Society for Invertebrate Pathology

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Membership
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Kerstin Jung

Founder’s Lecture
Dudley Pinnock (Chair)
David Ellar
John Vandenburg
Max Bergoin

Meetings
Mark Goettel (Chair)
Brian Federici
Lawrence Lacey
Flavio Moscardi

Endowment & Financial Support
Wendy Gelertner (Chair)
Juerg Huber
Michael Dimock
Pat O’Leary

Awards & Student Contest
Stephen P. Wraight (Chair)
Andreas Linde
Nguya K. Maniania
Bryony Bonning

Publications
David Onstad (Chair)
Margaret Rotstein
Brian Federici
Leellen Solter
Hisanori Bando
Albrecht Koppenhofer
Doreen Winstanley
Harry Kaya
Just Vlak
Suzanne Thiem

2004 ANNUAL MEETING ORGANIZING COMMITTEE

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Scientific Program Chair: prof. Jorgen Eilenberg (RVAU, Copenhagen, Denmark)
General Secretary: Dr. Leena Huldén (Univ. Helsinki, Finland)
Treasurer: Dr. Econ. I. Menzler-Hokkanen (Univ. Helsinki, Finland)

The support of the following organizations for the
37th Annual Meeting of the Society for Invertebrate Pathology and
The 7th International Conference on Bacillus thuringiensis
is gratefully acknowledged.

Jorgen Eilenberg, Susanne Vestergaard (DK),
Ingeborg Klingen (NO)
Bjarne Munk Hansen, Jorgen Eilenberg (DK)
Rudolf Wegensteiner (AT), Regina Kleespies (DE)
Ralf Ehlers (DE), Solveig Haukeland Salinas (NO)
Jorgen Eilenberg (DK), John Burand (USA)
Richard Meadow (NO)
Holger Philipsen (DK)

Fungi
Bacteria
Microsporidia
Nematodes
Viruses
Microbial Control
Posters

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Cover photograph © Heikki Hokkanen
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: 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\textsuperscript{nd}, 2004  
Time: 08:00 - 09:30, Lecture Room 1  

Plenary (Cross-Divisional)  
\textbf{Invertebrate pathogens as pests}  

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

Symposium (Division of Bacteria)  
\textbf{Second generation transgenic crops}  

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

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

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

Symposium (Division of Viruses)  
\textbf{Virus ecology}  

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

Symposium (Cross-Divisional)  
\textbf{Honeybee pathology}  

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

Contributed Papers (Division of Viruses)  
\textbf{virus / contributed paper session 1}  

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

Symposium (Division of Nematodes)  
\textbf{Nematodes and cold adaptations}  

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

Contributed Papers (Division of Microbial Control)  
\textbf{microbial control / contributed paper session 1}  

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

\textbf{Poster Session 1: Posters for fungi and bacteria}  

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

Contributed Papers (Division of Viruses)  
\textbf{virus / contributed papers session 2}  

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

Symposium (Division of Fungi)  
\textbf{Insect-fungal associations}  

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

Symposium (Division of Microbial Control)  
\textbf{Bringing pathogens from the laboratory to the field}  

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

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

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

\textbf{Helsinki University reception}  

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

\textbf{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: 10:15 - 12:00, Lecture Room 6

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

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

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: 19:00 - 24:00, Tolkkinen

BBQ
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<th>Date</th>
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<th>Location</th>
<th>Event Details</th>
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<tr>
<td>Wednesday, August 4th, 2004</td>
<td>09:00 - 12:00, Lecture Room 10</td>
<td>Contributed Papers (Division of Microsporidia)</td>
<td>microsporidia / contributed paper session 1</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>09:00 - 12:00, Lecture Room 12</td>
<td>Workshops (Division of Viruses)</td>
<td>Genome analysis methodology -workshop</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>13:30 - 15:30, Lecture Room 7</td>
<td>Symposium (Cross-Divisional)</td>
<td>Fungi and nematodes under unfavorable conditions</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>13:30 - 15:30, Lecture Room 6</td>
<td>Contributed Papers (Division of Fungi)</td>
<td>fungus / contributed paper session 2</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>13:30 - 15:30, Lecture Room 7</td>
<td>Contributed Papers (Division of Microbial Control)</td>
<td>microbial control / contributed paper session 2</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>13:30 - 15:30, Lecture Room 7</td>
<td>Symposium (Division of Bacteria)</td>
<td>Genomics and pathogenesis of invertebrate pathogens</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>16:00 - 18:00, Lecture Room 10</td>
<td>Poster Session 2: ALL other than fungi and bacteria</td>
<td></td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>16:00 - 18:00, Lecture Room 12</td>
<td>Contributed Papers (Division of Fungi)</td>
<td>fungi / contributed paper session 3</td>
</tr>
<tr>
<td>Wednesday, August 4th, 2004</td>
<td>20:00 - 22:00, Lecture Rooms 1, 12</td>
<td>Division meetings: MC, F</td>
<td></td>
</tr>
</tbody>
</table>
Symposium (Division of Bacteria)
New advances in research and development of insecticidal proteins

Workshop (Cross-Divisional)
Risk assessment

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

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

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

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

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

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

Workshop (Cross-Divisional)
SIP education workshop
Program
SIP 2004

**STU** indicates papers being judged for graduate student presentation awards
SIP Council Meeting

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

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, Thorvaldensesvej 40, DK-1871 Frederiksberg C, DENMARK

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.

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

Jaroslaw Weiser, Praha 4, Heraclea 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 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
Chair: Todd Udine

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

Monday, August 2nd, 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 2nd, 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 2nd, 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
<table>
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<tr>
<th>Time</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00</td>
<td>INFECTED HOST'S ROLE IN INFECTION DYNAMICS OF ENTOMOPATHOGENIC NEMATODES</td>
<td>Parwinder S. Grewal, Department of Entomology, Ohio State University, U.S.A.</td>
</tr>
<tr>
<td>10:20</td>
<td>INFECTED HOST INTERACTION WITH ANTAGONISTS</td>
<td>Harry Kaya, University of California-Davis, USA; Heidi Goodrich-Blair, University of Wisconsin -Madison, USA</td>
</tr>
<tr>
<td>10:40</td>
<td>EMERGENCE DYNAMICS FROM THE INFECTED HOST AND QUALITY OF EMERGED NEMATODES</td>
<td>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</td>
</tr>
<tr>
<td>11:00</td>
<td>RESPONSE OF SOIL FAUNA TO INUNDATIVELY AND CADAVER-APPLIED ENTOMOPATHOGENIC NEMATODES</td>
<td>Mary Barbercheck, The Pennsylvania State University, USA; C. Marie Greenwood, North Carolina State University, USA</td>
</tr>
<tr>
<td>10:00</td>
<td>FUNCTIONAL IMPORTANCE OF DELETION MUTANT GENOTYPES IN A NUCLEOPOLYHEDROVIRUS POPULATION</td>
<td>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</td>
</tr>
<tr>
<td>10:50</td>
<td>PERSISTENT INFECTIONS OF BACULOVIRUSES AND CYPOVIRUSES</td>
<td>Rosie Halls, John Burden, NERC Centre for Ecology and Hydrology, UK; Claire Nixon, School of Biological and Molecular Sciences, Oxford Brookes University, UK; Rob Graham, NERC Centre for Ecology and Hydrology, UK; Steve Sall, 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</td>
</tr>
<tr>
<td>11:00</td>
<td>MOLECULAR CHARACTERISATION OF THE EUROPEAN BUMBLE BEE MICROSPORIDIAN PARASITE NOSEMA BOMBI BASED ON RIBOSOMAL RNA AND BETA-TUBULIN GENES</td>
<td>W. T. Tay, School of Biology and Biochemistry, Queens University Belfast, UNITED KINGDOM</td>
</tr>
<tr>
<td>10:20</td>
<td>FUNGAL DISEASES IN BEES: A STORY OF ASCOSPHAERA</td>
<td>Rosalind James, Craig Huntzinger, Ellen Klinger, USDA, ARS, Bee Biology &amp; Systematics Laboratory, USA; Jeff Skinner, Oregon State University, USA</td>
</tr>
<tr>
<td>10:40</td>
<td>MOLECULAR AND BIOCHEMICAL DIFFERENTIATION BETWEEN PAENIBACILLUS LARVAE SUBSP. LARVAE AND PAENIBACILLUS LARVAE SUBSP. PULVIFACIENS.</td>
<td>Elke Genersch, Ainura Ashiralieva, Institute for Bee Research, GERMANY; Jochen Kiliwinski, SVUA Arnberg, GERMANY</td>
</tr>
<tr>
<td>11:00</td>
<td>SAMPLING OF ADULT BEES FOR DETECTION OF AMERICAN FOULBROOD (PAENIBACILLUS LARVAE SUBSP. LARVAE) SPORES IN HONEY BEE (APIS MELLIFERA) COLONIES</td>
<td>Anders Lindström, Ingemar Fries, Swedish University of Agricultural Sciences, Department of Entomology., SWEDEN</td>
</tr>
<tr>
<td>11:20</td>
<td>INVESTIGATING INTERACTIONS BETWEEN VARROA DESTRUCTOR, VIRUSES AND HONEY BEES</td>
<td>Brenda Ball, Judith Wilson, Norman Carreck, Rothamsted Research, UK</td>
</tr>
</tbody>
</table>
### Contributed Papers (Division of Viruses)

#### virus / contributed paper session 1

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

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Authors</th>
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<tbody>
<tr>
<td>13:30</td>
<td><strong>THE PERITROPHIC MATRIX AS A BARRIER TO FATAL BACULOVIRUS INFECTION IN COTTON-FED HELIOTHIS VIRESCENS</strong></td>
<td>Ruth Plymale, Diana Cox-Foster, Dan Jones, Kelli Hoover, Penn State University, USA</td>
</tr>
<tr>
<td>13:45</td>
<td><strong>SELENIUM IMPACTS THE INFECTIVITY OF ACMNVP IN TRICHIOPSIS NI</strong></td>
<td>Holly Popham, USDA ARS Biological Control of Insects Research Laboratory, UNITED STATES; Kent Shelby, USDA ARS Biological Control of Insects Research Laboratory, UNITED STATES</td>
</tr>
<tr>
<td>14:00</td>
<td><strong>INACTIVATION OF PHTHORIMAEA OPERCULELLA GRANULOVIRUS (POGV) DUE TO NATURAL RADIATION AND THE POTENTIAL OF UV-ADJUVANTS FOR VIRAL PROTECTION</strong></td>
<td>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</td>
</tr>
<tr>
<td>14:15</td>
<td><strong>HORIZONTAL AND VERTICAL TRANSMISSION OF WILD-TYPE AND RECOMBINANT HASNPV</strong></td>
<td>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; Zhilong Hu, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, Hubei, CHINA</td>
</tr>
<tr>
<td>14:30</td>
<td><strong>THE POLYHEDRIN GENE OF THE AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDRO-VIRUS IS A MOSAIC OF GROUP I AND GROUP II NPV POLYHEDRIN GENES</strong></td>
<td>Johannes A. Jehle, Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate, GERMANY</td>
</tr>
</tbody>
</table>

### Contributed Papers (Division of Microbial Control)

**microbial control / contributed paper session 1**

**Chair:** Lawrence Lacey; Shawn McLaughlin

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<tr>
<td>13:30</td>
<td><strong>EVALUATION OF COMMERCIAL FORMULATIONS OF THE CODLING MOTH GRANULOVIRUS AGAINST NATURAL CODLING MOTH INFESTATIONS IN PACIFIC NORTH-WEST APPLE AND PEAR ORCHARDS</strong></td>
<td>Steven Arthurs, Lawrence Lacey, USDA-ARS, USA</td>
</tr>
<tr>
<td>13:45</td>
<td><strong>CONTROL OF THE BROWNTAIL MOTH, EUPROCITIS CHRYSORRHOEA, IN THE UNITED STATES WITH A BACULOVIRUS</strong></td>
<td>James Slavicek, USDA Forest Service, USA; Joseph Elkin-ton, University of Massachusetts, USA; John D. Podgwaite, USDA Forest Service, USA</td>
</tr>
<tr>
<td>14:00</td>
<td><strong>DEVELOPMENT OF SPODOPTERA EXEMPTA NUCLEOPOLYHEDROVIRUS (SPEXMNPV) FOR THE CONTROL OF AFRICAN ARMYWORM IN EAST AFRICA</strong></td>
<td>David Grzywacz, Mark Parnell, Natural Resources Institute, UK; Wilfred Mushobza, Pest Control Services, TANZANIA; Ken Wilson, Lancaster University, UK</td>
</tr>
</tbody>
</table>

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Monday, August 2nd, 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 Boushila, 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 Brebèlin, EMPI, Unité INRA UMII 1133, Université de Montpellier II, FRANCE

**B-6** PARTIAL RESISTANCE OF PLUTEILLA XYLSTELLAE TO COMMERCIAL FORMULATES OF BACILLUS THURINGIENSIS IN AGRICULTURAL FIELDS IN MEXICO*

Artemisa Perea, Magdalena Iraichi-Cardenas, Facultad de Ciencias Biologicas/UANL, MEXICO; Rafael Bujuanos-Muniz, INIFAP-Celaya, MEXICO; Luis Galan-Wong, Benito Perez-y-Alerare, 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, Biotecnology & 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, Fukuoaka 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, Mujiang Tang, Yongxia Shi, Wei Xu, Jianxin 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, Mujiang 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

Takio Ichimatsu, Kazuhiho Higuchi, Fukuoaka Industrial Technology Center, JAPAN; Kuniko Kagoshima, Kyushu University, JAPAN; Eiichi Mizuki, Fukuoaka 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, Fukuoaka Industrial Technology Center, JAPAN; Yoshitaka Murata, Masako Nomaguchi, Kyurin Corporation, JAPAN; Hiroyuki Saitoh, Satoko Yamashita, Fukuoaka Industrial Technology Center, JAPAN; Yasuyuki Sasaguri, University of Occupational and Environmental Health, JAPAN; Michio Ohba, Kyushu University, JAPAN
LYOPHILIZATION OF LEPIDOPTERAN MIDGUTS: A PRESERVING METHOD FOR BACILLUS THURINGIENSIS TOXIN BINDING STUDIES

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

MOLECULAR STUDIES OF A BACILLUS THURINGIENSIS PUTATIVE VIRULENCE OPERON

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

A NOVEL TOXIN FROM A BRAZILIAN STRAIN OF BACILLUS THURINGIENSIS REPORTED TO KILL THE COTTON BOLL WEEVIL (ANTHOMOMUS GRANDIS)

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

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 Herzero, Petra L. Bakker, Ruud De Maagd, Plant Research International B.V., THE NETHERLANDS; Juan Ferré, Universidad de Valencia, ESPAA

CRY1C-TOLERANCE STUDIES USING SF9 CELLS AS A MODEL SYSTEM

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

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

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

PURIFICATION AND CHARACTERIZATIONS OF A NEW BACILLUS THURINGIENSIS VIRULENCE FACTOR: THE INHA2 METALLOPROTEASE

Myriam Hajaj, Unité Génétique Microbienn e et Environnement, INRA, la Minière, FRANCE; Michel Gohar, Unité Génétique Microbienn e et Environnement, INRA, Unité Microbiologie et Génétique Microbienn e, INRA, FRANCE; Sind a Fedhila, Unité Génétique Microbienn e et Environnement, INRA, la Minière, FRANCE; Didier Lerleus, Christina Nielsen-LeRoux, Unité Génétique Microbienn e et Environnement, INRA, Groupe Génétique et Physiologie des Bacill us pathogènes, Institut Pasteur, FRANCE

HOST RANGE EXTENSION OF 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 Xinjun, 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

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

Maria A. Ibargetxui, 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

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

Yuko Sakano, Department of Entomology, University of California, U.S.A.; Baoxue Ge, Department of Entomology, 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.

