Protein Sciences, Tsinghua University, CHINA*; Just M. Vlak, *Laboratory of Virology, Wageningen University, THE NETHERLANDS*; Zhihong Hu, *The Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*

Abstract: Budded virus of group II nucleopolyhedroviruses (NPV) enters host cells via an envelope fusion protein named F, which requires a cellular convertase cleavage to become active. Baculovirus F proteins share a similar arrangement of functional domains as paramyxovirus F proteins with cellular convertase cleavage site, fusion peptide, heptad repeat, transmembrane domain and cytoplasmic tail. Ha133 of Helicoverpa armigera single nucleocapsid NPV (HaSNPV) encodes an F protein. Three heptad repeats (HRs), HR1 (193A-230L), HR3 (241C-276R) and HR2 (540E-574I), were found in HA133. HR1, HR2 and HR1-6xGly-HR2 were expressed separately in E. coli DE3 as GST fusion proteins. They were purified by affinity chromatography and thrombin cleavage. Circular dichroism spectrometry analysis of HR1 and HR2 in PBS demonstrated predominantly an -helix content. HR1-6xGly -HR2 had a similar CD spectrum but its -helix level was temperature resistant. Gel filtration analysis of HR1-6xGly-HR2 showed that it presented both as monomer and multimer. After cross linking using EGS (ethyleneglyco bis), this peptide presented as a stable dimer in SDS-PAGE. In the cases of paramyxoviruses, coronaviruses and retroviruses, soluble HRs are potent inhibitors of membrane fusion and virus entry. In our experiments, however, neither HR1 nor HR2 was able to inhibit HaSNPV entry into H. armigera cells efficiently. In contrast to antibodies against the whole F protein, antibodies against HR1 and HR2 only weakly neutralized viral infection. The possible role of HR repeats in the F protein-mediated membrane fusion will be discussed.
This research is supported by NSFC grants (030210 and 30025003), a 973 grant (2003CB114202) and a grant from the KNAW (01CDP023). E-mail: ganglong58@hotmail.com

08:50 **STU** **HA-VP39 BINDING TO ACTIN AND MOLECULAR MECHANISM FOR HANPV TRANSPORTING TO THE HOST NUCLEUS**

Songya Lu, Guoqiong Ge, Yipeng Qi, *Wuhan University, CHINA*

Abstract: Ha-VP39 binding to actin and molecular mechanism for HaNPV transporting to the host nucleus
Songya Lu, Guoqiong Guo and Yipeng Qi*
Key laboratory of Ministry of Education in Virology, College of Life Sciences, Wuhan University, WuhanP.R. China 430072
Third instar larvae of cotton bollworm were fed with recombinan Helioverpa armigera nuclear polyhedrosis virus (HaNPV) carried green fluorescent protein (gfp) gene. GFP expression in various tissues showed the infectious course of HaNPV: fluorescence first appeared in midgud and hemocytes, then in tracheae system, and finally in fat body and epidermis. Our results supported the point that the virus could cause the primary infection through the tracheae system. Purified nucleocapsid protein Ha-VP39 of HaNPV could bind to actin in vitro without assistant factors assayed with Western blot and isothermal titration calorimeter. H and binding constants (K) assayed by isothermal titration calorimete suggested that Ha-VP39 first binds to actin to seed for actin to form the hexamer, then such hexamers link each other to form filaments, finally the actin filaments themselves twisted into cable structure. With confocal immunofluorecence microscopy and VP39 antibodies, it was detected that the released HaNPV nucleocapsids co-localized with the aggregated actin in the host cytoplasm (1 hr p.i.) and then the nucleocapsids were transported from the host cytoplasm to the nucleus (4 hr p.i.). Using cytochalasin DCD to prevent global actin from forming filamentous structures, HaNPV formed incomplete virions but the host actin concentration and the replication of viral DNA was not influenced. The transportation of HaNPV nucleocapsid from the cytoplasm to the nucleus was inhibited by the pretreatment of the host cells with cytochalasin D. The infection efficiency of the recombinant virus HaNPV/gfpp74 decreased to 7.34%, compared to 34.7% in normal host cells. While the infection efficiency in the cells whose microtubes were destructed by colchicin did not decrease (i.e.55.7%). The superslices revealed that HaNPV nucleocapsids could enter the cytoplasm of CD-treated cells but could not be transported to the nucleus, which resulted in the lower infection efficiency of HaNPV/gfpp74 in CD-treated cells. The transportation of the nucleocapsids was not inhibited in colchicin-treated cells. This result demonstrated that the transportation of HaNPV nucleocapsid from the cytoplasm to the nucleus was associated with actin filaments but not microtubes.

09:10 **IE1 AND IE0 HAVE SEPARATE ROLES IN THE REPLICATION OF AUTOGRAPHA CALIFORNICA MULTIPLE NUCLEOPOLYHEDROVIRUS IN SPODOPTERA FRUGIPERDA CELLS**

Taryn Stewart, Ilse Huijskens, *Faculty of Agricultural Sciences, University of British Columbia, CANADA*; Leslie Willis, David Theilmann, *Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, CANADA*

Abstract: Homologs of the baculovirus immediate early gene ie1gene have been identified in most baculoviruses. Studies have shown that IE1 of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is a potent transcriptional transactivator and is also vital for viral infection. IE1 contains 582 amino acid residues arranged into a number of different domains, including an acidic activation domain at the N-terminus, a DNA binding domain and, at the C-terminus, an oligomerization domain. At early times post-infection however, in addition to ie1 transcripts, the ie1 ORF also produces a spliced product ie0. The ie0 transcript is composed of 162 bp from the exon0 ORF spliced to the 5' end of ie1. Two translation products are made from the ie0 transcript, IE1 and IE0, a 52 amino acid N-terminally elongated form of IE1 called IE0. Our previous studies have shown that IE0 is expressed at higher levels than IE1 at early times and therefore may be more important more early infecton events than IE1. In this study we have investigated the function of IE0 and IE1 in virus infected cells. Through the use of AcMNPV bacmid technology, we have replaced the ie1 ORF with the Zeocin resistance gene, effectively knocking out both IE0 and IE1. This AcMNPV-IE0/IE1 knock out (KO) bacmid does not infect Spodoptera frugiperda (Sf 9) cells showing that IE0/IE1 is essential for viral infection. Repair viruses of the AcMNPV-IE0/IE1 KO were constructed that express only IE1, or IE1 and IE0 translated from the IE0 transcript, or a mutant ie0M-A, that is translated only as IE0. Analysis of the AcMNPV-IE0/IE1 KO-IE1 or -IE0M-A viruses revealed that both proteins can function to independently support replication producing infectious virus. However, observations of infected cells indicate that IE0 does not function equivalently to IE1. AcMNPV viruses expressing only IE0 or IE0M-A produced significantly fewer cells with polyhedra than their IE1 counterpart. These results suggest that IE0 does not activate late stage infection events as efficiently as IE1 and that the quantitative ratios of IE1 to IE0 seem to be integral to proper viral infection.

09:30 **INVOLVEMENT OF THE RING FINGER MOTIF OF ACMNPV EXON0 IN BUDDED VIRUS PRODUCTION**

Xiaojiang Dai, David Theilmann, *Pacific Agri-Food Research Centre, CANADA*

Abstract: We have recently identified the exon0 gene (orf141) of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) which is required for the production of budded virus in the AcMNPV life cycle. Budded virus production from Sf9 cells transfected with an exon0 knockout virus is reduced at least three orders of magnitude in comparison to wildtype virus. The C-terminus of EXON0 contains a RING finger domain whose consensus motif differs from most other known proteins of this type. The consensus RING motif of EXON0 is C3Y/FC4 compared to the normal motif of C3HC4 suggesting that the histidine is replaced by a tyrosine or phenylanaline. In addition, in EXON0 there is an additional highly conserved cysteine adjacent to the third cysteine of the RING motif. In this study we have focused on the RING finger to determine if this domain is essential for the function of EXON0 and if it is a novel RING finger. For the initial analysis we generated several exon0 mutants where the RING finger motif was removed by deletion or by creating a frame shift. Analysis of these mutants showed that loss of the RING finger resulted in the same phenotype as the exon0 knockout virus.

10:20 **ANALYSIS OF CF103, A ZINC-FINGER ORF FROM THE BACULOVIRUS CFMNPV**

Jondavid De Jong, *Department of Microbiology, University of Guelph, CANADA*; Basil Arif, *Canadian Forest Service, Sault Ste, CANADA*; Peter Krell, *Department of Microbiology, University of Guelph, CANADA*

Abstract: The Choristoneura fumiferana multicapsid nucleopolyhedrovirus (CfMNPV) is an ideal candidate as a bioinsecticide to control the eastern spruce budworm (C. fumiferana) due to its narrow host-range. We identified a CfMNPV ORF that encodes a putative DNA binding domain. Cf103 has an estimated molecular mass of 14.02 kDa and contains a putative zinc-finger domain. Cf103 is transcribed within the first six hours of viral infection. Northern analysis demonstrated two major transcripts peaking at approximately 72 hours post-infection. Using 5' and 3' RACE, we identified a single transcriptional start site and one transcriptional termination site. We were also able to successfully knockout Cf103 through homologous recombination with a GFP reporter indicating that Cf103 is not essential for viral replication in Cf-203 cells. The null-mutant was confirmed as such by PCR and lack of Cf103 transcripts was indicated by RT-PCR analysis. The ÄCf103 phenotype was comparable to that of wild-type CfMNPV infection in Cf-203 cells in terms of cytopathology. Viral growth curves and temporal expression analysis indicated that the behaviour of the Cf103 null-mutant was similar to that of wild-type CfMNPV. We are currently using Q RT-PCR to quantify any difference seen at the transcriptional levels of the immediate early, early, late and very-late transcription classes and to quantify any differences in DNA replication levels.

10:40 **IDENTIFICATION AND CHARACTERIZATION OF A DNA PHOTOLYASE-CONTAINING BACULOVIRUS FROM CHRYSODEIXIS CHALCITES**

Monique M. Van Oers, *Laboratory of Virology, Wageningen University, NETHERLANDS*; Elisabeth Herniou, *Department of Biological Sciences, Imperial College London, UNITED KINGDOM*; Awaluddin Junaid, Magda Usmany, *Laboratory of Virology, Wageningen University, NETHERLANDS*; Gerben J. Messelink, *Applied Plant Research, Naaaldwijk, NETHERLANDS*; Just M. Vlak, *Laboratory of Virology, Wageningen University, NETHERLANDS*

Abstract: A hitherto unknown single nucleocapsid nucleopolyhedrovirus with a unique property was isolated from larvae of the looper Chrysodeixis chalcites (Lepidoptera, Noctuidae, Plusiinae). Genetic characterization of this virus (ChchNPV) involved the random cloning of HindIII fragments into a plasmid vector and analysis by end-in sequencing. This revealed, among others, DNA polymerase, lef-3 and iap genes. The highest similarity was obtained with corresponding genes in class II NPVs. Polyhedrin, lef-8 and pif-2 gene sequences were obtained by PCR with degenerate primers. The sequences were used for in-depth phylogenetic analysis showing that this virus grouped with other class II NPVs. The polyhedrin sequnce was most similar to that of other group II NPVs of Plusiinae. The end-in sequencing also identified a gene so far unique to baculoviruses encoding a class II cyclobutane pyrimidine dimer (CPD) DNA photolyase (dpl). In pro- and eukaryotic organisms, except placental mammals, this enzyme is involved in DNA repair, i.e. the elimination of pyrimidine dimers as a result of UV damage. The transcriptional activity of this ChchNPV gene was demonstrated by RT-PCR and 5and 3RACE techniques using RNA isolated from infected T. ni High Five cells or C. chalcites hemocytes. The possible role of this gene in the biology of the virus is discussed.

11:00 **BACULOVIRUS INDUCTION AND SUPPRESSION OF APOPTOSIS OF SPODOPTERA LITTORALIS SL2 CELLS**

Qinghzen Liu, Nor Chejanovsky, *The Volcani Center, ISRAEL*

Abstract: Infection of Spodoptera littoralis SL2 cells with the Autographa californica nucleopolyhedrovirus (AcMNPV) induces apoptosis in contrast to infection with the Spodoptera littoralis nucleopolyhedrovirus (SlNPV). Induction of apoptosis of SL2 cells by AcMNPV-infection or UV-irradiation involved the activation of an effector caspase that we isolated and designed Sl-caspase-1. Sl-caspase-1 encoded a polypeptide of about 37 kDa. Comparison of Sl-caspase-1 amino acid sequence to those of Spodoptera frugiperda-, Trichoplusia ni- and Bombyx mori-caspase-1 revealed that these caspases display a high degree of homology, suggesting that their activation pathway is highly conserved in Lepidopterans. SlNPV-infected cells synthesized the apoptosis suppressor P49 and inhibited completely the maturation of Sl-caspase-1, suggesting that P49 might inhibit the apoptosis cascade in SL2-cells upstream of the apoptotic suppressor P35 of AcMNPV. This indication was further supported by data obtained studying Sl-caspase-1-processing in SL2 cells stably transformed to express the p35 gene challenged by apoptosis stimuli.

11:20 **MAPPING THE POLYPEPTIDE REGIONS OF P10 OF HASNPV THAT ARE REQUIRED FOR FILAMENT FORMATION**

Chunsheng Dong, Dan Li, Gang Long, Fei Deng, Hualin Wang, Zhihong Hu, *Oint Laboratory of Invertebrate Virology and the Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA*

Abstract: To study the filament formation function of polypeptide regions of P10 protein, a serial C-terminal truncated p10 genes of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus HaSNPVwere fused with green fluorescence protein (GFP) and transfected into HzAM1 cells. Western blot using antiserum against HaSNPV P10 revealed that all the P10-GST fusion proteins were expressed in HzAM1 cells. When observed under confocal microscopy, different truncated p10 genes appeared different structures in the transfected cells. Cells transfected with plasmid pN87-GFP, the full length of p10 tagged with GFP, formed green patches mostly in the cytoplasm. Plasmid pN80-GFP, which contains N-terminal 80 amino acids (aa) of P10 and tagged with GFP, formed network structures in the transfected cells. Plasmid pN66-GFP, which contains N-terminal 66 aa of P10 and tagged with GFP, appeared branch-like structures. However homogeneity fluorescence was detected in the cells transfected with plasmid pN60-GFP, pN49-GFP, pN43-GFP and pN36-GFP. These studies suggest that the N-terminal 66 aa, which contains coiled-coil domains are essential for the formation of filament structure. The 7 aa basic C-terminal, however, is not acquired for the formation of fibrillar structure.

11:40 **ANALYSIS OF THE CHITINASE GENE HOMOLOGUE OF THE BACULOVIRUS PLODIA INTERPUNCTELLA GRANULOVIRUS, PIGV**

Caroline Griffiths, Sukvinder Bharya, *Oxford Brookes University, UNITED KINGDOM*; John Burden, *NERC CEH-Oxford, UNITED KINGDOM*; Linda King, *Oxford Brookes University, UNITED KINGDOM*

Abstract: OX1 3SR.Plodia interpunctella (Hübner) (Lepidoptera; Pyralidae) is a significant pest of stored food products and the baculovirus Plodia interpunctella granulovirus (PiGV) demonstrates high virulence and specificity, making it a strong candidate for development as a bio-pesticide. Baculovirus populations in the wild are heterologous, comprising a number of isolates distinguishable by genomic differences, and we have demonstrated this to be true of PiGV. We have documented genetic variation of the virus during long-term association with hosts, and it appears that this variation may be least partially influenced by host and environmental factors. A dominant variant observed within the virus population when analysing genomes using SalI was mapped to a homologue of the AcMNPV chitinase gene, chiA. PiGV does not induce melting of infected host larvae, a feature common to many but not all baculoviruses, and as chitinase is crucial to host melting, it had been anticipated that PiGV would lack this gene. DNA Sequencing revealed that the promoter region of the PiGV chiA homologue contains a baculovirus consensus late promoter motif, TAAG, and the predicted amino acid sequence from this gene shows identity with other baculovirus chitinases, most closely with the granuloviruses of Cydia pomonella (65%) and Xestia c-nigrum (63%). RT-PCR of total RNA extracted from PiGV-infected P. interpunctella larvae detected chiA mRNA from 24hrs post infection and continuing throughout the infection to death

(approx. 21 days p.i.), indicating the gene is transcriptionally active in insect cells. Molecular cloning and expression of the PiGV chiA gene sequences using chiA- AcMNPV recombinant viruses has been achieved, in order to examine the enzymatic function of the predicted protein in vitro and in vivo. Melting of the host cuticle by chitinase is known to require the complementary action of a viral protease, such as the AcMNPV cathepsin gene, v-cath. To date, sequencing of the PiGV genome has not yet revealed a v-cath gene homologue, nor has one been detected by low stringency hybridisation, although a putative metalloprotease gene has been identified. The activity of PiGV chitinase, its role in PiGV infection and the implications of host life cycle on the genetic stability of non-essential genes will be discussed.

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 3

Chair: C. Nielsen-LeRoux; A. Bravo

13:30 **BACILLUS THURINGIENSIS EXTRACHROMOSOMAL MOLECULES: FROM SMALL LINEAR PROPHAGES TO LARGE CONJUGATIVE PLASMIDS**

Géraldine Van der Auwera, Delphine Forget-Hanus, Céline Verheust, Jacques Mahillon, *UCL, BELGIUM*

Abstract: Bacillus thuringiensis belongs the Bacillus cereus sensu lato family of Gram-positive sporeforming bacteria. This group contains six species that are genetically related but nonetheless display highly specialised lifestyles, especially with regard to their respective virulence spectra. Most notable are B. cereus sensu stricto, which is implicated in several human diseases, B. anthracis, the etiological agent of anthrax, and B. thuringiensis which unlike the first two does not affect mammals, but produces d-endotoxin crystals toxic to insect larvae. It has been shown that some of these species share different mobile genetic elements (transposons, insertion sequences and phages), and in synergy with various mechanisms of horizontal gene transfer (mainly conjugation and transduction), can exchange important genetic traits mostly pertaining to their extrachromosomal pool. In order to gather more insights into the mechanisms contributing to the B. thuringiensis genome flexibility and gene dispersion, we embarked on the detailed characterisation of extrachromosomal, linear and circular, molecules encountered in representative strains of this bacterium. The wide host-spectrum conjugative plasmid pAW63 was identified in B. thuringiensis serovar kurstaki, where it displayed a highly efficient ability to conjugate in liquid medium, for both its own transmission as well as that of small mobilizable plasmids. Moreover, heterologous conjugation experiments have shown that it is also capable of transfer to B. cereus as well as to some less closely related species. Sequencing and gene expression analyses have led to a functional map of this plasmid, yielding many insights into its conjugative apparatus, its relation to the pAMß1-type family and its resemblance to other large Bacillus plasmids, including pXO2 from B. anthracis. Similarly, the conjugation and mobilisation behaviours of pXO16, a 350 kb element from B. thuringiensis serovar israelensis, have also been studied under a wide spectrum of growth conditions and matrices. B. thuringiensis serovar israelensis also harbours a 15-kb linear molecule named pGIL01. Experimental evidences showed that this plasmid actually corresponds to the prophage state of a phage displaying structural and functional features reminiscent of those found in the tectiviridae family. Specifically, this 14,931 bp molecule harbours at least 30 ORFs, five of which displayed similarity with proteins involved in phage systems. The functional characterisation of these putative proteins is currently under investigation.