HIGH LEVEL OF CYT1A SYNTHESIS IN BACILLUS THURINGIENSIS SUBSP. ISRAELIENSIS IS DUE TO THREE PROMOTERS AND A STRONG 3’ MRNA STEM-LOOP STRUCTURE

The 20-KDA PROTEIN OF BACILLUS THURINGIENSIS SUBSP. ISRAELIENSIS ENCODES A CRY PROTEIN EFFECTIVE TO KILL THE COTTON BOLL WEEVIL (ANTHOMOMUS GRANDIS)

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 Swiecieka, Jacques Mahillon, UCL, BELGIUM

B-28 THE DEVELOPMENT OF AN ASPOROGENIC STRAIN OF BACILLUS THURINGIENSIS SUBSP. ISRAELIENSIS 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 Penttiäinen, Eija Kalliainen, 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 Schnepl, Independent, USA; Didier Lereclus, Institut Pasteur, FRANCE; Jim Baum, Monsanto, USA; Jereon 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

B-34 IMPROVEMENT OF MYCOINSECTICIDE BY SIMULTANEOUSLY OVEREXPRESSING A SUBTILISIN-LIKE GENE AND AN ENDOCITI-NASE 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 CHYTRIDIONYMECTE 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, Chiquito 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

Mitsuki 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

Marileena Aquino de Muro, Sarah Elliott, CABI Bioscience, UK; David Moore, CABI Bioscience, UK; Bruce Parker, Margaret Skinner, William Reid, University of Vermont, USA; Mustapha El Bouhssini, IICARDA, 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 NEOAPAPHIS REMAUDAIER 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 Flakkehøj, Department of Crop Protection, DENMARK; Jørgen Ellenberg, Department of Ecology, The Royal Veterinary and Agricultural University, DENMARK

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

Karsten Drömm, Jakob Magid, Jørgen Ellenberg, Peter Esbjerg, The Royal Veterinary and Agricultural University, DENMARK
BIOLOGICAL CONTROL OF VARROA Destructor DISSEMINATION AND IMPACT OF SPORE INOCULUM
Caroline Birchall, Rothamsted Research, UK; Gillian Davidson, Warwick HR, UK; Brenda Ball, Judith K. Pell, Rothamsted Research, UK; David Chandler, Warwick HR, UK

THE COST ACTION 842: STATUS OF RESEARCH ON ENTOMOPHTHORALES IN EUROPE
Siegfried Keller, Federal Research Station for Agroecology and Agriculture, SWITZERLAND

INFLUENCE OF ZN ON GROWTH AND PRODUCTION OF ORGANIC ACIDS BY PAECILOMYCYES 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

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; Anne Anderson, Donald Roberts, Department of Biology, Utah State University, USA

THE EFFECT OF AMMONIA ON CONIDIAL LONGEVITY OF BEAUVIERIA BASSIANA AND METARHIZIUM ANISOPLIAE
Tove Steenberg, Ole Kilpinen, Danish Pest Infestation Laboratory, DENMARK; Dave Moore, CABI Bioscience, UNITED KINGDOM

INVESTIGATION OF THE SURVIVAL OF CONIDIA OF ENTOMOPATHOGENIC FUNGI WITH POTENTIAL FOR CONTROL OF VARROA DESTRUCTOR IN HONEY BEE COLONIES
Gillian Davidson, Warwick HR, UNITED KINGDOM; Caroline Birchall, Judith K. Pell, Brenda Ball, Rothamsted Research, UNITED KINGDOM; David Chandler, Warwick HR, UNITED KINGDOM

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.

PRELIMINARY SURVEY OF ENTO-MOPATHOGENIC 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

CICADAPEPTINS, NEW AIB-CONTAINING PEPTIDES,
Stuart B. Krasnow, Department of Plant Pathology, Cornell University, U.S.A.; Donna M. Gibson, USDA, ARS, Plant Protection Research Unit, U.S.A.; Melissa Wagenaar, Ricardo Réategui, James B. Gloer, Department of Chemistry, University of Iowa, U.S.A.

STUDY OF THE SPORULATION OF PAECILOMYCYES FUMOSOROSEUS VARYING CARBON AND NITROGEN SOURCE
Ana Gabriela Osuna Paez, Consejo Estatal de Ciencia y TECHNOLOGY, MEXICO; Héctor Manuel Cárdenas Cota, Centro de Ciencias de Sinaloa, MEXICO; Rene Castro Monroy, Facultad de Fisicomatemáticas, Ciudad Universitaria, MEXICO

SUSCEPTIBILITY OF THE CEREAL APHID METOPOLOPHIDIRHODUM TO THE ENTOMOPATHOGENIC FUNGUS PANDORA NEOAPHIDIS ON GNA Wheat
Paresh A. Shah, Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM; A.M.R. Gathhouse, School of Biology, King George VI Building, University of Newcastle, UNITED KINGDOM; Judith K. Pell, Plant and Invertebrate Ecology Division, Rothamsted Research, UNITED KINGDOM

EFFECTIVENESS OF LOCAL FUNGAL ISOLATES FOR COLORADO POTATO BEETLE IN UZBEKISTAN
Kerim K. Ergashev, Erkin N. Abdullaev, Samarkand State University, UZBEKISTAN

DIFFERENTIAL SUSCEPTIBILITY BETWEEN DIAPAUSING AND NON-DIAPAUSING COLORADO POTATO BEETLES (Leptinotarsa Decemlineata) TREATED WITH BEAUVIERIA 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

METHODS FOR RISK ASSESSMENT OF BIOLOGICAL CONTROL PROGRAMS IN THE SAHELIAN REGION
Eva Norhe Fisker, Niels Elmegaard, The Danish National Environmental Research Institute, DENMARK; Jörgen Elenberg, The Royal Veterinary and Agricultural University, DENMARK; Christiana Kuyman, Jürgen Langwald, 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 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

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

Karim 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


F-31 IN VIVO PATHOGENICITY OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE ON CHROTOGONUS TRACHYPETERUS(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 LABOULBEINALES SPECIES ACTUALLY SPATIALLY MONOPHAGOUS SPECIES?

Larry Hulden, Finnish Museum of Natural History, FINLAND

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Monday, August 2nd, 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 REPLICAION OF A NOVEL PICORNALIKE VIRUS OF THE GENUS IFLAVIRUS

Juliette Ongus, Dick Peters, Just M. Vlak, Wageningen University, THE NETHERLANDS; Eberhard Bengsch, Centre de Biophysique Moleculaire, 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, Wuhan 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 DENVSVIRUSES (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 Félédère, Institut de Recherche pour le Développement, Faculty of Agriculture, Cairo University, EGYPT; François Couserans, Elizabeth Baquerizo, François-Xavie Jousset, Laboratoire de Pathologie Comparée, Université Montpellier II, FRANCE; Peter Tijssen, Mohamed El-Far, INRS-Institut Armand-Frappier, Université du Québec, CANADA

17:00 SPLITMNVP 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 (AGMNVP) GENOME
Jose Luiz Caldas Wolff, Laboratorio 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 García-Maruniak, James Maruniak, Entomology & Nematology Department, University of Florida, Gainesville, FL, USA; Flavio Moscardi, Embrapa/CNPSO, Londrina, PR, BRAZIL; Marilinda Lobo de Souza, Maria Elita Batista de Castro, Embrapa/CENARGEN, Brasilia, DF, BRAZIL; Paulo M. de A. Zanotto, Embrapa/CNPSO, Londrina, PR, USA; Juan-Pablo Elguizo, Instituto de Ciencias Biomédicas, 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 Naclacioglu, Just M. Viak, Wageningen University, THE NETHERLANDS; Zihlin Demirbag, Karadeniz Technical University, TURKEY; Monique M. Van Oers, Wageningen University, THE NETHERLANDS

Monday, August 2nd, 2004
Time: 15:00 - 18:00, Lecture Room 12
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 Bidoehla, 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 2nd, 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:
15:30 DEVELOPING A MICROBIAL: CHOOSING THE RIGHT FUNGAL STRAIN

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

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 2nd, 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 Bohe 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 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
Tuesday, August 3rd, 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 VAPORARIO-RUM IN MEDITERRANEAN TOMATO GREENHOUSE
Jacques Fargues, Thierry Boulard, Benoît Jeannequin, INRA, FRANCE

08:15 INFLUENCE OF TEMPERATURE PREFERENCE OF TWO RDNAA-ITS LINEAGES OF PAE-CILOMYCES FUMOSOROSEUS ON THEIR CO-INFECTION PATTERN
Jacques Fargues, INRA, FRANCE; Marie-Claude Bon, EBCL/USDA-ARS, FRANCE

08:30 EFFECT OF INITIAL HIGH HUMIDITY EXPOSURE ON THE EFFICACY OF LECANI-LICILLUM LECANII BLASTOSPORES AGAINST THE HEMLOCK WOOLLY ADELGID ADHELGE TSUGAE ANNAND (HOMOPTERA: ADELGI-DAE).
William Reid, Vladimir Gouli, Svetlana Gouli, University of Vermont, USA

08:45 GERMINABILITY OF METARHIZIUM ANISO-PLIAE AND BEAUVERIA BASSIANA CONIDIA IN THE PRESENCE OF COMMON SOIL AND PHYLOPLANE FUNGI
Richard Meadow, Norwegian Crop Research Institute, NOR-WAY; Linda Gordon Hjeljord, Agricultural University of Norway, NORWAY

09:00 DOSE DEPENDENT ACQUISITION OF BEAU-VERIA 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 REDUCING ADULT LIFE SPAN OF MALARIA (ANOPHELES GAMBIAE S.L.) AND FILARIA-SIS (CULEX QUINQUEFASCATUS) VECTORS USING THE ENCOMPATHOGENIC FUNGUS METARHIZIUM ANISOPLIAE: A FIELD STUDY IN TANZANIA
Ernst-Jan Scholte, Wageningen University and Research, THE NETHERLANDS; Kija Ng’abí, 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 POP-ULATIONS
Michael McGuire, Jarrod Leland, USDA-ARS, USA

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 RE-LEASE OF EUROPEAN GYPSY MOTH MI-CROSPORIDIA 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 ESTABLISHMENT OF PARANOSEMA (NOSEMA) LOCUS-TAE IN GRASSHOPPERS (ORTHOPTERA: ACRIDIDOIDEA) OF ARGENTINA.
Carlos Lange, CEPAVE, CIC-UNLP-CONICET, ARGENTINA; María Laura De Wysiecki, CEPAVE, UNLP-CONICET, ARGENTINA

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

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

Chair: Trevor Jackson; Suzanne Thiem

08:00 THE ORYCTES BACULOVIRUS: ITS DETEC-TION, 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
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

ORYCTES VIRUS TIME FOR A NEW LOOK AT A USEFUL BIOCONTROL AGENT
Trevor Jackson, Travis Glare, AgResearch, NEW ZEALAND

Tuesday, August 3rd, 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 HELIOTRIS 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 ISRAELIENSIS
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 (CYTICA) FROM B. THURINGIENSIS ISRAELIENSIS 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; Prim- itivo Caballero, Departamento de Produccion Agraria, Universidad Pública de Navarra, SPAIN

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 3rd, 2004
Time: 10:15 - 12:00, Lecture Room 1
Society General Meeting
Chair: Harry Kaya

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, Nuukoso
Excursion 1: Nuukoso 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, Nuukoso
Excursion 2: Nuukoso 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 15:00 by bus from UH Main Building

Tuesday, August 3rd, 2004
Time: 19:00 - 24:00, Tokkkinen
BBQ
Wednesday, August 4th, 2004
Time: 09:00 - 12:00, Lecture Room 10

Contributed Papers (Division of Microsporidia)

**microsporidia / contributed 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**

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; Jaroslav Weiser, Heraclea 964, 140 00 Praha 4, CZECH REPUBLIC; Z. Ziska, Electron Microscopy, Inst. of Microbiology, Acad. Sci., CZECH REPUBLIC

11:00 **THE CYST LIKE SPOREPHOROUS VESICLE OF CHYTIDIOPSIS TYPOGRAPHI**
Rudolf Wegensteiner, Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU - University of Natural Resources and Applied Life Science, AUSTRIA; Jaroslav Weiser, Emeritus, Institute of Entomology, Academy of Sciences of the Czech Republic, CZECH REPUBLIC