13:45 **INTRACELLULAR EFFECTS OF CYT1AA FROM BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS ON ESCHERICHIA COLI EXPRESSING CYT1AA**

Robert Manasherob, Mark Itsko, Olga Burgazliev, Eitan Ben-Dov, Sammy Boussiba, Arieh Zaritsky, *Ben-Gurion University of the Negev, ISRAEL*

Abstract: An endotoxic protein produced during Bacillus thuringiensis subsp. israelensis sporulation, Cyt1Aa (27 kDa) can also be defined as antibiotic. Upon expressing cyt1Aa, recombinant Escherichia coli lose colony-forming ability associated with arrest of DNA synthesis and nucleoid compaction in the cell's center. Cyt1Aa-affected nucleoid compaction is delayed but eventually more intense than chloramphenicol-induced compaction. Determinations of relative nucleoid length ruled out the possibility that the small, compact nucleoid in Cyt1Aa-expressing cells resulted in DNA replication run-out and segregation following cell division. Treatments with membrane-perforating substances other than the hydrophobic Cyt1Aa did not cause such compaction, but rather the nucleoid overexpanded to occupy nearly all of the cell volume. These findings support the suggestion that, in addition to its perforating ability, Cyt1Aa causes specific disruption of nucleoid associations with the cytoplasmic membrane. In situ immuno-fluorescence labeling did not demonstrate large amount of Cyt1Aa associated with the membrane. Clear separation between Alexa-labelled Cyt1Aa and 4',6-diamidino-2-phenylindole (DAPI)-stained DNA indicated that the nucleoid did not bind Cyt1Aa. In addition, the ability of P20 to abolish this intracellular effect was determined using molecular genetic tools.
Contributed Paper

14:00 **PATHOGENICITY OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS AND ENTOMOPATHOGENIC NEMATODES OF THE GENUS STEINERNEMA AGAINST TIPULA PALUDOSA**

Jesko Oestergaard, Ralf-Udo Ehlers, *Institute for Phytopathology, Christian-Albrechts-University Kiel, GERMANY*

Abstract: The LD50value of Bacillus thuringiensis subsp. israelensis (strain H14) was determined for the different larval instars of Tipula paludosa (Diptera: Nematocera). For the L2stage the LD50 is 10,42 :g rsp 73 ITUs (International Toxic Units determined with Aedes aegypti); for the L3 41,21 :g rsp. 289 ITUs and for the L4 440,94 :g rsp. 3087 ITUs. Bt clones, which encode for only one of the four proteins were tested and their synergistic effects investigated. Different nematode strains were tested in a laboratory assay against T. paludosa L1 at 15C. A mortality of 75% was reached with 50 infective juveniles (DJs) of S. carpocapsae/larva. The efficiency of S. feltiae reached 37%. At 8C, S. feltiae caused an efficiency of 26% and S. carpocapsae of 6%. Positive synergistic effects between S. feltiae and Bti were detected at 8C for L1 and at 15C for L4 of T. paludosa. In a field trial an efficiency of 79% was reached against L1/L2 with an amount of 1,3 g/m2 Bti, whereas S. feltiae caused 15% mortality after application of 50 million DJ/m and the same amount a week later. In a second field trial an application of 1 g Bti/m caused an efficiency of 43% against L2/L3 at a soil temperature of 7C.

14:15 **NEW ENTOMOPATHOGENIC BACTERIA FOR THE CONTROL OF WHITE GRUBS (COLEOPTERA:SCARABAEIDAE)**

Zitlhally Rodríguez Segura, Francisco Javier Villalobos, *Facultad de Ciencias Agropecuarias, Universidad Nacional Autónoma de México, MEXICO*; Luciano Hernández, *Universidad Autónoma del Estado de Morelos, Facultad de Química, Universidad Nacional Autónoma de México, MEXICO*; Eduardo Aranda, *Centro de Investigación en Biotecnología, Universidad Nacional Autónoma de México, MEXICO*; Maria Eugenia Núñez-Valdez, *Facultad de Ciencias Agropecuarias, Universidad Nacional Autónoma de México, MEXICO*

Abstract: According to the principles of sustainable agriculture, the use of entomopathogenic bacteria as bioinsecticides has been a promising alternative to the use of chemicals for the control of insect pests. Larvae of insects belonging to the scarab family known as white grubs (Coleoptera:Scarabaeidae) are soil pests of many crops including Graminae, Leguminosae, vegetables and ornamentals in Mexico and other countries. The larvae may feed on roots of plants causing severe damage. In México, there are about 68 different species of white grubs (Phyllophaga spp) reported as potential pests and there is no effective biological control agent to cop with them. The aim of this work was the search for entomopathogenic bacteria active against Phyllophaga spp larvae for their future use in a program for Integrated Pest Management. Bacteria were isolated from the haemocoel of dead larvae previously showing disease symptoms. Ninety isolates were obtained from 785 larvae collected from the field. Isolates were propagated at 30 C on nutrient broth-agar plates. Thirty eight isolates showing homogeneous and good yields were selected for oral bioassays. Pathogenic bacteria were selected by their ability to cause anti-feeding effect (AFE) and mortality (M) by two rounds of oral bioassays. For this purpose, healthy larvae of Phyllophaga blanchardi were fed with small pieces of carrot coated with the selected isolates. Uncoated carrot was used to feed control larvae. Similar treatments were applied during an inoculation period of 6 days and the percentage of consumed carrot was daily evaluated. After the inoculation period, all larvae were fed with uncoated carrot. Differences in the percentage of consumed carrot among control and experimental groups were evaluated by ANOVA. Mortality was evaluated by the statistical test X square. Eleven isolates caused significant AFE ranging from 45 to 92% during the inoculation period and from 42 to 78 % after that period. These isolates were selected as pathogenic strains. No significant mortality was observed during the bioassays. Phenotypical and biochemical tests used for bacterial taxonomy and also, sequencing of 16S DNAr, showed that the selected bacteria were Serratia marcesens (4 isolates), Enterobacter agglomerans, Bacillus sphaericus, Enterobacter cloacae (3 isolates), Enterobacter aerogenes and Alcaligenes faecalis. The

potential of these strains as biocontrol agents to prevent crop damage will be discuss in terms of the reduction of selecting pressure of resistance and the induction of the beneficial activity of the larvae.

14:30 **THE RISK EVALUATION OF THE GENETICALLY ENGINEERING BACILLUS THURINGIENSIS WG-001 IN SOUTH CHINA VEGETABLE FIELDS**

Zhang Zhenyu, Li Lin, Sun Ming, Yu Ziniu, *State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Huazhong Agriculture University, P.R. CHINA*

Abstract: To evaluate the risk of the genetically modified Bacillus thuringiensis WG-001 in the vegetable fields of South China, we have proceeded several releasing experiments from July 25th, 2002 to August 19th, 2003 in Bajia village, Doumen region of Zhuhai city in Guangdong province. The following five categories have been evaluated:The ability of surviving in the fields, spreading and transmitting in the wild environment, influencing the indigenous microorganisms, the ability of mutating and horizontal transferring of the characteristic genes cry1Aa and cry1Ac to the indigenous microorganisms. The results indicated that: (1)B. thuringiensis WG-001 could live up to 9 days on the vegetable leaves, but survive for much longer in the soil with low level of the count(0.72104cfu/g dry soil sample), which indicates the weak ability of surviving in the fields. (2)B. thuringiensis WG-001 could spread 50m in the air but survive for less than 3hrs. Spreading on the vegetable leaves within 30m but survive for less than 24hrs. Transmitting in the soil is uncountable. These indicate that it has certain ability of spreading and transmitting in the nature, but could not survive for a long time. (3)B. thuringiensis WG-001 can not influence the indigenous microorganisms observably. (4)The characteristic genes of cry1Aa and cry1Ac have genetically stable. (5)We did not detect the horizontal transferring of the characteristic genes cry1Aa and cry1Ac to the indigenous microorganisms. We have drawn some primary conclusions: the genetically modified B. thuringiensis WG-001 does not have the evident ability to influence the ecosystem of fields in the vegetable fields of South China, and it can apply with high safety.
* This research was supported by: Chinese National Natural Science Fundation(30170032); Chinese National Programs for High Technology Research and Development(2001AA212301)