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Wednesday, August 4th, 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 S˜ ao Paulo, USP, Sao Paulo, SP, BRAZIL; Ricardo Pereira, Instituto de Matemática e Estatística - IME, Universidade de S˜ ao 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

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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 ENТОMOPATHOGENIC NЕМАТОDES 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; Edla Sigurðís Óddísdottr, Gudmundur Hallidóttsson, Iceland Forest Research, Mogilsa, IS 116, Reykjavik, ICELAND; Tróndur Leivsson, Forestry Service of the Faroe Islands, Hvítanesvegur 5, 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 4th, 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 Polytechnical University, UK; Judith K. Pell, Rothamsted Research, UK; Alison Thomas, Anglia Polytechnical University, UK

13:45 BIOCHEMICAL, MORPHOLOGICAL AND PATHOGENICITY VARIATIONS IN BEAUVERIA BASSIANA ISOLATES
Reza Talaei Hassanlou, Aziz Kharazi Pakdel, Dep. Plant Protection, College of Agriculture, University of Tehran, IRAN; Mark Goettel, Lethbridge Research Centre, CANADA; Javad Monaffari, 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 4th, 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
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 4th, 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 ENDSYMBIONT OF ENTO-MAPATHOGENIC NEMATODES

Eric Duchaud, Atelier de Bioinformatique, 12 rue Cuvier, 75252 Paris Cedex 05, FRANCE; Alain Givaudan, Noël Boemare, Laboratoire de Pathologie Comparée, 2, 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 Sasaki, 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

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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 MAGNANINA
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 ENTHOMOPOXVIRUS FIELD ISOLATES FROM JAPAN
Jun Takatsuka, Shohei Okuno, Takayoshi Ishii, Yuko Takehashi, 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 ENTHOMOPOXVIRUS
Aki Fujimoto, Tokyo University of Agriculture and Technology, Japan; Shohei Okuno, Takayoshi Ishii, Jun Takatsuka, Kazuko Nakanishi, Madoka Nakai, Yasuhisa Kunimi, University 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, Xiuliian 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?
Serafin Gutierrez, Miguel Lopez-Ferber, Laboratoire de Pathologie Comparee INRA/CNRS/Universite 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 Moecey, 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 spp. NUCLEOPOLYHEDROVIRUSES IS NOT DETERMINED BY VIRUS ENTRY OR THE PRIMARY INFECTION CYCLE
Oihane Simon, Trevor Williams, Departamento de Produccion Agraria, Universidad Publica de Navarra, Spain; Miguel Lopez-Ferber, 2Laboratoire de Patologie Comparee, UMR 5087, INRA-CNRS-Universite de Montpellier II, France; Primitivo Caballero, Departamento de Produccion Agraria, Universidad Publica de Navarra, Spain

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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, 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; Trevor Williams, Primitivo Caballero, Departamento de Producción Agraria, Universidad Pública de Navarra, SPAIN

NUCLEOPOLYHEDROVIRUS (SEMNPV) AND OPTICAL BRIGHTENER FORMULATIONS FOR CONTROL OF SPODOPTERA EXIGUA IN GREENHOUSES IN SOUTHERN SPAIN

Rodrigo Lasas, 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

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

IDENTIFICATION OF A NOVEL SHRIMP PROTEIN PHOSPHATASE AS THE INTERACTING PARTNER FOR LATENCY-ASSOCIATED PROTEIN ORF427 OF WHITE SPOT SYNDROME VIRUS

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

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

EUROPEAN LEUCOMA SALICIS MNPV IS CLOSELY RELATED TO ORGYIA PSEUDOT-SUGATA 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. Ylak, Laboratory of Virology Wageningen University, NETHERLANDS

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

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

DANISH CENTRE FOR BIOLOGICAL CONTROL

Jørgen Elenenberg, Department of Ecology, The Royal Veterinary and Agricultural University, Thorsøervej 40, DK-1871 Frederiksberg C, DENMARK; Annie Enkegaard, The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkehøj, 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, Thorsøervej 40, DK-1871 Frederiksberg C, DENMARK; Jørgen Brøchner Jespersen, The Danish Institute of Agricultural Sciences, Danish Pest Infestation Laboratory, Skovbrynet 14, 2800 Lynghøj, DENMARK; John Laren, The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkehøj, 4600 Slagelse, DENMARK; Anne Mette Madsen, The National Institute of Environmental Health, Department of Chemical Working Environments, Lerkparkallé 105, 2100 Kbh., DENMARK; Hans-Peter Ravn, Forest and Landscape, Department of Applied Ecology, Horsholm Kongevej 11, 2970 Hørsholm, DENMARK

QUALIFICATION AND QUANTIFICATION OF CULTURABLE MICROORGANISMS IN MARKETED MICROBIAL PEST CONTROL AGENTS

Anne Winding, Bjarne Munk Hansen, Dept. Env. Chem. Microbiol., NERI, DENMARK; Anita Enkegaard, The National Institute of Environmental Health, Department of Chemical Working Environments, Lerkparkallé 105, 2100 Kbh., DENMARK; John Larsen, The Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkehøj, 4600 Slagelse, DENMARK; Niels Bohse Hendriksen, The National Environmental Research Institute, Department of Environmental Chemistry and Microbiology, Frederiksborgvej 399, 4000 Roskilde, DENMARK

EFFECTS OF SEVERAL ABIOTIC FACTORS ON THE VIRAL ENHANCING ABILITY OF THE SPINDLE OF ANOMALA CUPREA ENTOMOPOXVIRUS

Wataru Mitsuhashi, Ritsuko Murakami, Kazuhisa Muto, National Institute of Agrobiological Sciences, JAPAN
MC-6 EFFECT OF BEAUVERIA BASSIANA, VERTICILLIUM LECANII, BACILLUS THURINGIENSIS SUBSP. TENEBRIONIS AND AZADIRACHTIN COMPOUNDS ON SITOPHILUS ORYZAE (L.) AND TRIBOLIUM CONFUSUM DU VAL IN STORED RYE

Dimitris Kontodimas, Nicholas Karallieratos, Spyridon Mantzoukas, Benaki Phytopathological Institute, GREECE; Christos Athanassiu, Agricultural University of Athens, GREECE; Maria Anagnostou-Veromikis, 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; Francilene Angelotti, Universidade Estadual de Maringá, BRAZIL; Ivan T.V. Licursi, Fundação Djalma Giacometti, BRAZIL; Eduardo Poletto, 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, Hannelie Joonde, Astrid Jankelsohn, 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; Yuyong He, Laboratory of Insect Ecology, CHINA; Chuck Quimby, USDA Agriculture Research Service, US Plant, Soil and Nutrition Laboratory, U.S.A.; Stephen P. Wraight, Department of Entomology, Cornell University, U.S.A.

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

Yuriy Tokarev, Maxim Levchenko, Anton Naumov, George Lednev, All-Russian Institute 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 ANTHOCYANISMA 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. Grosi de Sa, Maria Cristina Mattar da Silva, Rafael Perseghini Del Sarto, Marise Ventura Coutinho, Edson Luiz Zangrando Figueira, Embrapa Recursos Genéticos e Biotecnologia. Parque Estação 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, Heikki M. T. Hokkanen, Applied Zoology, University of Helsinki, FINLAND; Lasse Smidt, National Institute of Occupational Health, DENMARK; Niels Bohse Hendriksen, National Environmental Research Institute, DENMARK; Jørgen Eilenberg, Agricultural University, DENMARK; Bjarne Munk Hansen, National Environmental Research Institute, DENMARK; Zhiming Yuan, Wuhan Institute of Virology, CHINA; Gert Bolander Jensen, National Institute of Occupational Health, DENMARK; Xiaomin Hu, Wuhan Institute of Virology, CHINA; Bjarne Munk Hansen, National Environmental Research Institute, DENMARK; Jørgen Eilenberg, Agricultural University, DENMARK; Lasse Smidt, National Institute of Occupational Health, DENMARK; Niels Bohse Hendriksen, National Environmental Research Institute, DENMARK; Jørgen Eilenberg, Agricultural University, DENMARK; Bjarne Munk Hansen, National Environmental Research Institute, DENMARK; Zhiming Yuan, Wuhan Institute of Virology, CHINA; Gert Bolander Jensen, National Institute of Occupational Health, DENMARK; Xiaomin Hu, Wuhan Institute of Virology, CHINA; Bjarne Munk Hansen, National Environmental Research Institute, DENMARK; Jørgen Eilenberg, Agricultural University, DENMARK; Lasse Smidt, National Institute of Occupational Health, DENMARK; Niels Bohse Hendriksen, National Environmental Research Institute, DENMARK; Jørgen Eilenberg, Agricultural University, DENMARK.
MC-20 THE INTERACTION BETWEEN ROOT HERBIVOROUS LARVAE AND BENEFICIAL SOIL ORGANISMS IN NURSERY PEAT VS. FOREST SOIL - A POT EXPERIMENT.
Edda Sigurðís Oddsdóttir, Icelandic Forestry Research, ICELAND; Jørgen Eilenberg, The Royal Veterinary and Agricultural University, DENMARK; Robin Sen, Department of Biosciences, University of Helsinki, FINLAND; Gudmundur Hallgríms, Icelandic Forestry Research, ICELAND

MC-21 NATURALLY OCCURRING INSECT PATHOGENIC FUNGI ON KEY COFFEE PESTS, AND THE INFLUENCE OF MANAGEMENT PRACTICES
Arnulfo Monzón, 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, Heikiki M. T. Hokkaiden, Laboratory of Applied Zoology, FINLAND

N-1 ECOLOGICAL CHARACTERIZATION OF HETERORHABDITIS SP. (CABORCA STRAIN) (NEMATODE: HETERORHABDITIDAE), A NATURAL PATHOGEN OF DICEROPOCCTA 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 Meta 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, Institute of Cytology Russian Academy of Sciences, St.Petersburg, RUSSIA; Irma Isi, Yuriy Tokarev, Institute for Plant Protection, St.Petersburg, RUSSIA; Elena Morzhina, Institute 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, Toshihiko Hukuhara, Nihon University, JAPAN

CA-1 TEMPERATURE AND THE NORTHERN RANGE OF PLASMODIUM VIVAX IN EUROPE
Lena Huldén, University of Helsinki, FINLAND

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

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

16:00 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 Bioscatalysis 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 the University of California-Riverside, USA; Alejandra Bravo, Mario Soberón, Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO

16:15 ANALYSIS OF THE INTERACTION BETWEEN CRY11A AND CYTIA 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 "Résponses des Organismes aux Stress Environnementaux, FRANCE; Frédéric Luton, IPMC, CNRS-UMR 6097, FRANCE; Claude Castella, INRA, UMR 1112 "Résponses des Organismes aux Stress Environnementaux, FRANCE; Jean-François Charles, Institut Pasteur, Unité de génétique des génomes bactériens, FRANCE; David Pauron, INRA, UMR 1112 "Résponses des Organismes aux Stress Environnementaux, FRANCE

16:45 UNFOLDING EVENTS IN THE MONOMERIC CRY1AB TOXIN DURING TRANSITION TO MEMBRANE INSERTED OLMGOMIC 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
16:45 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

17:00 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

17:15 RECENT RESEARCH ON FUNGUS PATHOGENS OF MITES (ACARI) IN POLAND

Cezary Tkaczuk, Ryszard Miśkiewski, University of Podlasie, POLAND; Stanisław Balazy, Research Centre for Agricultural and Forest Environment, POLAND

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

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

17:45 INVESTIGATIONS OF COLORADO POTATO BEETLE MORTALITY FOLLOWING FOLIAR APPLICATIONS FOR MULTI-SPECIES BIOCONTROL

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

16:00 INTERACTIONS BETWEEN FUSARIUM OXYSPORUM F. SP. ASPARAGI (ASCOMYCOTA: PYREMOMYCETES) AND HETERORHABDITIS CABORCA STRAIN (HETERORHABDITIDAE) IN GALLERIA MELLONELLA LARVAE

Jennifer Bauman, Department of Plant Sciences, University of Arizona. Tucson AZ 85721-0036, 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

16:15 CONTROL OF PLUTELLA XYLOSTELLA USING NOVEL FORMULATION TECHNIQUES TO IMPROVE PERFORMANCE OF EN TOMOPATHOGENIC NEMATODES ON THE FOLIAGE


16:30 CONTROLLING THE QUALITY OF EN TOMOPATHOGENIC 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

16:45 IMMUNE SYSTEMS (I. FAYE)

I. Faye, -

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

Richard Glatz, University of Adelaide, AUSTRALIA; Sasan 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 PICORNAVIRUS INFECTIONS

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

Wednesday, August 4th, 2004

Time: 20:00 - 22:00, Lecture Rooms 1, 12

Division meetings: MC, F

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, -
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 French-Constant, Biology and Biochemistry, University of Bath, UNITED KINGDOM

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

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 MICRO-ORGANISMS IN EU
Anita Fjelsted, Ministry of Environment, Danish Environmental Protection Agency, DENMARK

10:15 NONTARGET EFFECTS OF ENТОMOPATHOGENIC FUNGI: ARE WE FINALLY ABLE TO GENERALIZE?
Jørgen Ellenberg, Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldensesvej 40, DK-1871 Frederiksberg C, DENMARK; Siegfried Keller, Federal Research Station for Agroecology and Agriculture, 8046 Zurich, SWITZERLAND; John D. Vandenbergh, 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

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 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 Huysmans, Faculty of Agricultural Sciences, University of British Columbia, CANADA; Leslie Willis, David Thelamann, 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 Thelamann, Pacific Agri-Food Research Centre, CANADA
10:20 ANALYSIS OF CF103, A ZINC-FINGER ORF FROM THE BACULOVIRUS CFMNVP

Jon David 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. Messelein, Applied Plant Research, Naaldwijk, NETHERLANDS; Just M. Vlak, Laboratory of Virology, Wageningen University, NETHERLANDS

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

Qinghze 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, Wakan 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, Suvinder Bharya, Oxford Brookes University, UNITED KINGDOM; John Burden, NERC CEH, Oxford, UNITED KINGDOM; Linda King, Oxford Brookes University, UNITED KINGDOM

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

Contributed Papers (Division of Bacteria)

bacteria / contributed paper session 3

Chair: C. Nielsen-Le Roux; A. Bravo

13:30 BACILLUS THURINGIENSI S 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 THURINGIENSI S SUBSP. ISRAELENSIS ON ESCHERICHIA COLI EXPRESSING CYT1AA

Robert Manasherob, Mark Itako, Olga Burgazliev, Eitan Ben-Dov, Sammy Boussila, Arieh Zaritsky, Ben-Gurion University of the Negev, ISRAEL

14:00 PATHOGENICITY OF BACILLUS THURINGIENSI S 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)

Zithally 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-Valdés, Facultad de Ciencias Agropecuarias, Universidad Nacional Autónoma de México, MEXICO

14:30 THE RISK EVALUATION OF THE GENETICALLY ENGINEERING BACILLUS THURINGIENSI S 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 CHOLEREA

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

15:00 TARGETED DRUG DELIVERY OF CYT1AA PROTEIN FROM BACILLUS THURINGIENSI S 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, Rikva Cahin, Michael Finer, 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 5th, 2004
Time: 13:30 - 15:30, Lecture Room 12

Contributed Papers (Division of Fungi)

fungi / contributed paper session 4

Chair: Jorgen Eilenberg; F. Vega

13:30 BEAUVIERA 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. Vandenbergs, USDA-ARS, US Plant, Soil & Nutrition Laboratory, UNITED STATES

14:00 DISTRIBUTION AND OCCURRENCE OF ENTO-MOPATHOGENIC FUNGI IN THE SOIL IN A SINGLE AGROECOSYSTEM IN DENMARK

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

14:15 PROTECTION OF ENTO-MOPATHOGENIC 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 ENTO-MOPATHOGENIC HYMENOPTERA FUNGI.