14:45 **THE ASSOCIATION OF CHIRONOMIDS AND VIBRIO CHOLERAE**

Meir Broza, Malka Halpern, *Faculty of Science and Science Education, University of Haifa, ISRAEL*; Hanan Gancz, Yechezkel Kashi, *Faculty of Biotechnology, Israel Institute of Technology, ISRAEL*

Abstract: Gelatinous egg masses of Chironomus (Diptera, Chironomidae) collected in a pond of rehabilitated water in Israel and left overnight in the lab were completely disintegrated. Single unhatched eggs were found on the bottom. Vibrio cholerae was isolated and identified as the cause of this phenomenon. Haemaglutinin/ protease secreted by those bacteria caused the degradation of the gelatinous matrix. The matrix was found to be composed mainly of glycoprotein. V. cholerae exist as natural inhabitant of aquatic ecosystems. Yet its natural reservoir is unknown and the ways of its dissemination during pandemics is not fully understood. Three years of continuous observations in four types of water bodies in northern Israel, and sporadic collections in 16 other sites in Israel, India and Africa, revealed a frequent adherence of free living Vibrio bacteria to egg masses surface. More than 35 different serogroups of V. cholerae were isolated from chironomid egg masses. The two pathogenic strains, O1 and O139, were not isolated yet, because we never collected egg masses during cholera epidemics. Laboratory experiments with pathogenic and non pathogenic bacteria showed no differences between the two groups regarding the use egg masses as food resource. Vibrio cholerae was also isolated from adult chironomids. Simulations, both in the lab and in field experiments, shows that flying adults can transfer the bacteria from one water source to another. We suggest that aerial transfer by flying chironomids may play a role in continental and inter-continental dissemination of V. cholerae.

15:00 **TARGETED DRUG DELIVERY OF CYT1AA PROTEIN FROM BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS**

Shmuel Cohen, *Department of Life Sciences, Ben-Gurion University of the Negev, 2Department of Chemical Engineering and Biotechnology, College Judea and Samaria, ISRAEL*; Eitan Ben-Dov, *Department of Life Sciences, Ben-Gurion University of the Negev, ISRAEL*; Marina Nisnevitch, Rivka Cahan, Michael Firer, *2Department of Chemical Engineering and Biotechnology, College Judea and Samaria, ISRAEL*; Arieh Zaritsky, *Department of Life Sciences, Ben-Gurion University of the Negev, ISRAEL*

Abstract: Bacillus thuringiensis subsp. israelensis Cyt1Aa synergies Bt mosquitocidal proteins in vivo. However, upon proteolytic activation, the protein has also hemolytic and cytolytic effect against variety kind of cells in vitro. This activity is mediated by a non-specific binding to unsaturated phospholipids and consequently destruction of the membrane integrity. Since the destruction mechanism is rapid and internalization is not required this protein is highly attractive for therapeutic purposes. Moreover, the lack of requirement for specific receptor on the membrane and uptake into the cytosol may be of importance in preventing development of drug resistant. Previous investigators targeted Cyt1Aa to receptor presenting tumor cells through chemical conjugation to an appropriate ligand. While this conjugate was highly specific to target cells it did not show the same rapid high cytolytic effect as the free Cyt1Aa. Furthermore, the purification procedure of the conjugate was very tedious and the final product was not homogenous. In order to avoid these problems we linked a specific ligand to either the N or C termini of activated Cyt1Aa fragment by genetic engineering. The chosen ligand is a fragment of Myelin Basic Protein (MBPp), 12 amino acids in length. The MBPp is recognized by B-cell hybridoma cells that express surface IgG1 and are being used as a clonotypic model of Multiple Myeloma. The recombinant construct is composed of the endogenous promoter of cyt1Aa, cyt1Aa-MBPp and the helper gene p20. The chimeric gene was expressed in an acrytstalliferous Bti strain and the effect of the purified product was examined on hybridoma cells. This model enables us to determine the specific and non-specific activity of the Cyt1Aa conjugate.

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 6

Contributed Papers (Division of Fungi)

fungi / contributed paper session 4

Chair: Jorgen Eilenberg; F. Vega

13:30 **BEAUVERIA BASSIANA AS A KEYSTONE SPECIES IN PINE ECOSYSTEM**

Zengzhi Li, Meizhen Fan, Bin Wang, Degui Ding, *Department of Forestry, Anhui Agricultural University, CHINA*

Abstract: In Southern Anhui, Southeastern China, Beauveria bassiana was applied inoculatively against the Massons pine caterpillar, Dendrolimus punctatus, followed by a biodiversity investigation in the experiment plots in an anniversary year. Paecilomyces cateniannulatus, P. farinosus, Metarhizium anisopliae were revealed from 32 insect species, but the most abundant is B. bassiana, with 127strains isolated from 30 insect species. Based on esterase analysis, all these strains were attributed into 32 esterase types, which were substantially virulent on the caterpillars, with very LT50 difference at quite a few times. The result suggests that B. bassiana persists and disperses along more than one route. Each esterase type accounts for at least one chain in a food web. Some strains of B. bassiana with very wide host range can connect different food chains, making food web more complicated. Fifteen RAPD polymers were used for PCR amplification and analysis on 92 strains and 388 bands were amplified, showing that each strain is of specific genotype. Based on a clustering analysis, they were clustered into 7 different clades, each with specific host chain. Similar to esterase analysis result, different host chains shared or monopolized some interconnecting points. Niche overlapping analysis showed that some clades shared over 50% overlapping, while some clades were completely independent. Through overlapped host niche, i.e. parasitism on the same host insects, strains of different genotypes finishes gene exchange; in the meantime, parasitism of some clades on different hosts provides possibility for keeping their respective genetic stability in the ecosystem. Complicated host chains also suggests that B. bassiana can survive by infecting other hosts when the caterpillars are at low level. Host chains of different clades of B. bassiana consist of different species and amounts of hosts indicate that they have different host ranges and specificities. Among various host insects, a few species associated with some different B. bassiana strains are obviously keystone species which keep B. bassiana populations stable in forests. The species number are very limited, but their amounts account for large proportion. Two weevils, Brachyderes incanus and Sympiezomias veletus and the pine caterpillar are such keystone species which maintain infection of B. bassiana in the forest ecosystem, each accounts for 64.7 and 60.0%, respectively of coleopeteran and lepidopteran cadavers and play important roles for maintaining inocula of B. bassiana in the forests. All the above proofs suggest that B. bassiana is a keystone species for maintaining stability of insect community in the pine plantation ecosystem.

13:45 **FIELD RELEASES OF BEAUVERIA BASSIANA STRAIN GHA AFFECT GENETIC DIVERSITY OF INDIGENOUS CONSPECIFIC POPULATIONS**

L. A. Castrillo, *Department of Entomology, Cornell University, UNITED STATES*; P. Mishra, L. Annis, Eleanor Groden, *Department of Biological Sciences, University of Maine,, UNITED STATES*; John D. Vandenberg, *USDA-ARS, US Plant, Soil & Nutrition Laboratory, UNITED STATES*

Abstract: Risk assessment studies of field releases of a microbial control agent typically focus on beneficial organisms that may serve as an alternate host of the pathogen. Little is known about the effects of microbial agents on indigenous conspecific strains in agricultural fields. In this study we are evaluating the effects of mass releases of a commercial formulation of Beauveria bassiana strain GHA on naturally occurring conspecific strains by comparing prevalence of and genetic diversity within indigenous populations of B. bassiana in fields with no history of GHA treatment and in fields representing a range of GHA application histories. Genetic diversity in B. bassiana isolates collected from soil core samples from four potato farms in Maine and two in New York, representing different treatments, was examined using amplified fragment-length polymorphisms and random amplified polymorphic DNA markers. Our data show greater diversity among populations in untreated fields than in GHA-treated fields, with displacement of indigenous strains in treated fields by GHA or GHA-similar haplotypes. This displacement, however, appears to be temporary with recovery of native strains over time since the last GHA application. The potential for recombination between GHA and indigenous strains, which could result in novel haplotypes, is also being investigated by determining vegetative compatibility groups among the more predominant native strains and GHA.

14:00 **STU** **DISTRIBUTION AND OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN THE SOIL IN A SINGLE AGROECOSYSTEM IN DENMARK**

Nicolai Vitt Meyling, Jørgen Eilenberg, *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40 DK-1871 Frederiksberg C, DENMARK*

Abstract: The natural occurrence and the horizontal distribution of entomopathogenic fungi were investigated in soil samples from an organically grown field (17 ha) and the adjacent hedgerow in Denmark. Soil samples were collected from the field 2001 (n=274), 2002 (n=270) and 2003 (n=70) from points, 25 m apart, based on Geographical Information Systems (GIS) and Global Positioning System (GPS) ensuring that each point could be relocated accurately in consecutive years. In addition, samples were collected from hedgerow soil along transects in 2002 (n=70) and 2003 (n=70). Fungi were isolated using the Galleria bait method. The most common fungi in the field soil were Beauveria bassiana, Metarhizium flavoviride, Paecilomyces farinosus in both 2001 and 2002. Soil samples from the hedgerow were dominated by P. fumosoroseus, B. bassiana and P. farinosus in descending order. Metarhizium anisopliae was only found occasionally in the field soil. Sample points that yielded positive or negative samples in both 2001 and 2002 gave no difference as regard to the frequency of occurrence of B. bassiana when they were resampled in 2003. The occurrence of this fungus in specific points seemed thus to be dynamic. The effect of sampling scale was investigated by isolating fungi from quadrates with sampling points spaced 5 m and 1 m apart, respectively. Quadrates in a high-density area confirmed high density at a lower scale, as did quadrates in a low-density area based on points 25 m apart. This indicated that the original sampling scale gave a good indication of the natural occurrence.

14:15 **PROTECTION OF ENTOMOPATHOGENIC FUNGI AT THE LANDSCAPE SCALE**

Stanislaw Balazy, *Research Centre for Agricultural and Forest Environment PAS, POLAND*

Abstract: Entomopathogenic fungi have generally been considered as desirable components in agroecosystems due to their suppressive effects on noxious arthropods. Studies in terrestrial habitats have shown, however, strong impoverishment of their frequency and diversity, especially in one-year cereal and row-crops as compared with perennial fodder plant cultures, meadows, arboreous areas and swamps. From among about 210 entomopathogenous species of Entomophthorales, Hypocreales and Hyphomycetes found in a number of investigated countryside areas in Poland, France, Germany and Romania only less than 20 (about 10%) have been pretty regularly recorded in one-year crops. They are mostly represented by the common polyphagous hyphomycetes and a number of widespread entomophthoralean aphid and fly pathogens of the genera Beauveria, Paecilomyces, Metarhizium, Entomophthora, Pandora and Zoophthora. Modern agriculture systems of huge homogenous cultures protected mostly by the use of chemical pesticides cause progressive decline of arthropod diversity and a decreasing number of their pathogens. Most endangered are the host selective (monophagous or narrowly obligophagous), obligatorily biotrophic species. In order to efficiently protect the diversity of entomopathogenic fungi, networks of natural or seminatural refuge habitats should be maintained among the arable fields that ensure conditions for the persistence of their potential hosts and differentiated vertical humidity gradient which allows these pathogens to develop in particular layers of vegetation cover. Even the presence of weeds and grassy balks or roadsides enrich the communities of these fungi by about 10 to 15 species mostly pathogenic to mites, plant-hoppers, flies and thysanopterans, whereas real biodiversity refuges such as woodlots, shelterbelts, perennial crops, swamps and rushes scattered among arable fields increase species diversity up to about 60 species. This number makes about 30% of the total list of species and seems characteristic for the Polish Lowlands agricultural areas of the diversified landscape structure. There appears a growing tendency to protect biodiversity refuges by law because of their functions comparable with nature reserves and other forms of territorial protection.

14:30 **THE ABILITY OF COLLEMBOLANS TO ACT AS NON-HOST VECTORS OF ENTOMOPATHOGENIC HYPHOMYCETE FUNGI.**

Karsten Dromph, *The Royal Veterinary and Agricultural University, DENMARK*

Abstract: The aim of the study was to test the ability of soil dwelling collembolans to act as non-host vectors of entomopathogenic hyphomycete fungi and thereby cause infections in susceptible coleopterans. It was found that uninfected specimens of the three collembolan species Folsomia fimitaria, Hypogastrura assimilis and Proisotoma minuta were all able to transmit sufficient inoculum of the entomopathogenic fungi Beauveria bassiana, B. brongniartii and Metarhizium anisopliae to directly cause infection in larvae of Tenebrio molitor after exposure to soil containing conidia. However, their ability differed significantly, with transmission by P. minuta causing the lowest mortality (up to10% mortality) while there was no significant difference between F. fimetaria and H. asssimilis (up to18% mortality) The mortality of T. molitor increased for all combinations of fungi and collembolan species studied with increasing concentration of conidia in the soil and duration of exposure. When the potential of the three species as vectors was compared by transferring a range of numbers of F. fimitaria, H. assimilis and P. minuta previously exposed to sporulating fungal material, P. minuta was found to cause a significantly lower infection levels than equal numbers of F. fimetaria and H. assimilis. It was further documented that transmission of Metarhizium anisopliae to the predatory ground beetle Anchomenus dorsalis through its collembolan prey, F. fimetaria, was possible. Exposure of A. dorsalis for 24 h to 10 living adults of the collembolan previously fed M. anisopliae resulted thus in 8% mortality of A. dorsalis due to M. anisopliae, wile exposure to the substrate after the collembolans had been removed only resulted in 2% mortality due to M. anisopliae. When A. dorsalis was exposed to 10 freeze killed uninfected collembolans, or surface sterilised freeze killed uninfected collembolans, the resulting mortalities of A. dorsalis due to M. anisopliae was respectively 20% and 6%. The present study, therefore, demonstrates that viable conidia in the gut content of collembolans may be an important source of infection of both soil dwelling larvae and adult predators by entomopathogenic fungi.

14:45 **SENSITIVITY OF FOLSOMIA CANDIDA (COLLEMBOLA) TO BEAUVERIA BASSIANA GHA STRAIN AND METARHIZIUM ANISOPLIAE VAR. ACRIDUM IMI 330189**

Michael Brownbridge, *University of Vermont, Entomology Research Laboratory, U.S.A.*

Abstract: The goal of maintaining high levels of agricultural productivity while reducing pesticide use presents a significant challenge. Fungal entomopathogens have proven potential for use as biopesticides, where they can effectively replace chemical insecticides. Beauveria bassiana GHA strain (Emerlald BioAgriculture; formerly Mycotech Corp.), and Metarhizium anisopliae var acridum IMI 330189 (Green Muscle; CABI BioScience) are two of the best known and researched strains that are produced commercially for control of a variety of insect pests. Non-target studies have largely focused on their effects against beneficial species such as predators, parasitoids and bees. No less important, though, are soil organisms which are essential components of a healthy soil biota. Comparatively little effort has been directed towards an assessment of effects of fungi on beneficial detrivores such as Collembola (springtails). Collembola are abundant in robust and productive soils, and play a vital role in the removal and breakdown of microbes and crop residues. Folsomia candida is frequently used in laboratory evaluations. Cultures of this collembolan species are easy to maintain and its short reproductive cycle makes it ideal for ecotoxicological experiments. In addition, since F. candida is parthenogenetic, tests may be done against a population of continuous genetic uniformity. While data from these 'standardized' tests cannot be directly extrapolated to the field, they facilitate the assessment of direct toxic and chronic sub-lethal effects of test materials under controlled conditions. The purpose of the current project was to assess effects of B. bassiana GHA and M. anisopliae var.

acridum against F. candida. Collembola were exposed to the pathogens in a number of different ways: via direct sprays of conidia; they were fed conidia and mycelia incorporated into or growing on a standard diet; reared in soil contaminated with conidia; and they were fed on diet containing crude filtrates recovered from liquid cultures of the fungi. Although resistant to infection via sprays or feeding, some side-effects were observed when constantly fed on actively-growing cultures. Results of the tests will be presented and discussed.

15:00 **BEAUVERIA BASSIANA AS A COFFEE ENDOPHYTE.**

Francisco Posada, Fernando Vega, *Insect Biocontrol Lab., USDA, ARS, Bldg. 011A, Beltsville, MD 20705, USA*

Abstract: The coffee berry borer Hypothenemus hampei (Ferrari) (Curculionidae: Scolytinae) causes severe losses to coffee throughout the world. The insect's development inside the coffee berry makes it very difficult to control using conventional methods. Alternative pest management strategies are therefore needed, such as the establishment of fungal entomopathogens as fungal endophytes. To assess whether this would be possible with coffee plants, we attempted inoculation with three highly virulent Beauveria bassiana strains selected from fifty that were previously evaluated in bioassays. The methods used for inoculations consisted of injection, soil drenching, spraying, and seed soaking. All methods were effective for introducing B. bassiana into the plant based on subsequent B. bassiana isolation from leaves, stem and roots. Sampling for endophytes in coffee plants collected in Colombia, Hawaii, Puerto Rico and Mexico revealed the presence of B. bassiana from coffee leaves and seeds obtained from red berries from Colombia. One of the endophytic B. bassiana strains was bioassayed against coffee berry borer adults and compared with the three B. bassiana isolates used to inoculate coffee plants with all strains causing 100% mortality by dipping and spraying at 1x10 7 spores ml-1. Our endophyte survey revealed the presence of various entomopathogenic fungi, e.g. Aschersonia sp. and Cladosporium sp. In addition, we isolated Clonostachys rosea (= Gliocladium roseum) as a coffee endophyte (first report for this genus), which caused 82% mortality to the coffee berry borer.

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 10

Contributed Papers (Division of Nematodes)

nematodes / contributed paper session 2

Chair: Arne Peters; David Shapiro-Ilan

13:30 **ORAL TOXICITY OF PHOTORHABDUS TEMPERATA AGAINST THRIPS SPECIES**

Lonne Gerritsen, *Plant Research International, P.O. Box 16, 6700 AA Wageningen, NETHERLANDS*; Jana Georgieva, *University of Sofia, Dept. of Biology, Dragan Tzankov Boulevard, Sofia, BULGARIA*; Rob Van Tol, Gerrie Wiegers, *Plant Research International, P.O. Box 16, 6700 AA Wageningen, NETHERLANDS*

Abstract: The oral toxicity of excretion products of several Photorhabdus and Xenorhabdus strains was tested on two thrips species: Frankliniella occidentalis and Thrips tabaci. Out of 46 Photorhabdus isolates and 6 Xenorhabdus isolates only 6 North American P. temperata isolates were toxic to the thrips species. After 7 days of drinking from P. temperata supernatant a mortality of 90% could be reached. Thrips were also killed after sucking from leaves covered with the toxins. Toxins have a negative effect on thrips fecundity. Possibilities of using P. temperata in the control of thrips will be discussed.