Karsten Dromph, The Royal Veterinary and Agricultural University, DENMARK

14:45 SENSITIVITY OF FOLSOMIA CANDIDA (COLEMBOLA) 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 5th, 2004

Time: 13:30 - 15:30, Lecture Room 10

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

Chair: Jean-Louis Schwartz; Patricia Stock

13:30 USE OF ENTO-MOPATHOGENIC NEMATODES IN THE NORDIC COUNTRIES

Haukeland Salinas Solveig, Norwegian Crop Research Institute, NORWAY

13:50 THE EFFECT OF HOST PLANT ON THE EVO-LUTION OF BT RESISTANCE IN GREENHOUSE TRICHOPLUSIA NI POPULATIONS

Alida Jannaat, 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?**

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 GREENHOUSE WHITEFLY TO INFECTION BY ENOMOPATHOGENIC FUNGUS PAECILOMYCES FUMOSOROSEUS**

Ayhan Gökçé, University of Gaziosmanpaa, TURKEY; Mehmet Kubilay Er, University of Sütçü İymam, TURKEY

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

Michael Brownbridge, Entomology Research Laboratory, Univ. of Vermont, U.S.A.; Stephen Goodwin, W.G. Liang, Marilyn Y. Steiner, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldseenvej 40, DK 1871 Frederiksberg C, DENMARK; Ken Fry, Alberta Research Council, Vegreville, CANADA

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**Thursday, August 5th, 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 5th, 2004**

**Time: 16:00 - 18:00, Lecture Room 1**

**Workshop (Cross-Divisional)**

**SIP education workshop**

*Chair: Helen Roy; Jørgen 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, Thorvaldseenvej 40, DK-1871 Frederiksberg C, DENMARK; Dan Funck Jensen, John Hockenhull, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldseenvej 40, DK 1871 Frb. C, DENMARK; Holger Philipsen, Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Thorvaldseenvej 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
STU indicates papers being judged for graduate student presentation awards
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, Thorvaldensenvej 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, 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 snowy 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

Jaroslav Weiser, Praha 4, Velehradka 964, CZECH REPUBLIC

Abstract: For Microsporidologists 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 co-operation with the SIP Vavra, Sprague and others published monographs on Biology and Taxonomy of microsporidia (1976, 1977). A recent continuation 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. Lou 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 Irina Issi's and Voronin's laboratory at St. Petersburg. In Africa the laboratory of Toguebaye in Dakar is in good contact with Marchand and Boux 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 efinmans 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 codkhafer, 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 fresseni in the US. The involvement of governments and community consultations should 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 benzid 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, Ephesia kuhniiella. 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 pesticidal 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, 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 product based on B. thuringiensis HD1 isolate of Bt. 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 Isreal in 1976, a subspecies highly toxic to mosquito and blackfly larvae, and later the discovery by A. Huger and his colleagues of the tenebriosis 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. Eilar'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 phenomenon 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. Vandenbarg, 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.
Invertebrate pathogens as pests

Presenter: Heikki Hokkanen

Epidemiology in honey bees

Ingermar 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 fusion 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 adaptations in this system it is necessary to study, and to quantify, parasite transmission rates (horizontal as well as vertical) at colony level.

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 Pacifastacus 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.
Monday, August 2nd, 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 occurs 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 heterogenetic infections. Steinernema glaseri were more attractive to hosts colonized by conspecific nematodes than to uninjured insects. Infective juveniles S. carpocapsae were repelled from the 24-hr-old conspecific infection, whereas S. glaseri that turned less attractive to 24- than 4-hr. old conspecific infections. Experiments with insects infected with bacteria from the nematodes suggested the bacteria as a source of active volatiles. Recruitment of conspecific 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 neematophagogous 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 121°C for 20 min) and retained its activity after passage through a 0.45 Ym 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/larvae) 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.


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 and a brief discussion of the relative importance of Cry1F of Cry1F may have an antibiotic effect on black cutworm, the predominant 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 (BBMV) isolated from whole WCWR 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. BBMV 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 WCWR larval BBMV 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.
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 heterogeneous 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 UNDATERVLY AND CADAVER-APPLIED ENTO-MOPATHOGENIC 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


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 2nd, 2004
Times: 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 Vermeecken, Wageningen University, NETHERLANDS; Tran Phuc 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 Southeast Asia and beyond. WSSV is a sole member of the family Nimaviridae and contains a large, circular, double-stranded DNA molecule with 180 computational open reading frames (ORF). There is evidence that several ORFs 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 (Jh’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 suggest 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

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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.

FUNCTIONAL IMPORTANCE OF DELETION MUTANT GENOTYPES IN A NUCLEOPOLYHEDROVIRUS POPULATION

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

Abstract: A nucleopolyhedrovirus (Baculovirus) that attacks the fall armyworm, Spodoptera frugiperda, survives as a complex mixture of genotypes. In vitro cloning and endonuclease analysis revealed that all variants present segregated genomic deictions, except variant B (complete genotype), whereas variants C and D were not infectious per se. All pure genotypes were less pathogenic than the wild-type isolate. Previous studies demonstrated a significant positive interaction between genotypes B and C, when co-occluded 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 co-occluded genotype mixtures (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.

PERSISTENT INFECTIONS OF BACULOVIRUSES AND CYPOVIRUSES

Rosie Halls, 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 Salt, 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 cytopoviruses 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 implications for our understanding of host pathogen interactions in the field.

Honeybee pathology

Chair: Ingemar Fries

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 European 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
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 is 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 present, 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 Megachile 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.

MOLECULAR AND BIOCHEMICAL DIFFERENTIATION BETWEEN PAENIBACILLUS LARVAE SUBSP. LARVAE AND PAENIBACILLUS LARVAE SUBSP. PULVIFICANS

Elke Genersch, Anuria Ashiralieva, Institute for Bee Research, GERMANY; Jochen Kilwinski, SVUA Arnberg, 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. pulvificans (P. l. pulvificans). 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. pulvificans only. These 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. l. larvae reference strains (DSM 7030 for P. l. larvae; DSM 3615, DSM 8442, DSM 8443 for P. l. pulvificans) 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. pulvificans 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. pulvificans 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. pulvificans and unequivocally identify P. l. larvae.

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.
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.

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**Inactivated Viruses**

**Monday, August 2nd, 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 Autographica californica nucleopolyhedrovirus (AcNPV) experience decreased viral mortality compared with larvae fed on lettuce or artificial diet. We investigated whether dietary selenium levels do impact the infectivity of Autographica californica nucleopolyhedrovirus (AcMNPV) in Trichoplusia Ni. 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 PHTHORIMAI A 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, Phtthorimaea 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 different 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 erythmalight (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 (bimodal) model incorporating two separate exponential curves. Initial inactivation curtailed when approximately 98% of the virus was inactivated. Thereafter, inactivation was 4.4 times
HORIZONTAL AND VERTICAL TRANSMISSION OF WILD-TYPE AND RECOMBINANT HASNPV

Xuilian Sun, Mingzhe Zhou, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, Hubei, CHINA; Work done at the Crop and Weed Ecology Group, Wageningen University, THE NETHERLANDS; Just M. Vlak, Laboratory of Virology, Wageningen University, THE NETHERLANDS; Zhilong 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 densities 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 that of HasNPV-wt (12.6 ± 2.0%). Data obtained in this study provide a basis for building an epidemiological model of wild-type and recombinant HasNPVs in cotton and aids in the risk assessment of using genetically modified baculoviruses as biopesticides.

This research is supported by 863 grants (2001AA214031 and 2001AA212031), CAS grants (KSCX2-1-02 and Kscx2-SW-301-09), an NSF grant (30025033) and a grant from the KNWW (01CDP243). E-mail: xiulian.sun@wur.nl.

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 nematodes 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 (0°C) within either the cadaver of a dead host or in a live host. At 5°C, Steinernema carpocapsae survived in the cadaver of the wax moth Galleria mellonella for at least 12 days beyond the emergence time at 25°C (9 days post-infection). On transfer to 25°C, 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 -4°C have been recorded for six steinernematid and heterorhabditid species. Infective juveniles of S. riobravin, S. carpocapsae S. feltiae, H. bacteriophora, and S. glaseriand S. anomali survived 19,15, 6, 5, and 2 days at -4°C for periods respectively. S. riobravin, S. carpocapsae were still pathogenic after 6 days freezing, S. feltiae, S. glaseri and S. anomali were pathogenic for 4, 3 and 2 days respectively. 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.
Contributed Papers (Division of Microbial Control)  

**14:10 SEASONAL DYNAMICS OF ENTOPHATHOGENIC NEMATODES OF THE GENERA STEINERNEMA AND HETEROHABDITIS 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 Budíjovice. 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 the insects were quantified using Tulgrin’s apparatus. Four entomopathogenic nematode species were found during the investigation. Steinernema affine dominated in both habitats. Moreover, oak wood was inhabited by S. krausei and S. weiseri while meadow by Heterorhabditis bacteriophora. The mean abundance of total EPN community was 28 000 ind.m-2 in oak and 11 000 ind.m-2 in meadow. The host range of entomopathogenic nematodes in both habitats was formed predominantly by larvae of dipteran and coleopteran families, particularly Asiliidae or Em- pididae (Diptera) and Carabidae or Curculionidae (Coleoptera). Seasonal dynamics of entomopathogenic nematodes in both habitats was character- istic 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 parasitization probably played ma- jor role in nematode and suitable insect seasonal dynamics: high nematode and low insect densities at the beginning of the season probably led to se- vere competition and nematode density decreased. Then insect numbers arose and the balance between nematode and insect numbers in the fol- lowing 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 ex- plained partly by overwintering of nematodes in insect bodies. However, further investigation is needed.

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**13:30 EVALUATION OF COMMERCIAL FORMULATIONS OF THE CODLING MOTH GRANULOVIRUS AGAINST NATURAL CODLING MOTH INFESTATIONS IN PACIFIC NORTH-WEST 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 targets larvae, were assessed in 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 (0 250 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 (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.

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**13:45 CONTROL OF THE BROWNTAIL MOTH, EU- PROCTIS CHRYSORROHOEA, IN THE UNITED STATES WITH A BACULOVIRUS**

**James Slavicek, USDA Forest Service, USA; Joseph Elkin- ton, 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. Ec- NPV 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 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. Larvae 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 dead 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 EnNPV 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 Gryzwacz, 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 higher inclusion bodies of SpexMNPV per hectare to two morphologically distinct 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 Biomedical 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 Ancistrocama 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 2nd, 2004**

**Poster Session 1: Posters for fungi and bacteria**
B-2 INTERACTION BETWEEN P20 AND CYT1AA IN VIVO USING THE TWO-HYBRID SYSTEM OF SACCHAROMYCYES CEREVISIAE

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

Abstract: The insecticidal crystal proteins of Bacillus thuringiensis subsp. israelensis (Bti) include four major polypeptides, Cry1Aa, 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 hemolytic and cytotoxic in vitro. Expressing cyt1Aa in Escherichia coli arrests biomass growth and reduces viability by 4 orders of magnitude. This lethal effect of Cyt1Aa on E. coli is abolished by co-expression with p20. P20 is a Bti-encoded helper polypeptide that stabilizes Cry1Aa. Testing the hypothesis that the two proteins physically interact in vivo, the yeast two-hybrid interaction trap was used. As corollaries, the effect of Cyt1Aa 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 THURINGIENSISS SUBSP. ISRAELENSIS

Nadine Sela-Baranes, Robert Manasherob, Elian Ben-Dov, Arieh Zarishtky, 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, Cry1Aa is less active than two similar proteins, Cry1Ba (of Bt jagathee) 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-pesticide. Such a new composite may, in addition, contribute to regain 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 cry1-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, cry1Aa, 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 THURINGIENSISS TO SUSCEPTIBLE LEPIDOPTERA

Maria Minin, 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 Zarishtky, Ben-Gurion University of the Negev, ISRAEL

Abstract: The gene cry1Ac of Bacillus thuringiensis subsp. kurstaki was introduced into previously constructed Escherichia coli clones expressing cry1Aa 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. Cry1Aa, produced with and without P20 in E. coli, lacks toxicity towards susceptible larvae of Cotton Bollworm (H. armigera) and Pink Bollworm (Pectinophora gossypella). Expression of p20 is necessary for toxicity. Toxicity of Bti-encoded Cry1Aa towards susceptible Lepidopteran larvae, but the question with p20-cry1Aa, respectively, displayed toxicity to Lepidopteran larvae. Toxicity of pMVER-A and pMVER-ARCyt, expressing cry1Ac without and with p20-cry1Aa, respectively, displayed toxicity to Lepidopteran larvae. Toxicity of pMVER-ARCyt, expressing cry1Ac without and with p20, is significantly increased. Cry1Ac was also toxic to the Gram-negative bacterium Escherichia coli and to the yeast Saccharomyces cerevisiae. The analysis of binding reveals that there is no diminution in the adhesion of bacteria onto hemocytes when incubated in presence of immunoglobulin or 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 laminarin. We show that the specific scavenger receptor inhibitor, the polynosinic 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.

We show that the formation of membrane ruffling and filopodia, respectively, as 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.

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 Zumblib, Fabienne Vigneux, Noel Boemare, Michel Brechlin, EMIP Unité INRA UMH 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 plasma 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, laminarin 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 laminarin. We show that the specific scavenger receptor inhibitor, the polynosinic 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.

Student poster

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

Artemis Perea, Magdalena Irageta-Cardenas, Facultad de Ciencias Biologicas/UANL, MEXICO; Rafael Bujanos-Munínez, INIFAP-Celaya, MEXICO; Luis Galan-Wong, Benito Pereyra-Allereza, 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 formulations. 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= 0.284 for XentariT and 0.216 for JavelinT. Resistance ratio (RR) (LC50 field colony / LC50 of laboratory colony) showed higher differences among formulations, 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 10X JavelinT (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 proteins. 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 di-amondback moth to B. thuringiensis formulates in México could be due to both phenomena, binding and protoxin processing. *CONACYT 38040-N

**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.

**Abstract:** A novel -endotoxin gene of the Lepidoptera-specific Bacillus thuringiensis strain were toxic to Bombyx mori and Plutella xylostella, while nontoxic to Culex pipiens molestus. Spodoptera litura, S. exigua, Plodia interpunctella, Helicoverpa armigera, and Brinckerhojia armigera, but nontoxic to Culex pipiens molestus and Anopheles stephensi.

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

Tokio Ichimatsu, Kauzhiho 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 663 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 aer-ation 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, yellowish-colored, and smooth and circular colonies with no endospore 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, Kyusun Corporation, JAPAN; Hiroyoshi 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 cytotoxic 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 MIDGEUTS: A PRESERVING METHOD FOR BACILLUS THURINGIENSIS TOXIN BINDING STUDIES

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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 the risk associated with shipping dry ice, lyophilization is often used 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. 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. Comparison of equilibration 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

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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, insertion 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 BROCHONIA KN STRAIN OF BACILLUS THURINGIENSIS REPORTED TO KILL THE COTTON BOLL WEEVIL (ANTHONOMUS GRANDIS)

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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 ST75 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 ST75 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 Cry9B proteins. PCR analysis of total DNA revealed a cry9-like and a cry1Ab 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 Crys1 proteins. The Cry9-like protein was expressed in an acrystallous strain of Bt subsp. israelensis, purified and analysed by scanning electron microscopy, in vitro processing assays, and immunofluorescence 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 SPODOTERA EXIGUA (HÜBNER).

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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 hybrid toxins were used to study the role of these domains in the mode of action of Cry 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

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Abstract: The Spodoptera frugiperda SF9 cell line is highly and specifically sensitive to the Bacillus thuringiensis α-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 photography of normal SF9 control 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

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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 Diazadegma 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 CRY1C IN DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA

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Abstract: Crucifer crops have an important pest, Plutella xylostella (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-labelled 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 CHARACTERIZATION OF A NEW BACILLUS THURINGIENSIS VIRULENCE FACTOR ; THE INHA2 METALLOPRO-TEASE

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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 that contribute to the overall virulence of these bacteria toward insects. The importance of the Bt pleiotropic PloR regulator was demonstrated by reduced mortality in larvae of the greater wax moth Galleria mellonella infected with spores from a Bt 407 cry- plR mutant (Salamitou et al., 2000). PloR governs many putative virulence factors (phospho-
pases, enterotoxins, hemolysins, proteases etc.), and recently the putative PcrR-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 migidt cells). InhA2 is found as a 72 kDa polypeptide in the secretomes of Bt 407 cry- cultures in early stationary phase. InhA2 has 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 pPH315 Omega (papha3-inha2) where inhA2 is placed downstream of the constitutive promoter of alpha3 resulting in high level expression. Following precipitation by 85% ammonium sulphate, InhA2 was fully purified by anion-exchange chromatography in the presence of Cs2+ which is required for stability. Using azo-casein as a colorimetric substrate for measuring the enzymatic activity, InhA2 was found to be active in a large range of temperatures, from 25 to 55°C, with an optimum at 45°C, 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 reguron 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 metalloc Proteinase of Bacillus thuringiensis strain 407 is required for pathogenicity in insects via the oral route. J. Bacteriol. 184: 2926-3304. Fedhila, S., Gobar, M., Slamti, L., Nel, P., Lereclus, D., 2003. The Bacillus thuringiensis PcrR-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)

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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 have not been properly tested. We show here that the Cry-type genes from Bacillus thuringiensis strain 407 (Cry1Aa, Cry1C, Cry1Ea, Cry1Fa, Cry1J, Cry2Aa, and Cry2Ab) were non-toxic to Earias insulana. Insect bioassays were performed under high toxin concentration (100 g/ml). A second experiment involved degradation of Cry2 or Cry9) for larvae of E. insulana. Insect bioassays were performed under high toxin concentration (100 g/ml). A second experiment involved degradation of Cry2 or Cry9) for larvae of E. insulana. Insect bioassays were performed under high toxin concentration (100 g/ml). A second experiment involved degradation of Cry2 or Cry9) for larvae of E. insulana. Insect bioassays were performed under high toxin concentration (100 g/ml). A second experiment involved degradation of Cry2 or Cry9) for larvae of E. insulana. Insect bioassays were performed under high toxin concentration (100 g/ml).

B-22

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

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Abstract: Spodoptera exigua is a very important pest with that damage to many plants is getting more and more serious in China. Bacillus thuringiensis (Bt) cry-13 strain, one of 37 Bt isolates in Hebei, China, appeared more toxic against S. exigua than that from Bt HD-1 with mortality of 97.94±9.47% and 68.83±35% at concentration of 5.0×10⁶ 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 cry-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. ISRAELIENSIS IS DUE TO THREE PROMOTERS AND A STRONG 3' MRNA STEM-LOOP STRUCTURE

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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 the 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. ISRAELIENSIS ENHANCES

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Abstract: The mosquitoicidal bacterium Bacillus thuringiensis subsp. israelensis (BtI) produces four major mosquitoicidal proteins of Cry4A (128 kDa), Cry4B (135 kDa), Cry11A (72 kDa) and Cry11A (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 the Cry11A synthesis. This protein was originally shown to be required for efficient Cry1A 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 Cyt1A toxin in Bacillus 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 Q7 acrystalliferous strain of Bti. Using the latter construct, a 1.3-fold increase in the yield of Bin was obtained in comparison to the wild type operon.

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

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Abstract: Bacillus cereus, B. 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 cytolyisin 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 tract, 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 cerulolysine O was found in all isolates, the frequencies of hemolysin HBL, hemolysin 2, and cytolyisin 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 pulotypes 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 TO CONTROL THE COTTON BOLL WEEVIL, ANTHONOMUS GRANDIS


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 electron microscopic characterization showed the presence of spherical and bipyrramid 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 deduced 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 in 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, FAPAL, FIALGO, CNPq.

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

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Abstract: The so-called greenhouse 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 greenhouse 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 80°C degrees. The selected heat-resistant bacteria were spread on LB medium and incubated at 30°C. In total, 451 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: microscopical observation of endotoxin crystal inclusions in sporangia, haemolytic activity on sheep blood agar and growth on MVY agar (Maanetal 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 characterisation 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. ISRAELIENSIS BY DISRUPTING THE SIGK GENE AFFECTS CRYSTAL PROTEIN EXPRESSION AND TOXICITY

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Abstract: Commercial preparations of bioinsecticides based on Bacillus thuringiensis contain viable spores and recent environmental concerns have appeared in the spread of living spores to the environment. In this respect, 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 form 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, 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 spo-strain 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 to be B. cereus sensu stricto, with only one isolate of Bacillus mycoides but no strain of Bacillus thuringiensis. Our analysis of that sequence showed that it shares a homology between 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 gossypii populations. ECB cadherin-like cDNA sequence has been reported in a patent (WO01/36639) as a Cry1A 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. gossypii suggests that both genes are orthologous. 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-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 gossypii 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. gossypii suggests that both genes are orthologous. 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

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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. Macrotestriction profiles (MRP) of the isolates were characterised by pulsed-field gel electrophoresis (PFGE). Strains were characterised by biotyping (nitrate reduction, mannatol and salicin fermentation). To study this 145 P. l. larvae isolates were selected. They were isolated 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 mannatol 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 be seen in our results.

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

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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, cryl 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 family cryl family genes which consist of 50-mer oligonucleotide probes targeting cryl 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
A VIP NOMENCLATURE?

Neil Crickmore, University of Sussex, UK; Dan Ziegler, Bacillus Genetic Stock Center, USA; Alejandro Bravo, National University, MEXICO; Ernest Schnepf, Independent, USA; Didier Lereclus, Institut Pasteur, FRANCE; Jim Baum, Monsanto, USA; Jeroen Van Rio, 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.

INHIBITORY EFFECT OF THE ENTO-MOPATHOGENIC BACTERIUM PHOTOBHAR-DUS LUMINESCENS ON MANUDA SEXTA PHENOLOXIDASE

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Abstract: Photobahdus luminescens is a motile Gram-negative bacterium that lives in symbiosis with nematodes of the family Heterorhabdiidae. The bacteria produce a variety of toxins (e.g. melf1 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 (phenoloxidase 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 spectrophotometric 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 cosmids clone F12/2B, which inhibited the active form of PO in vitro. Inhibition was dose-dependent and heat stable. The inhibitor was effective regardless of the method of phenoloxidase (PO) activation. PPO activators used were the detergent CPC, E. coli lipopolysaccharides (LPS) and 1% 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.

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

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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 terminar TtrpC. These two expression cassettes were then ligated into pBANF-bar to form a recombinant plasmid, pBANF-bar-pAN52-qCDEP-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. Three 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 activity. Using third instar larva of Pieris rapae as target insect, we compared the virulence of CG5 and CG7, and CC2, a transformant 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 B. bassiana can enhance the killing speed of B. bassiana.

Fungi Myiofungus ucrainicus, a Chytridomycte Fuscal Pathogen of Spodoptera frugiperda in Non-Irrigated Rice in Colombia

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Abstract: The fungus Myiofungus 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 Myiofungus 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 Myiofungus and about how a watermelon can operate successfully against terrestrial insects. The production of posteriorly unflagellated 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 Myiofungus in the order Blastocladiales within the Chytridiomycetes is discussed.

A NOVEL TECHNIQUE TO INOCULATE CONIDIA OF ENTOMOPATHOGENIC FUNGUS AND ITS APPLICATION FOR INVESTIGATION OF SUSCEPTIBILITY OF THE JAPANESE PINE SAWYER TO BEAUVERIA BASSIANA

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Abstract: Field observations allowed many insights about the life history of Myiofungus and about how a watermelon can operate successfully against terrestrial insects. The production of posteriorly unflagellated 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 Myiofungus in the order Blastocladiales within the Chytridiomycetes is discussed.
Abstract: The Japanese pine sawyer, Monochamus alternatus (Coleoptera: Cerambycidae) is the most important pest insect of pine forests in Japan, because it vectors the pine wool nematode, Bursaphelenchus xylophilus, 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 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 100°C 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 ± 106 conidia/individual for adults for over 10 days after emergence (aged adults) and 1.9 ± 106 conidia/individual for those within 4 days (young adults), and those at 30 d were 2.8 ± 105 conidia/individual for aged adults and 2.4 ± 104 conidia/individual for young adults. The number of conidia produced on a nonwoven fabric strip was 3.5 ± 108 conidia/cm², and adult beetles which walked on the strip got 8.5 ± 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 Bioscience, UK; Bruce Parker, Margaret Skinner, William Reid, University of Vermont, USA; Mustapha El Bouhissini, 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 mauro, and Aelia, Carpocoris and Dolychoris (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 bronniartii) were obtained from Sunn Pest (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), AFLFP (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 AFLFP analyses gave clear indications of genetic diversity among the isolates and revealed some intra-specific groups 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

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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: Coleytyinae), 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

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Abstract: Erynia neoaphidis (Zygomycales, 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, procymidine, sulphur and triadimefon were chosen from fungicides, alfa-cypermethrin, deltamethrin, fenitrothion, fozalan and pirimicarb from insecticides and chlazolep-P-ethyl, glifosat, MCPA and pendimethalin 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, 25 and 30 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. neoaphidin in vitro. Among all the fungicides used in the experiment, mancozeb, copper and sulphur showed the strongest inhibiting effect on the growth of fungus. Procymidine 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. Chlorothalolin and mancozeb were particularly toxic and prevented germination of spices in all concentrations. Procymidine and sulphur seem to be the most selective to E. neoaphidis. Insecticides fozalan and fenitrothion 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.
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 cereal aphids: Sitobion avenue and Rhopalosiphum padi (hosts), entomopathogenic fungi from Entomophthorales (horizontal transmission) and the endosymbiotic bacteria Buchnera aphidicola (vertical transmission). The specific aims are: 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 endosymbiotic 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-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: The project aims to assess the susceptibility to infection with entomopathogenic fungi of aphids and mites. Aphids and mites can serve as natural biological control agents 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-8 EFFECTS OF DETRIVORES ON A PLANT HERBIVORE SYSTEM
Carsten 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, has 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 to 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 trophic tracers of nitrogen and carbon to study the impact of the soil fauna on decomposition of isolated 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 isolate labels. The applied methodology 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
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Abstract: The 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 bee viruses. This may result in a reduction in the size and health of the colony and consequent 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 of V. destructor on the development of fungi and fungi on Varroa 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
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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 run until 15th December 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 thysanopteran larvae. They possess 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 example methods 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 PAE-CILOMYCYES FUMOSOROSEUS IN SOLID AND SUBMERGED CULTURE
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Abstract: Paecilomyces fumosoroseus was cultivated in submerged (SmF) and solid statefermentation (SSF) using a chemically defined medium. The main metabolites produced in both cultures were 2,6-pyridindicarboxylic 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 25mL
Conidial Color is Important for Solar Radiation Tolerance in the Entomopathogenic Fungus Metarhizium Anisopliae Var. Anisopliae
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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 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 129, 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 germination was assessed after 12, 24 and 36 h of incubation. In general, tolerance was least in the white mutants, greatest 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 60 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 mutants 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.