13:45 **ENTOMOPATHOGENIC NEMATODES FOR CONTROL OF THE PINE WEEVIL**

Haukeland Salinas Solveig, *Norwegian Crop Research Institute, NORWAY*

Abstract: The large pine weevil, Hylobius abietis is a major pest of young conifers throughout Europe and Asia. This pest causes severe damage and mortality to conifer seedlings that have not been protected. In consequence it is the only forest pest for which prophylactic chemical treatment is routine in many countries, including Norway. Damage caused by H. abietis in Norwegian forestry is currently estimated to cost more than 2 mill euro per annum. Adult weevils cause damage to conifer seedlings by feeding on the bark. When a site is felled, the volatiles released attract adult weevils from a large area to lay their eggs in the stumps of the felled trees. It takes at least 1.5 years for the larvae to develop and mature, so that when the site is re-planted there are many adults present. The adults feed on the vulnerable transplanted seedlings and in the absence of protection, about 96% of transplanted trees die. The use of prophylactic chemical treatments against large pine weevil is environmentally undesirable. In view of this there is an urgent need for alternative methods of control. In the United Kingdom several field experiments using entomopathogenic nematodes to treat stumps and have proved quite successful and is being used in practice in some forest sites. In Norway our preliminary studies have shown the presence of several naturally occurring entomopathogenic nematodes in forest soils that can be tested against H. abietis. A research program is currently investigating the possibility to reduce damage caused by the large pine weevil by treating stumps to target the developing larvae.

14:00 **USE OF STEINERNEMA CARPOCAPSAE FOR POST HARVEST CONTROL OF NAVEL ORANGEWORM (AMYELOIS TRANSITELLA) IN FALLEN PISTACHIOS**

Joel Siegel, Lawrence Lacey, *USDA/ARS, USA*; Bradley Higbee, *Paramount Farming Company, USA*; Robert , Jr. Fritts, *CertisUSA, USA*

Abstract: The navel orangeworm (NOW), Amyelois transitella, is an important pest of California almonds and pistachios. Previous USDA researchers demonstrated that high concentrations of Steinernema carpocapsae were infectious to NOW larvae in almonds on trees, but did not investigate the susceptibility of NOW in fallen pistachios to infection. The mild San Joaquin Valley winter and its accompanying rains provides an opportunity for the use of nematodes for post harvest control of the NOW in pistachios, either to augment current sanitation practices or as a replacement. Our target NOW population infests nuts on the soil surface or nuts that are shallowly buried (soil depth down to 3 cm). Studies conducted in 2003 during February, March, and April demonstrated that nematodes applied at a concentration of 10 cm2 and an application rate of 3,740 liters/ha followed by 3,740 liters/ha of water caused substantial mortality (>50%) in infested pistachios and almonds used as sentinels. Steinernema carpocapsae was more effective than Steinernema feltiae and produced > 72% mortality at a concentration of 10 cm2 when nighttime temperatures were above freezing. This species was equally effective in bare and leaf-covered plots and had the potential to multiply in the field. Studies were initiated in Fall 2003 and in 2004 to determine the minimum application rate of water, minimum and maximum soil temperature, and minimum soil moisture necessary for successful use of nematodes. Abiotic factors such as soil moisture and soil temperature played an important role in determining the successful outcome after nematode treatment. A large disparity in soil moisture was noted between the berm (2-5% relative saturation) and the drive-row between berms (50%). Soil temperature fluctuated daily as much as 26C in the winter and the maximum soil temperatures were as much as 12C lower when the canopy filled out. The implications of these abiotic factors on the use of nematodes will be discussed.

14:15 **CAN FOLIAR APPLICATIONS OF ENTOMOPATHOGENIC NEMATODES BE ADOPTED FOR COMBATING THRIPS?**

Nasser Halaweh, Christian Borgemeister, Lemma Ebssa, Hans-Michael Poehling, *Hannover University, GERMANY*

Abstract: Entomopathogenic nematodes (EPNs) can successfully control western flower thrips (WFT) Frankliniella occidentalis in the soil, where the majority of the thrips pupate. However, WFT spends most of its lifetime on the leaves or flowers where they feed, reproduce and cause the economic losses. Therefore, we are testing the use of EPNs for control of the foliar-feeding life stages of WFT. In leaf disc bioassays we screened several EPN species/strains,conducted dose rate studies and tested adjuvants to ameliorate the formulations. The Heterorhabditis indica strain LN2 at a concentration of 1,000 IJs/ml formulated in a 0.05% Tween solution proved to be the best combination. Presently we are investigating in greenhouse experiments the potential of H. indica LN2 to control WFT infestations on Chrysanthemum and African violet Saintpaulia ionatha.
*e-mail: nasser@bioinf.uni-hannover.de. Phone: +49 511 762 19259; Fax: +49 511 7623015

14:30 STU **DOES IT MATTER FOR ENTOMOPATHOGENIC NEMATODES IF THRIPS PUPATE AT DIFFERENT SOIL DEPTHS, AND FOR THE THRIPS TO DECIDE WHERE TO PUPATE IF NEMATODES ARE AROUND?**

Lemma Ebssa, Christian Borgemeister, Jörg Semrau, Hans-Michael Poehling, *Hannover University, GERMANY*

Abstract: To study effects of western flower thrips (WFT) Franklin-

iella occidentalis (Pergande) pupation depth on the efficacy of entomopathogenic nematodes (EPNs), Heterorhabditis indica strain LN2 and Steinernema bicornutum were applied at concentrations of 100 and 400 infective juveniles (IJs) cm-2 to WFT that had pupated at 0.5, 1.0, 2.0, 3.0, and 4.0 cm soil depths. Additionally, effects of EPN concentrations of 100 and 400 IJs cm-2 and densities of 20, 50, and 70 second instar larvae of WFT per arena were tested on the pupation depth of WFT. The results indicate that a higher concentration of H. indica was required when the thrips pupated at deeper soil depth. Yet applications of S. bicornutum even at a high concentration resulted in a significantly lower WFT mortality at deeper depths. Under no or low EPN concentrations, up to 80% of WFT pupated at the deepest depth of 3-5cm. However, at higher thrips densities and EPN concentrations, 45-48% of WFT pupated in the medium depth of 1-3 cm. Hence, except for H. indica at a higher concentration it actually matters where thrips pupate. The thrips themselves change their pupation preference in the presence of EPNs. Therefore, the depth of pupation is an important factor when using EPNs for WFT control.
*email: ebssa@ipp.uni-hannover.de. Phone: +49 511 7623553; Fax: +49 511 7623015

14:45 **SCREENING AMONG ENTOMOPATHOGENIC NEMATODE STRAINS FOR VIRULENCE AGAINST DIABROTICA VIRGIFERA VIRGIFERA**

Stefan Toepfer, Ulrich Kuhlmann, Christine Gueldenzoph, *CABI Bioscience Switzerland Centre, SWITZERLAND*; Ralf-Udo Ehlers, *2 Institute for Phytopathology, Department of Phytopathology, Christian-Albrechts-University Kiel, GERMANY*

Abstract: As part of a management strategy against the invasive maize pest, Western Corn Rootworm (Diabrotica virgifera virgifera LeConte, Coleoptera: Chrysomelidae), augmentive biological control against root - feeding larvae or silk - feeding adults with entomopathogenic nematodes (EPN) would be an option. Strains of Steinernema glaseri, S. arenarium, S. abassi, S. bicornutum, S. feltiae, S. kraussei, S. carpocapsae and Heterorhabditis bacteriophora were screened for virulence against second instars and adults of D. v. virgifera in petri-dishes with sand at concentrations of 0.5, 0.8, 7.9 and 15.9 infective juveniles/ cm2. All strains were able to invade and propagate in D. v. virgifera larvae, but adults were rarely found parasitised. At concentrations of 7.9 and 15.9 EPN/cm2, S. glaseri, S. arenarium, S. abassi and H. bacteriophora caused the highest mortality of D. v. virgifera larvae, i.e. 63 and 77 %, 63 and 53 %, 60 and 30 % and 27 and 70 %, respectively. Progeny of S. abassi, S. bicornutum, S. carpocapsae and H. bacteriophora were i.e. 5595 811 (SEM), 5970 779, 6854 1187 and 4039 1025 nematodes per larva, respectively. Significantly less but reasonable propagation of 3336 234 (SEM) nematodes per larva was recorded for S. arenarium, whereas for S. glaseri, S. feltiae and S. kraussei very low nubmers of offspring were recorded. Steinernema glaseri, S. arenarium, S. feltiae, S. kraussei, and Heterorhabditis bacteriophora were screened for their virulence against second instar D. v. virgifera larvae in sand - filled trays with maize plants atconcentrations of 16.7 EPN/cm2. Larval mortality obtained with S. arenarium, S. feltiae, and H. bacteriophora were 67% 3.5 (SEM) , 57 % 17.1 and 77 % 16.6, respectively. S. arenarium and H. bacteriophora are of first priority to be further tested as biological control agents under field conditions.