The Effect of Ammonia on Conidial Longevity of Beauveria Bassiana and Metarhizium Anisopliae
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Abstract: Entomopathogenic fungi are currently being developed for control of chicken mites (Dermanyssus gallinae) and other arthropod pests in poultry production systems. Increases in ammonia levels are generally conducing for conidial germination, while the persistence of fungus inoculum might prove problematic, especially when conidial powders are applied in large quantities for several days. 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-16 PRELIMINARY SURVEY OF ENTO-MOPATHOGENIC FUNGI ASSOCIATED WITH THE AFRICAN ROOT AND TUBER SCALE STICTOCoccus VAYSSIEREI RICHARD (HEMIPTERA: STICTOCoccidae)

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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 anisoplaiae and Paecilomyces fumosoroseus 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 anisoplaiae 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 CICADAPETTINS, 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 Ungaruna, Ricardo Reitegui, James B. Gleer, Department of Chemistry, University of Iowa, U.S.A.

Abstract: Fermentation extracts of an undescribed Tolyphocladium 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, cicadapetin I and II, was accomplished by amino acid analysis as well as mass and NMR spectral studies. Amino acid sequences of minor cicadapetins were deduced from mass spectra. Cicadapetins display insecticidal activity as well as antibacterial activity against both gram positive and gram negative bacteria.

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

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Abstract: The spores of entomopathogenic fungus Paecilomyces fumosoroseus have been used successfully in the control of diverse insect pests. The rice mixed with husk, maize, wheat and soybean 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 difficult to detect the problems in the production. For this reason the optimal sporulation of the Pfrdi isolate P. fumosoroseus was determined in the solid medium varying carbon and nitrogen sources. The composition of the solid medium (Fargues Medium) in g/L was: KHOPO 0.39, NaHPO4.12 H2 O 1.06, MgSO4.7H2O 0.60, KCl 1.0, NH4NO3 0.70 and bacteriological agar 20. The concentrations in g/L of glucose and yeast extract were of 30, 60, 90 and 120 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 the medium with 95% 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°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 the center, varying the design with the methodology of response surface to 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 of 5 x 106 spores/mL and 0.06 mL 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 differeent when increasing th and the concentration in g/L of glucose and yeast extract (α = 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 METROPOLYPHIUM DIRHODUM TO THE EN- TOMOPATHOGENIC FUNGUS PANDORA NEOAPHIDIS ON GNA WHEAT

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Abstract: Studies were carried out to determine if susceptibility of the cereal aphid Metopolophium dirhodum to the fungal biocontrol agent Pandora neoaphidis differed between transgenic and non-transgenic lines of wheat. Aphid infection did not differ significantly between transgenic GNA and non-transgenic lines (91% and 82%, respectively). Feecundity was similar between P. neoaphidis-treated and untreated aphids with both transgenic and non-transgenic lines. 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

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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 used for preparing of the soil suspension with 5 double dissolutions. Each suspension was placed on three petri dishes with potato-dextrose medium containing 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 anisoplaiae (6 isolates), and Paecilomyces fumosoroseus (14 isolates). Frequency of isolations was followed: B. bassiana 85 - 95, M. anisoplaiae 85 - 95, P. fumosoroseus 60 - 70. The most effectiveness was showed some isolates of the fungi B. bassiana and M. anisoplaiae. These isolates will be recommended from 60 to 70%. The most effectiveness was showed some isolates of the fungus Pandora neoaphidis from 15 to 100%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%. The most effectiveness was showed some isolates of the fungus Pandora neoaphidis from 15 to 100%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%. The most effectiveness was showed some isolates of the fungus Pandora neoaphidis from 15 to 100%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%. The most effectiveness was showed some isolates of the fungus Pandora neoaphidis from 15 to 100%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%. The most effectiveness was showed some isolates of the fungus Pandora neoaphidis from 15 to 100%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%. The most effectiveness was showed some isolates of the fungus Pandora neoaphidis from 15 to 100%; M. anisoplaiae from 85 to 95%; P. fumosoroseus from 60 to 70%.
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 29.5 ml 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 another food source in an incubator at 17°C and 16.8:1.2 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 beetles 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. A similar study among the non-diapausing beetles and 21% among the diapausing beetles were placed in a moist chamber to determine infection. Results show a higher percentage of mortality among the non-diapausing beetles 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. 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 among the non-diapausing beetles and 21% among the diapausing beetles were placed in a moist chamber to determine infection. Results show a higher percentage of mortality among the non-diapausing beetles 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. 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.

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 biological control strategies. The component of this strategy 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 possibility 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 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, Pyrgomorphia cognata and Pseudocoryphum duvali were evaluated using classical, PCR and microsatellite analysis. Immediate effects of microbial pesticide application 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 and 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

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Abstract: Beauveria bassiana was isolated frequently from leaves of common hedgerow plants throughout the growing season in a Danish agro-ecosystem. A quantitative inoculum production was carried out in 2004 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. The diametrical 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 valid tool for two important large collections 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-25 INSECT PATHOGENIC FUNGI AND PARASITIDS AS NATURAL CONTROL AGENTS OF THE AP- PLE APHIDS APHIS POMI AND DYSAPHIS PLAN- TAGINEA

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Abstract: Insect pathogenic fungi and parasitoids are important control agents of aphids. In a survey on one conventional and four organic apple orchards in Norway insect pathogenic fungi and parasitoids as natural enemies of aphids were recorded. The surveyed apple aphids 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 freseni, Erynia neoaphidis and Conidiobolus obscurus. The fungus N. freseni 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. planiteginea. Mortality caused by parasitoids was more important in A. pomi than in D. planiteginea and the highest parasitization recorded in A. pomi was 30 %. Four species of primary parasitoids hatched from A. pomi: Binnodoxys angelae, Liposelsis gracilis, Praon sp. and Ehederus sp. Hyperparasitoids that hatched from A. pomi were: Dendrocerus carpenteri, Alloxysta pleuralis, Pheanoglyphis villosa and Asaphes suspensus. Only one individual of D. planiteginea was parasitized and this parasitoid was Ehederus nigripes.

F-26 THE EFFECT OF METHOD USED ON OBSERVED INFECTION LEVEL OF NEOZYGITES FLORIDANA ON A TETANYCHUS URITCAE POPULATION IN STRAWBERRY

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Abstract: The Entomophthoralean fungi Neozygites floridana is an important natural enemy of the psyllid Tetranychus urticae and of several other tetanychid 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, lactophenol-aniline blue or acetocein for observation of hyphal bodies, restingspores or capilllaconidia; c) Incubation of fungal infected mites visible on the leaf surface of strawberry or species growing in the same Petri dish. Some isolates significantly inhibited the growth of T. urticae in washed out alcohol of the T. urticae population level in strawberry was used for the estimation of N. floridana infection level in T. urticae. In our study, the N. floridana infection level in 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 infection level in phylloplane samples; b) Observation of N. floridana infection level. Washed out T. urticae were kept in 80% alcohol before looking adult females were mounted in LPCB and chemeted 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 grown for the observation of one or more capillaconidia attached to the hosts surface. These 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**

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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. Characterisation 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 temperature optimum for growth of P. blunckii was between 20 and 25°C. Zoophthora radicans generally showed better growth at 25°C. In the in vitro interaction experiment, the growth of three isolates from each species was affected 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 this instar P. xylostella larvae at 20 and 25°C. 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 identify each species in the in vivo interaction experiments when identification by sporulation is not possible.

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

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Abstract: Pandora neohapidis (aphid-specific) and Beauveria bassiana (generalist) are commonly found species of entomopathogenic fungi that are being developed as biological control agents against aphids. These species require successful colonization of the 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 18°C, P. neohapidis 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 sporulation could only occur after a period of approximately 4 days during the infection process of both species of fungus and continues to produce apparently healthy progeny until immediately prior to death. Potentially, the nympha 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. neohapidis and B. bassiana on the reproductive potential of A. pisum. Infection by P. neohapidis and B. bassiana resulted in 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 uninoculated control aphids. Subsequent experiments indicated that infection by either P. neohapidis 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 effect on the fitness of the host’s progeny. Implications for the use of P. neohapidis and B. bassiana as biological control agents are discussed.
F.31 IN VIVO PATHOGENICITY OF BEAUVERIA BASSIANA AND METARZHIUM ANISOPLIAE ON CHIROGONUS TRACHYPUS (ORTH.: PYRGMORPHIDAE).


Abstract: The Sugarcane grasshopper (Chrogonus trachypus) 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 the Metarzhium anisopliae and Green Muscle, a commercialized formulation of Metarzhium anisopliae var. acridum, were investigated on the Chrogonus trachypus, several, closely related hosts. Polyphagous Laboulbeniales may be questionable 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 Patrobos (Carabidae) in southern Florida. The four Patrobos species have quite different distribution patterns, but are coinciding with the distribution of Patrobos atomorus where the host is common in southern Florida. L. fasciculata has been found on the other Patrobos species only when P. atomorus 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 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 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 or the host populations are too small north of a certain latitude.

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, closely related hosts. Polyphagous Laboulbeniales may be questionable 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 Patrobos (Carabidae) in southern Florida. The four Patrobos species have quite different distribution patterns, but are coinciding with the distribution of Patrobos atomorus where the host is common in southern Florida. L. fasciculata has been found on the other Patrobos species only when P. atomorus 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 2nd, 2004
Time: 15:00 - 18:00, Lecture Room 6
Contributed Papers (Division of Viruses)

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 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 JRI Bioscience). 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 TB-168 and IPBL-Tecon). In another SFM (such as IBL-LdEp, IBL-Ld652y) which grows rather slowly there was no noticeable difference compared to growth on normal medium, while others grew much better than on their normal medium (such as IBL-Ld-Tcon and IBL-Tecon). The three lines chosen for these experiments fall into one of 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 AgSp 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, endpoints always suggest that cells grown in serum-containing medium are more susceptible to virus infection 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 LdEp-21 cell lines, although in that case, both of the cell lines that were tested grew much faster 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: Reproductive productivity 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 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 infected females creates conditions which favors virus replication in the insect host and the transmission of virus between individuals in the field.
Abstract: Aggregations of 27-nm virus-like particles were observed in electron microscope images of sectioned VDVs infected with an oocyte destructor virus. 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 mitochondria. 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 similarity sequence to members of the genus Densovirus. Phloem loading of the virus showed symptoms similar to those observed and described previously in relation to the homologous P74 protein. The two of the structural proteins showed the greatest divergence from DWV and KV and 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 honeybees 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 mice, 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 mouse 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 (ASCORVIRIDAE) FROM AUSTRALIA

Ian Newton, University of Queensand, AUSTRALIA

Abstract: Ascoviruses (Ascorviridae) 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). Uapaphleia puparia virus and Oophaga ascosoma virus both have a high incidence (>50%) in populations of Helicoverpa armigera (Hübner) and Helioverpa 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 NRLFPs, Southern blot hybridisation and sequencing the polymerase gene), the host range of the virus and the histopathology. The Australian ascovirus was similar to the Heliothis virensascovirus (Fabricius) ascovirus (RvAV) and the Trichoplusia ni (Hübner) ascovirus (TnAV). The Australian ascovirus (AqAV) was described from pupae of 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 parasitoid Helicoverpa armigera and the parasitoid H. armigera. The Australian ascoviruses were found to be vectored by the parasitoid M. demolitor. By using PCR and sequencing, ascovirus was detected in the haemolymph of parasitised H. armigera (Morley) and Helicoverpa armigera (Wilkinson). The virus was transmitted from a contaminating ooviposit (or even a pin), meaning any larval parasitoid that probes an ascovirus infected host could vector the virus. Ascoviruses have a network of co-infection. In order to investigate the development of the parasitoid larvae within the ascovirus infected host, I examined another alternative modes of transmission and its field prevalence in Australia. The Australian ascovirus appears to be an opportunistic pathogen. It may rely on multiple vectors and multiple modes of transmission for dissemination and persistence in the field.
translated from an unspliced mRNA for NS-3 (5' most ORF) and by alternative initiation from a spliced mRNA for NS-2. This work demonstrated that SpltMNPV genome sequence is similar to that of group one ORFs. 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 alternative initiation from a spliced mRNA for 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.