15:00 **SURVIVAL PATTERNS OF HETERORHABDITIS BACTERIOPHORA IN WATER AND IN FORMULATED PACKAGES**

Arne Peters, *E-nema GmbH, GERMANY*

Abstract: The survival of entomopathogenic nematodes in commercial packages and in aerated flasks was assessed at different incubation temperatures. Two alternative models were fit to the number of surviving nematodes over time, a negative exponential model with a constant mortality rate r and a Normal-Equivalent model with a Normal-distributed probability for dying over time. Infective juveniles of H. bacteriophora decreased with a constant rate when formulated in diatomaceous earth and stored at temperatures of 5, 10 and 15C. At higher incubation temperatures, however, the Normal-equivalent model fitted the data better than the negative exponential. When stored in tap water at 25C, survival was best described with the Normal equivalent model. The different survival patterns might reflect different causes for mortality.

Thursday, August 5^{th}, 2004
Time: 13:30 - 15:30, Lecture Room 1

Symposium (Cross-Divisional)

Microbial control in greenhouses and nurseries

Chair: Jean-Louis Schwartz; Patricia Stock

13:30 **USE OF ENTOMOPATHOGENIC NEMATODES IN THE NORDIC COUNTRIES**

Haukeland Salinas Solveig, *Norwegian Crop Research Institute, NORWAY*

Abstract: There have in recent years been many advances on beneficial organisms for control of pests and an interesting group of organisms in this respect are entomopathogenic nematodes, which are small microscopic worms that kill only insects. Today several commercial products containing different species of entomopathogenic nematodes are available for control of insect pests particularly in protected crops in Europe, USA, Australia, Asia and some other countries.
One of the first reports regarding these nematodes in the Nordic countries was given by Prosper Bovien in Denmark in the early 1930's who described a nematode parasitizing bibionid flies. In late 1970, early 1980, Martin Burman and Albert Pye reported on studies using entomopathogenic nematodes for control of the pine weevil (Hylobius abietis) in Northern Sweden. Since then there have been some reports on the distribution of entomopathogenic nematodes and their use for control of insects from most of the Nordic countries.
Steinernema feltiae, a commonly used entomopathogenic nematode species, also in most Nordic countries, is successfully used to control sciarid flies (Bradysia paupera) in glasshouse crops like Poinsettia. A few other species, Heterorhabditis megidis, H. bacteriophora and more recently S. kraussei are marketed for vine weevil (Otiorhynchus sulcatus) control in protected crops in Europe. In some Nordic countries H. megidis has been used against vine weevil in strawberries and nurseries, but not on a very large scale. The use of these nematodes in Nordic countries, particularly in protected crops, will be reviewed including future prospects.

13:50 **THE EFFECT OF HOST PLANT ON THE EVOLUTION OF BT RESISTANCE IN GREENHOUSE TRICHOPLUSIA NI POPULATIONS**

Alida Janmaat, Judith Myers, *University of British Columbia, CANADA*

Abstract: The microbial insecticide, Bacillus thuringiensis (Bt), has become the mainstay of nonchemical control of Lepidopteran pests either as sprays or through the incorporation of Bt toxins into transgenic crops. Given the wide use of Bt, it is striking that currently only one pest species, Plutella xylostella, has been reported to have developed significant resistance to Bt outside of the laboratory. In contrast, we have observed the frequent and rapid development of resistance to Bacillus thuringiensis kurstaki (Dipel, Abbott) in cabbage looper populations, Trichoplusia ni, in commercial greenhouses.
The current lack of Bt resistance in the field may be due to an inherent instability of resistance in the absence of Bt exposure. Newly arisen resistance traits are often assumed to be associated with a fitness cost. Resistance to Bt does appear to be costly in T. ni as there is a rapid decline of resistance in T. ni populations collected from greenhouses and maintained in the laboratory without selection. However, the repeated and rapid evolution of resistance observed in greenhouse T. ni populations suggests that resistance alleles are maintained in T. ni populations in the absence of Bt sprays. Therefore, it is possible that fitness costs are not as deleterious in the wild as in the laboratory.
The host plant is one important environmental factor that impacts insect herbivores and may play an important role in the evolution of Bt resistance. T. ni is a pest of three different crops grown in commercial greenhouses and larval growth rates vary considerably between crops. The effect of crop on the resistance trait and associated fitness costs was examined to determine how resistance evolution and stability varies between each cropping system. Knowledge of such genotype-environment interactions will allow us to develop crop specific Bt resistance management strategies and to further our understanding of how herbivorous insects adapt to new circumstances.

14:10 **FIELD EFFICACY OF EPNS IN NURSERY AND TREE APPLICATIONS**

Rob Van Tol, *Plant Research International, Wageningen-UR, NETHERLANDS*; Michael Raupp, *University of Maryland, Central Maryland Research and Education Center, USA*

Abstract: Although EPNs have become an increasing successful mean to control several soil borne pests in ornamental tree production, results in the field are still variable. In contrast to the reliable and only limited varying efficacy of agrochemicals, this variation in EPN efficacy is an important limiting factor for the large-scale acceptance and use in pest control. To determine what factors influence this variation we analysed a large number of field data where EPNs are applied in ornamental trees and shrubs as well as information available about growers' perception to EPN use and economic factors. The results reveal that several management and economic aspects like protocols for monitoring and total pest control,

qualified support by extension service, labour costs for pest monitoring and visibility of control as well as reliability of the commercial EPN products are important limiting factors. Important factors causing variable field efficacy by the EPN products are quality variation of the products, limited persistence of activity after application, EPN species/strains used in the products, plant species, application timing (autumn vs. spring) and pot or field application. The field results indicate that the tritrophic interaction between plant species, insect and EPN species/strain used is more important for control than assumed before. Many of these and other field factors need more research to understand their influence on efficacy and improve the product reliability.

14:30 **STU** **DOES BEAUVERIA BASSIANA DISRUPT GREENHOUSE BIOLOGICAL CONTROL?**

Roselyne Labbé, Jacques Brodeur, Conrad Cloutier, *Université Laval, CANADA*; David Gillespie, *Pacific Agriculture and Agri-Food Canada Research Centre, CANADA*

Abstract: Interactions between natural enemies of the greenhouse whitefly, Trialeurodes vaporariorum, may influence the population dynamics of the pest. The predator Dicyphus hesperus, the parasitoid Encarsia formosa and the hyphomycete Beauveria bassiana (Botanigard, Emerald Bio, Lansing MI) were evaluated for concurrent control of the greenhouse whitefly. Experimental greenhouses were divided into eight compartments of 10 tomato plants each, four of which were randomly assigned for Botanigard treatment and the remaining four to be left untreated. Whitefly introductions were made to establish preliminary populations of prey, after which parasitoids and predators were also released. Three applications of the entomopathogen were made over 27 days. The pathogen was applied using a hand-held pressurized sprayer at a deposition rate of 5.133 x 103 conidia/mm2. Seven days following the first Botanigard application, whitefly populations in treated compartments were significantly reduced. All whitefly stages including eggs, N1-2, N3-4 and adults were susceptible to infection. Early instars (N1-2) were the most susceptible and adult whiteflies were the least. In addition, the entomopathogen treatment had a significant and negative impact on predator abundance as well as on the rate of predation within compartments. Predator abundance and rate of predation were 25.0% and 81.8% lower in treated compared to untreated compartments respectively. The within-plant distribution of the predator indicates that D. hesperus does not avoid sites where the number of infected whitefly are high. These results suggest an antagonistic interaction between the pathogen and the predator. In contrast during the course of our experiment, parasitoid densities were unaffected by the fungal application, suggesting that the pathogen does not interfere with the parasitoid.

14:50 **SUSCEPTIBILITY OF VARIOUS DEVELOPMENT STAGES OF GLASSHOUSE WHITEFLY TO INFECTION BY ENTOMOPATHOGENIC FUNGUS PAECILOMYCES FUMOSOROSEUS**

Ayhan Gökçe, *University of Gaziosmanpaa, TURKEY*; Mehmet Kubilay Er, *University of Sütçü Ýmam, TURKEY*

Abstract: Virulences of five Paecilomyces fumosoroseus isolates, (ARSEF 2658, 4400, 4406, 4408 and 4415), were investigated on egg, nymphal, pupal and adult stages of Trialeurodes vaporariorum with single dose (107spores/ml) treatments under laboratory conditions. Two millilitres of condial suspensions of each isolate were applied to excised tomato leaflets bearing eggs, nymphs or pupa or to directly adults using a Potter spray tower. The results of this single dose screening on different developmental stages of glasshouse whitefly showed that all the tested isolates of P.fumosoroseus were able to kill whiteflies under the experimental conditions employed here. The time taken to achieve a kill was often as short as 3 days, but after 6 days of incubation a large proportion of the population was usually infected. The test also revealed that there was intraspecific variation in virulence of the five isolates of P.fumosoroseus on each developmental stage of glasshouse whitefly. Moreover, the susceptibility of the insect to fungal infection varied depending on its developmental stage. On all the stages, except the third stage nymphs, P.fumosoroseus isolate 4415 was the most pathogenic at the end of 6-day incubation. isolate 4400 was the most pathogenic on the third stage nymphs. Several isolates of P.fumosoroseus were virulent on specific stages of the life cycle.