17:00 SPLITMNPV 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 Sc301 cells with SemNPV (S. exigua multiple nucleopolyhedrovirus) resulted in successful infection as shown by the drop in cell growth and OD. The second round infection can be seen when Si-szu-w cells, which derived from Spodoptera lutita, inoculated with SplitMNPV (S. litura multiple nucleopolyhedrovirus). Here we report that inoculation with SplitMNPV resulted in no OB production, however, Si-szu-w cells infected with SemNPV show some characteristics of apoptosis, including detachment 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 Si-szu-w infected with SemNPV. Si-szu-w cells and infected with SemNPV at different input m.o.i. of 1.5, 10 or 20 without 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. Oligonucleotide DNA ladder was detectable by 24 h p.i. without any significant changes in Si-szu-w infected with SpltMNPV at 24 h p.i. and 48 h p.i. in the detection of Si-szu-w cell with SplitMNPV at least 24 hours prior to infection with SemNPV can prevent apoptosis-like cell death. The cells infected with SplitMNPV 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 that block apoptosis in Si-szu-w cells. Three antiapoptotic types of genes, iap, p35 and p49, were found in baculovirus and SplitMNPV poses p49 and iap. Functional analysis with Sl-zsu-w cell transiently expression SplitMNPV, p49 further showed that SplitMNPV P49 exhibited clear apoptosis suppressing activity. It is possible that the accumulation of SplitMNPV P49 in Si-szu-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, Laboratorio 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; Alejandro Garcia-Maruniak, Departamento de Biologia, Universidad de Antioquia, Medellin, COLOMBIA; Just M. Vlak, Wageningen University, Wageningen, THE NETHERLANDS; Remziye Nalcacioglu, Laboratory for Biocontrol & Institute of Entomology, Zhongshan University, THE NETHERLANDS; Just M. Vlak, Wageningen University, TURKEY; Joao Morais, Universidade de Brasilia, Brasilia DF, BRAZIL; Bergmann Morais Ribeiro, Departamento de Biologia Celular, Universidade de Brasilia, Brasilia DF, BRAZIL; Jose Luiz Caldas Wolff, Laboratorio de Virologia Molecular, Nucleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes Mogi das Cruzes, 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 the 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 previous study determined a 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 (CDEFNPV) and, to a lesser degree, to Epiphysis postvittana 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 NPV 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 Insectebrate Pathology, Wukan 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 90-170 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. (CNV and (ii) phylogenetic association. 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 base 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 analysis of more than 130 baculovirus isolates will be presented and the necessity of an unambiguous nomenclature will be discussed.

17:45 DETERMINATION OF THE PROMOTOR REGION OF THE CHILo IRIDESCENT VIRUS DNA POLYMERASE GEnE

Remziye Nalcacioglu, Just M. Vlak, Wageningen University, THE NETHERLANDS; Zihni Demirbag, University of Florida, Gainesville, FL, USA; Zihni Demirbag, Wageningen 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 functional analysis of 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 mRNAs. 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 scholarships from the Wageningen Graduate School PEERG. E-mail: remziye.nalcacioglu@wur.nl
15.10 PHYLGENETICS OF THE INSECT PATHOGENIC FUNGUS BEAVIERA

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

Abstract: The entomopathogen Beaviera 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 Beaviera, details of the evolution, natural history and reproductive biology of species in this genus remain poorly understood. A multi-locus phylogenetic analysis for Beaviera will be presented which demonstrates that the main lines of evolution within Beaviera 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. scarabaeaeae, C. staphylinicola) are placed within Beaviera, corroborating that these groups 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 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 recombinating (i.e., sexual) reproductive mode. Together these phylogenetic and population genetic insights suggest most species of Beaviera 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 that are consistent with a recombining (i.e., sexual) reproductive mechanism. The genetic relationships and recombinational potential of Metarhizium strains are particularly relevant in the evaluation of field applications that are consistent with a recombining (i.e., sexual) reproductive mechanism. The main finding is that Cordyceps sexual states (e.g., C. bassiana, C. scarabaeaeae, C. staphylinicola) are placed within Beaviera, corroborating that these groups 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 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 recombinating (i.e., sexual) reproductive mode. Together these phylogenetic and population genetic insights suggest most species of Beaviera reproduce sexually and hence may be amenable to manipulation through conventional genetic approaches.

16.00 INTERACTIONS AMONG INSECT PARASITOIDS, ARTHROPOD PREDATORS AND ENDOCHEMICAL 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 arthropod fungous 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 coexistence 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 monocotyledons and dicotyledons. Most 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 invasion of healthy hosts, 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, and other fungi, illustrates 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 phylogenetically and biogeographically transmitted endophytes in tropical forests represent 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

Durr K. Aanen, Jacobs I. Boomessa, Biological Institute, University of Copenhagen, Denmark

Abstract: The "agriculture" symbiosis between termites (subfamily Macrotermiinae, Isoptera) and fungi (genera Termitomyces, Basidiomycota) is one of the most spectacular examples of mutualistic symbiosis. The "fungus-farming" termites cultivate their crops in special fungus gardens. These are commonly provided with externally derived plant material (e.g., wood, dry grass, leaf litter), while the older parts of the gardens consist of partially degraded plant material and fungal mycelium, are consumed. The large colonies of 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 independent descendents. This 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 BIOTROPIC PARASITES OF INSECTS AND OTHER ARTHROPODS

Alex Weir, Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York, USA; Beth 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 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 Basidiomycota and Hypocreales. The term is classed independently. 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. Laboulbe- niales, now firmly linked to filamentous ascomycetes, is one of the few fung- gal groups that has lost the ability to reproduce asexually. Loss of sexual reproduction is in the more common loss among other arthropod parasitic and fungi in general. Other fungi (Termitaria, Mattirolla, and Termi- tariopsis; Antennopsis, Hormiscium, Muogone, Muiaia, 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, Kathistides) and reproduction asexual (Termitaria) and sexual (Kathistes). Intimate morphological character state modifications and innovations in life histo- ries (e.g., loss of sexual or asexual state, dramatic host shifts), now can tracked in well-founded phylogenetic studies.

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 etologi- cal agent soon was identified as a virus linked to the presence of polye- hedral bodies found inside dead larvae. At the time, workers suggested that this virus, i.e., the gypsy moth nucleopolyhedrovirus (LeNPV), 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 chemi- cal 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 po- tential of LeNPV as a biopesticide. The rationale for this was twofold. First, the virus was naturally occurring and responsible for wholesale col- lapses 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 dom- inant. Research was conducted through a wide-reaching collaboration be- tween government, academia and industry. The goal was registration with the U.S. Environmental Protection Agency (EPA) of a safe and efficacious biostate. The research was focused on finding and characterizing a vir- ulent LeNPV isolate, assessing its safety for man and wildlife, developing cost effective methods for its mass production and formulation, and test- ing various ground and aerial systems for its delivery to the target pest. The product of this research, Gypchek, a wettable powder produced from LeNPV-killed larvae and mixed prior to use with a lignonfamate-based formulation, was registered with EPA in 1978. Since its registration Gypchek has been used to treat and control pest problems and research efforts have focused on improving the product through the development of an in vitro produced viral strain of enhanced potency and a ready-to-use for- mulation 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.

Monday, August 2nd, 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, NUCLE- OPOLYHEDOVIRUS PRODUCT GYPCHEK:
John D. Podgwaite, USDA Forest Service, Northeastern Re- search Station, U.S.A; Vincent D’Amico, USDA Forest Ser- vice, Northeastern Research Station, Department of Entomol- ogy and Applied Ecology, University of Delaware, U.S.A.

Abstract: The literature is full of studies in which numerous strains of entomopathogenic fungi are tested against a pest to find the most viru- lent 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. Efforts to eradicate this beetle in North America have been intensive. B. brongniartii is sold as cul- tures grown in non-woven fiber bands that are placed around orchard trees in Japan for control of cerambycid adults that self-inoculate during premat- urational wandering and our goal has been to develop this novel method- ology 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 promis- ing 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 research has been the development of a product that is highly efficacious against this species. 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 LAB- ORATORY 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. Se- lection of the appropriate nematode species and strain for specific insect targets, temperature ranges and habitat types has optimised nematode effi- cacy and persistence. A multitude of insects attack fruit tree and nuts and many of these that are 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 (Amylopis transitella) and root weevils in citrus (Pach- nacrus annipalpis 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 potential of irrigation for application of injective juveniles (IJ’s) of S. riobrave to or- ange groves facilitated 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 mainten- ance of moisture that enables the survival 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 1980’s, a novel Serratia spp. was found associated with a disease condition of the New Zealand grass grub (Costelytra zealandica, Coleopsetra: Scarabaeidae). Pathogenic strains of bacteria were isolated and characterized as a new species S. entomophila. Selected strains of bac- teria could be cultured, applied to healthy field populations and induce dis- ease outbreaks. S. entomophila was registered in New Zealand as a micro- bial 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 biological control system can be overcome by using a combination of strategies such as 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.

Abstract: Insecticidal bacteria 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 use 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 dramatic significant differences occurred in South Carolina when Bollgard field and the corresponding conventional cotton field. However, 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 human infections followed spray campaigns. Reports of human infection in the late 1990’s are problematic because the reports did not distinguish between B. cereus and B. thuringiensis. These research findings regarding infections due to both of these genera as evidence for the need for strain promiscuity of B. thuringiensis by 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. When using these lilly, adenosid, drenz, granular formulation 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 recombiant 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 will discuss the research used to develop several commercially promising recombinant strains of B. thuringiensis subsp. israelensis and B. sphaericus 2802. 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.

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 human infections followed spray campaigns. Reports of human infection in the late 1990’s are problematic because the research findings regarding infections due to both of these genera as evidence for the need for strain promiscuity of B. thuringiensis by 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. When using these lilly, adenosid, drenz, granular formulation 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 recombiant 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 will discuss the research used to develop several commercially promising recombinant strains of B. thuringiensis subsp. israelensis and B. sphaericus 2802. 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.

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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.

17:15  
Tuesday, August 3rd, 2004  
Time: 08:00 - 10:00, Lecture Room 12  
Contributed Papers (Division of Fungi)

Chair: Cezary Tkaczkul, Richard Meadow

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

Jacques Fargues, Thierry Boulard, Benoit 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 18S-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 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 13°C to 35°C, 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 20-25°C and 25-25°C. 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.
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 its host. 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 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. When applied prophylactically to soil or plant surfaces, Beauveria bassiana, Coniothyrium minitans, B. bassiana, M. anisopliae and C. fumago conidia were applied in LC(50) mixtures, their colonies inhibited mycelial growth of all the other fungi; M. anisopliae isolates produced clear antibiosis zones against Cladosporium sp., Botrytis cinerea, and Gliocladium sp. Results showed that the insect pathogens germinated as quickly as the environmental isolates only at temperatures >=25^oC. Although germination speed of the insect pathogens varied with the isolates on a range of agar media at 20^oC, growth of the insect pathogens was inhibited by 88.8% (on average for all isolates and species) at 20^oC. Fifty days after infection of HWA with M. anisopliae, mortality was assessed, and mortality was 100%. 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 its host. 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 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. When applied prophylactically to soil or plant surfaces, Beauveria bassiana, Coniothyrium minitans, B. bassiana, M. anisopliae and C. fumago conidia were applied in LC(50) mixtures, their colonies inhibited mycelial growth of all the other fungi; M. anisopliae isolates produced clear antibiosis zones against Cladosporium sp., Botrytis cinerea, and Gliocladium sp. Results showed that the insect pathogens germinated as quickly as the environmental isolates only at temperatures >=25^oC. Although germination speed of the insect pathogens varied with the isolates on a range of agar media at 20^oC, growth of the insect pathogens was inhibited by 88.8% (on average for all isolates and species) at 20^oC. Fifty days after infection of HWA with M. anisopliae, mortality was assessed, and mortality was 100%.
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-Poecia, 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 35°C, 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 further tested in 2004, both on alfalfa weevil (MS). Molecular markers will be used to distinguish isolated isolates from natural infection.

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?
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 hosts. 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 a 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, which 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 connecticus and Edhazardia aedis. Incorporation of E. aedis as a classical biological control agents for Aedes aegypti will be discussed. Much of the information concerning the life cycles and evaluations of polymorphic microsporidia has been derived from studies on 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 insect host will be discussed with respect to mosquito control. The 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 a higher specificity. Investigations 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 of these 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 ESTABLISHMENT OF PARANOSEMA (NOSEMA) LOCUSTAE IN GRASSHOPPERS (ORTHOPTERA: ACRIDIOIDEA) OF ARGENTINA.
Carlo Lange, CEPAVE, CIC-UNLP-CONICET, ARGENTINA; Maria Laura De Wysiecki, CEPAVE, UNLP-CONICET, ARGENTINA

Abstract: Paranoesma 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: Guajaina in north-western 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 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 the colonization 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 observed this initial concept, and future 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 3rd, 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 in vivo and in vitro and 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 auto-dissemination 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 genome studies of Oryctes rhinoceros virus. During this time it was accepted that this virus was a non-occluded baculovirus but now forms part of a separate group of dsDNA viruses. Early molecular studies included the development of virus molecular genotypes and a PCR detection system, 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 Maldives 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
Ramlie Musiem, Norman Kamcurdin, Wahid Mohd Baari, 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 the host insects 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 disease to palms. This has been especially intense in SE Asia following the introduction of no-burn policies 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 virulent 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.
INHERITANCE OF CRY-RESISTANCE AND CROSS-RESISTANCE IN CULEX QUIQUEFACIATUS SELECTED WITH TOXINS FROM BACILLUS THURINGIENSIS ISRAELIENSIS

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 partial sequences of five lines of B. t. israelensis Cry toxins combinations. Reciprocal mass-crosses were performed between the selected colony and a laboratory susceptible colony and back-crosses were performed using F1 offspring with the appropriate parental colony. In the C. quiquefasciatus colony selected with Cry4A + Cry4B, inheritance of resistance to Cry4A + Cry4B was immediate in resistance phenotype, in the F1 offspring and offspring of the back-cross 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 E. coli were investigated. Finally, 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 resistance mechanisms. Furthermore, the in vivo binding tests suggest that Cry11A cross-resistance in this colony from B. t. israelensis Cry toxins.