15:10 **VARIABILITY IN RESPONSES OF DISCRETE LABORATORY POPULATIONS OF WESTERN FLOWER THRIPS, FRANKLINIELLA OCCIDENTALIS (PERGANDE) TO ENTOMOPATHOGENIC FUNGI**

Michael Brownbridge, *Entomology Research Laboratory, Univ. of Vermont, U.S.A.*; Stephen Goodwin, W.G. Liang, Marilyn Y. Steiner, *NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford, AUSTRALIA*; Ken Fry, *Alberta Research Council, Vegreville, CANADA*

Abstract: A collaborative research project was undertaken to evaluate entomopathogenic fungi against geographically-discrete populations of western flower thrips, a major pest of greenhouse and field vegetable and ornamental crops. Using a standardised laboratory bioassay technique involving a single spore dose, a common collection of promising fungal isolates of Beauveria bassiana, Metarhizium anisopliae and Verticillium lecanii are being tested against second instar and adult female western flower thrips in Australia, Canada and the USA. Data illustrate a variability in response by different developmental stages, and according to the geographic location of the target organism. Results of the research to date and future directions in the development of these fungi as effective microbial control agents are presented.

Thursday, August 5^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 12

Workshops (Division of Microbial Control)

Status of microbial control products

Chair: Wendy Gelernter; Jeff Lord

Thursday, August 5^{th}, 2004
Time: 16:00 - 18:00, Lecture Room 1

Workshop (Cross-Divisional)

SIP education workshop

Chair: Helen Roy; Jorgen Eilenberg

16:00 **TEACHING ASPECTS OF MICROBIAL CONTROL AS A COMPONENT OF UNDERGRADUATE COURSES**

Helen Roy, *Department of Life Sciences, APU, UK*

Abstract: Microbial control is included as a component of several courses (modules) taught within the Department of Life Sciences, Anglia Polytechnic University including: Commercial Applications of Microorganisms, Insect Natural History and applied Ethology and Animal Welfare. The aims, objectives and learning outcomes of these modules are very varied. Both formal lectures and practical classes are included in these programmes and the emphasis varies depending on the module requirements.

As the title suggests in Commercial Applications of Microorganisms the emphasis is on the development of microorganisms for commercial ventures and microbial control is taught alongside many other examples of biotechnology. In this module I contribute lectures covering introductory entomology and biological control followed by more specific detail on fungal, bacterial and viral control measures. I have designed a practical class to assess growth of two different isolates of B. bassiana at different temperatures, humidities and on different media. A different perspective is given on Insect Natural History where the ecology of insect pathogens and the interactions with their hosts and other natural enemies are considered. The interactions between three aphid natural enemies are assessed within a practical class. Applied Ethology and Animal Welfare is a very diverse module with considerable attention given to vertebrate welfare issues and my contribution is on applied and theoretical aspects of microbial control as a component of manipulating insect behaviour to enhance invertebrate pest control.

Each teaching situation presents particular constraints and challenges. I will describe these concentrating my discussions on the problems (and solutions) of running undergraduate practical classes using entomopathogenic fungi. I will consider in detail a laboratory class titled: Aphid Natural Enemy Interactions: A Laboratory Experiment in which the mortality of aphids due to single or multiple natural enemies (predator, parasitoid and entomopathogenic fungus) is assessed and compared. The schedule for this practical class has been published in the Ecological Projects Compendium, a new British Ecological Society initiative which could be a useful resource for University lecturers.

16:20 **EXPERIENCE WITH A LECTURE COURSE AND TWO EXPERIMENTAL LABORATORY COURSES IN BIOLOGICAL CONTROL**

Jørgen Eilenberg, *Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*; Dan Funck Jensen, John Hockenhull, *Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871 Frb. C, DENMARK*; Holger Philipsen, *Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK*

Abstract: At our university biological control has for a long time been an element of the teaching courses in applied entomology and plant pathology. Since 1988 we have, however, developed courses with the focus on biological control. The expected background is that participants have passed courses in applied entomology and plant pathology. Today we offer three English spoken courses in biological control:
1) A lecture course covering biological control of pests and plant diseases and to a limited extent also weeds 2) An experimental laboratory course on biological control of insects 3) An experimental laboratory course on plant diseases
Students from Denmark, EU and abroad are attending the courses. Most students have a background in agronomy or horticulture, but also students from other areas attend (forestry for example). This range of previous experience offers a challenge since the students know different cropping systems and thus different insects and plant diseases.
We need in the lecture course to pay attention to biological control as a concept, which applies to many areas, and at the same time pay less attention to specific names of species. Parts of the lecture course are analytical: the students read scientific articles and the articles are discussed in the class. The terminology used in plant pathology and entomology differs. In our mutual lecture course we spend initially some time discussing a uniform terminology with the students, allowing them (and us!) to see biological control as a universal concept for all plant protection, but of course with discipline specific terms.
Our laboratory courses are organised as projects, executed by teams of four-five students, aiming to produce new results rather than as a series of planned exercises. A typical project team in insect biocontrol will, for example, first sample insect pathogens from a field. The students then decide which sort of experiments they will perform: characterization ? sampling over time ? bio-assays ? microscopy ? An important point is that the team has its own, unique isolates and obtains novel results. Of course this needs extensive supervision, and we cannot at the same time guarantee that each team gets the chance to try all relevant techniques. Some teams pass critical moments ('what are we aiming to achieve?). But in the final evaluation the students always stress that at the bottom line they feel that have gained both subject specific knowledge on biological control and considerable experience in scientific thinking.
We are continuously developing the teaching of biological control for example, we have recently decided to start an internet-based basic course ('e-learning') on biological control.

16:40 **MICROSPORIDIA AND BIOLOGICAL INVASIONS**

Alison Dunn, *University of Leeds, UK*; Calum MacNeil, *Queens University, Belfast, UK*; Jolene Slothouber-Galbreath, *University of Leeds, UK*; Jaimie Dick, *Queens University, Belfast, UK*

Abstract: Biological invasions are global threats to biodiversity and resources. Recent studies have highlighted the role of parasites in determining invasion outcomes. We present a study of the role of microsporidia in freshwater invasions in the UK. We find that Pleistophora mulleri infects the native amphipod crustacean Gammarus duebeni but is absent from 3 species of invader. Although the parasite has no direct effect on host survival, parasitised hosts show altered behaviour. They show reduced predatory abilities and are more vulnerable to predation by invaders. By altering predation patterns the parasite can determine the outcome of biological invasions. Practical and field projects using microsporidia can highlight the need to consider the impact of parasites on community structure and biodiversity.

17:00 **THE ROLE OF ROTHAMSTED RESEARCH IN EDUCATION AND TRAINING IN MICROBIAL CONTROL**

Judith K. Pell, Paresh A. Shah, Judy Mann, Brian R. Kerry, Brenda Ball, *Rothamsted Research, UK*

Abstract: Rothamsted scientists make regular contributions to seminar programmes and courses at universities. Although formal taught courses are not offered at Rothamsted, we play a prominent role in the training and education of postgraduate students through research. These students (currently 75) may be based for part or all of their training at Rothamsted and registered with universities. Students follow a formal training programme approved by the academic partner which provides a broad base of transferable skills in preparation for postdoctoral employment. Their progress is tracked and monitored at regular intervals and they are mentored throughout. Projects vary from fundamental epidemiological studies to applied research. In addition, Fellowships are available through the Rothamsted International Fellowship Scheme. This is a charity supported entirely by donations. These fellowships are unique and provide opportunities for mid-career scientists from developing countries to work at Rothamsted. Fellows contribute to research projects and are involved in both providing and receiving research training in areas that are appropriate for application on their return home. This helps to build research capacity and ensure that the benefits of sustainable productive agriculture are available to all. Projects emphasise the quality of science and the potential to achieve impact in the developing country concerned by continued collaboration through post-fellowship projects. Rothamsted also plays a significant role in public access to science both in the UK and overseas and is committed to the transfer of technology and knowledge developed in research to the end-users. Rothamsted has its own technology transfer organisation, Rothamsted Research Association (RRA), which aims to link arable crop farmers to research scientists and the latest information on pests and diseases. We are involved in the training and education of lay people who do not necessarily have a background in science or an understanding of the principles and practice of microbial control. This includes institute open days, farmer events (e.g. 'Cereals' the Home Grown Cereals Association (HGCA) annual event, Honey Show), events for the general public (e.g. the Royal Show), meetings for specialist organisations (e.g. British Beekeepers Association (BBKA), Farming and Wildlife Advisory Group (FWAG)) and workshops (e.g. farmers/ microbial production companies in developing countries).

17:20 **TEACHING PEST MANAGEMENT AND BIOLOGICAL CONTROL TO THE END-USER**

Wendy Gelernter, *PACE Consulting, USA*

Abstract: Teaching complex scientific ideas to a lay audience, without falling into the trap of over-simplification, challenges the communication skills of many scientists. And when the lay audience is composed of end-users growers, plant production managers or agricultural policy-makers the challenge grows to include ethical and legal considerations that can sometimes conflict with the responsibility to provide practical, bottom line recommendations. To illustrate the interplay among these competing concerns, printed materials, website publications and Power Point presentations will be reviewed. Suggestions for improved communications with end-users will be proposed.

Thursday, August 5^{th}, 2004
Time: 19:00 - 24:00, Marina Congress Center

Banquet

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