RESTORATION OF ANTI-BACTERIAL ACTIVITY OF A CRYPTIC ORF (CYT1CA) FROM B. THURINGIENSIS ISRAELIENSIS 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: receptor- 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. israeliensi. Its predicted product displays a two-domain fusion protein: N-terminal half comprising the common Cyt toxins, and C-terminal half similar to the receptor binding domain of several unrelated ricin-like toxins. Neither larval 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) and four amino acid differences between its Cyt-like (N-terminal) moiety and Cyt1Aa (2), the 3'-end of cyt1Ca was truncated (replacing the C-terminal domain), and four single bases in the remaining domain were appropriately site-directed mutagenized to replace the non-polar by appropriate resistance management strategies. Furthermore, genetic studies can promote the identification of resistance mechanisms. Here we report the partial sequences of five lines of B. t. israelensis Cry toxins combinations. Reciprocal mass-crosses were performed between the selected colony and a laboratory susceptible colony and back-crosses were performed using F1 offspring with the appropriate parental colony. In the C. quiquefasciatus colony selected with Cry4A + Cry4B, inheritance of resistance to Cry4A + Cry4B was immediate in resistance phenotype, in the F1 offspring and offspring of the back-cross 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 E. coli were investigated. Finally, 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 resistance mechanisms. Furthermore, the in vivo binding tests suggest that Cry11A cross-resistance in this colony from B. t. israelensis Cry toxins.

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5 k Fun Run

Time: 12:00 - 14:30, Solvalla

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

Excursion 1: Nuuksio National Park (off-path)

Host: Larry Huldén

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

Excursion 2: Nuuksio National Park (easy)

Host: Lena Huldén

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

Excursion 3: Marimekko factory outlet

Host: Ingeborg Menzler-Hokkanen

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

BBQ

Tuesday, August 3rd, 2004

Time: 10:15 - 12:00, Lecture Room 1

Society General Meeting

Chair: Harry Kaya

Time: 12:00 - 14:30, Solvalla

Excursion 2: Nuuksio National Park (easy)

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

Contributed Papers (Division of Microsporidia)

3. Microsporidia / contributed paper session 1

Chair: Rudolf Wegensteiner; Regina Kiespies

Time: 09:00 - 12:00, Lecture Room 10

Wednesday, August 4th, 2004

MICROSPORIDIAN PARASITES OF AUSTRALIAN FRESHWATER AMPHIPODS: HOST-PARASITE INTERACTION DURING INVASIONS

Johanna Slotshouber-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 crangonicus (Slotshouber-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 microsporidia 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. crangonicus. We demonstrate that F. crangonicus 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. crangonicus 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 MICR0SPORIDIAN PARASITES OF AUSTRALIAN FRESHWATER CRAYFISH, CHERAX DESTRUCT- OR 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. montivirulorum and V. ceracis. Ultrastructural features, patterns of spore production and SSU rDNA sequence similarities indicated T. montivirulorum 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. ceracis 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/Nosemi group of species. PCR assays based on SSU rDNA were developed for the detection of T. parastaci, T. montivirulorum and V. ceracis in crayfish tissues. Assays based on the ITS region of the rDNA of T. parastaci and T. montivirulorum were also developed. PCR methods proved to be more sensitive in detecting the presence of microsporidia than microscopic examination for spores in the same tissue sample.
Mycosporidia Suppress Melanization Reaction and Phenoloxidase Activity of the Haemolymph of Their Insect Hosts


Abstract: Melanization, mediated by phenoloxidase (PO) system, is a primary defense reaction of insect haemolymph, and many parasites suppress or avoid action of POs. We have shown previously, that mycosporidia (M) Parasemosea (=Noosea) grylly suppress PO in haemoocytes of cricket Gryllus bimacculus 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. bimacculus - P. grylly; locust Locusta migratoria - P. locustae; and wax moth Galleria mellonella - Vairimorpha ephestiae, and to find out possible effects on host PO activity and melanization in three parasite-host systems: Gryllus bicuculatus at the acute stage of microsporidiosis (Sokolova et al., 1999, 2000).

The presence of PO activity both in plasma and in haemoocytes. Whole haemolymph uptaken with cricket haemocyte monolayers caused 3-fold and 30-fold decrease in the injection of V. epithelialis 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-inoculation of P. locustae and P. grylli 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 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. Ability to suppress PO synthetic pathway might be conserved, that suppression of PO activity took place in all studied systems, 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.

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. The infection is localized only in the midgut. The infection is localized only in the midgut. The parasite causes evidently reduced peristaltics of the midgut. Further studies have shown that suppression of PO activity took place in all studied systems, 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.

11:00 THE CYST LIKE SPERMIOPHOREOUS VESICLE OF CHYTRIDIOPSIS TYPOGRAPHI

Rudolf Wegenersteiner, Institute of Forest Entomology, Forest Pathology, University of Natural Resources and Applied Life Science, AUSTRIA; Jaroslav 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 instances it infects more than 20% beetles. Furthermore, this mycosporidium infects several other bark beetle species living associated with I. typographus on Norway spruce. The infection is transmitted with persistent spore which are enclosed in a resistant spermiophorous vesicle which is a typical structure of the genus Chytridi-
Chytridiopsis typographi. with analogous structures in other Chytridiopsidae. The role of persistent the same way as it is in young spores of other microsporidia in the cre- nate 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 4th, 2004
Time: 09:00 - 12:00, Lecture Room 1

Workshops (Division of Viruses)

Genome analysis methodology -workshop

Chair: Johannes Jehle

09:00 GENOME SEQUENCING AND ANALYSIS

Claudio L.Afonso, Geraldo 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 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

Paulo M. de A. Zanotto, Instituto de Ciências Biomedicas II, Universidade de São Paulo, USP, São Paulo, SP, BRAZIL; Ricardo Pereira, Instituto de Matemática e Estatística - IME, Universidade de São Paulo USP, São 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 ances- tral 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 like- lihood 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 presence of genes or their order in the genome are hard to integrate with inferences based on gene alignments. Basically quantitative gene content analysis can be expressed as either adimensional quantities or by parsi- monious 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 concatameter (proteon) in a given order. Then, incongruencies may inform about gene transfer, etc. However, at low levels of synergy the details 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 ge- nomic features. The distribution moments, 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 addi- tion, strengths and limitations of various approaches will be considered in relation to their use for the analysis of baculovirus gene expression.

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 sequ- encing should lead to the generation of the first large sets of phylogeny. Beyond reconstruction of species trees based on complete genomes, the applica- tion 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 Uni- versity, U.S.A.; Erik D. Burnett, Boyce Thompson Institute, Cornell University, Lawrence Livermore National Laboratory, U.S.A.; Warren F. Lamboy, Center for Agricultural Bioinfor- matics, 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 mod- erately complex genomes, including those of large viruses such as bac- uloviruses. 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 dis- tance 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 adja- cent 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 addi- tion, 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, Agricul- ture and Agri-Food Canada, CANADA

Abstract: There has been a recent rapid increase in the number of bac- ulovirus 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 natu- ral clusters of genomes that are more highly related. This new wealth of molecular data along with biological data is providing new tools for de- termining 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 4th, 2004
Time: 09:00 - 12:00, Lecture Room 1

Symposium (Cross-Divisional)

Fungi and nematodes under unfavorable condi- tions

Chair: Solveig Haukeland-Salinas; Ingeborg Klingen
Improvement of the Desiccation and Temperature Tolerance of Heterorhabditis Bacteriophora

Ralf-Udo Ehlers, Olaf Strouch, Jesuk Oostergaard, Institute for Phytopathology, Department of 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 determined. Nematodes were dehydrated in polyethylene glycol (PEG) with defined water activities (Aw-values). Decreasing water activity at favourable temperatures, however at temperatures below 10-12°C effective control is more difficult. The vine weevil larvae feed actively on plant tissue until soil temperatures rise. This means plant damage is done before the mean tolerated temperature increased to 6.7 degrees Celsius. A screening of several protective substances like glycerol, trehalose and proteins. In early 1990 one of the first commercial EPN products was introduced. The vine weevil (Otiorhynchus sulcatus) can be controlled successfully with entomopathogenic nematodes under cold conditions. Several studies have shown that the vine weevil (Otiorhynchus sulcatus) can be controlled successfully with entomopathogenic nematodes at low temperatures. Results from berries. One of the objectives is to investigate the efficacy of Norwegian research project entitled Reduced use of pesticides in field grown straw.

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 10-12°C. In early 1990 one of the first commercial EPN products was introduced. The vine weevil (Otiorhynchus sulcatus) can be controlled successfully with entomopathogenic nematodes under cold conditions. Several studies have shown that the vine weevil (Otiorhynchus sulcatus) can be controlled successfully with entomopathogenic nematodes at low temperatures. Results from berries. One of the objectives is to investigate the efficacy of Norwegian research project entitled Reduced use of pesticides in field grown straw.

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 2°C. The nematode parasitoid 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.

How to Find Fungi in Extreme Environments

Marilena Aquino de Muro, Julian Smith, Paul Cannon, CAB International, 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 temperatures, 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. For example 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 characterisation 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 characterisation of fungal diversity in samples from extreme environments hold particular promise, and standard bacterial techniques such as DGGE 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.

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, Guðmundur Hallósdóttir, Iceland Forest Research, Mógilsa, IS 116, Reykjavik, ICELAND; Tróndur Leivsson, Forestry Service of the Faroe Islands, Høltanesvegur 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 occurrence of this fungus in Iceland has been documented in many circumstances. An overview of our present knowledge on the natural occurrence of insect pathogenic fungi in cold temperature, subpolar and polar regions in Iceland, Faroe Island and Greenland based on surveys of insects 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: Panodora, Strongwellsea, Entomophthora, 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.
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 CPRB larvae showed isolates more virulent than the parents (12-10, 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 isolates 12-10, 18-5, 18-8 and 18-9, and of comparable virulence (19-6, 19-7, 19-10) to their parent strains. Three times each were 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.

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

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

Abstract: Entomophaga maimaiaka 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 northen United States. E. maimaiaka was originally described from Japan and is thought to be native to Asia where it causes epizoicosis among gypsy moth populations, suppressing outbreak populations. Although E. maimaiaka was re-introduced into the US in 1989 as a control agent against E. kuehnellii, and later E. kuehnellii and E. davidiana from Japan and Korea in 1991, no gypsy moth populations were reinfested with it. Since 1989 the fungus spread throughout the gypsy moth range in the US originating 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. maimaiaka was present in the US in an effort to control the gypsy moth, it may have been released into the environment and then re-established by a more virulent strain. This study was conducted in 1989 and 1991 from Japan, 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 through the gypsy moth range in the US. The origin of E. maimaiaka is still unknown. Several hypotheses for its origin and establishment have been proposed. One hypothesis propose that the fungus primarily in the US originate 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. maimaiaka 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. maimaiaka, and to determine the origin of the North American population. We used AFLPs to assay the genetic diversity of E. maimaiaka 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 10 loci were polymorphic among 16 Asian isolates and 29 loci were polymorphic among the 14 isolates from Japan. Average gene

13:45 BIOCHEMICAL, MORPHOLOGICAL AND PATHOGENIC VARIATIONS IN BEAUVERIA BASSIANA ISOLATES

Reza Talebi Hassanlou, Aziz Khrazi Pakdel, Dep. Plant Protection, College of Agriculture, University of Tehran, IRAN; Mark Goettel, Lethbridge Research Centre, CANADA; Javad Mozzaffari, 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 geographic and biological origins. There is considerable interest in research on intraspecific variations for beneficial exploitation 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 5DAY plates and PDA broth and radial growth. A split-plot factorial GLM showed significant differences among isolates. Micrometry of over a thousand conidia by IMAGE PRO and of arthroclava on 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 Spodoptera frugiperda, one of the most important pests in North American population. We used AFLPs to assay the genetic diversity of E. maimaiaka 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 10 loci were polymorphic among 16 Asian isolates and 29 loci were polymorphic among the 12 isolates from Japan. Average gene

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

Contributed Papers (Division of Fungi)

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

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

Abstract: The entomopathogenic fungus Beauveria bassiana is a commercially important biocontrol agent and is used worldwide 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 Internal Spacer (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 fingerprints, 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.
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 698Z that exhibited characteristic symptoms of an obligate entomopathogen. A spontaneous 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 A698Zconins. Extensive biochemical characterization revealed that A698Zconins was a high mannose auxotroph (manE). A spontaneous revertant (frequency was 1 in 2x10^6) of A698Zconins 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.

14:30 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, Cronoecia tsugae, collected in different districts of the New England. The identification of the fungus was confirmed by Dr. Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China). The pathogen provokes explosive epizootics because only this species has the black stromata. A preliminary identification of fungus was confirmed by Dr. Zengzhi Li (Anhui Agricultural University, China)

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 a complex interaction between fungal cells and substrate with B. bassiana conidia and blastospores displaying differing adhesive qualities. Whereas conidia adhered readily 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 rodot (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.
ing of adults and parasites in laboratory. Four major parasite species were taken into account, including Itoplectis maculata P., Phaeogonius invisor Thumb., 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 ($\chi^2 = 13.3$ and 10.9; d.f.=2; P<0.01 in control and experiment respectively). Also there was the difference in the contribution for parasites to total pest mortality (2 = 12.4; d.f.=3; P<0.01). In experimental variants the efficiency of specialized parasitized - 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. maculata showed the opposite tendency (2 = 5.4; d.f.=1; P<0.02). The experimental variants 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 grasshoppers, whereas, in controls the opposite trend revealed. In subsequent experiments 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 parasitized - Ph. invisor varied significantly among the control variants (2 = 14.3; d.f.=3; P<0.01). Application of the Bt formulation erased these differences. At the same time, males showed significant variation in total parasitism (2 = 26.5; 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 evidence 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, Světlana 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 fungal sporulation. The first method has numerous modifications in inoculation of the substratum can be realized with conidia and blastospores. Possible fungal propagules, and then maintenance of the optimal physical conditions for fungal 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 1.2-5.9x10^9 on millet (Sikura, Primak, 1970); 1.5-3.0x10^9 on corn (Télanga, 1959); 5.0-9.0x10^9 wheat grain with bran; Metarhizium anisopliae 1.5-5.0x10^9 and Verticillium lecanii 6.0-9.5x10^9 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 0.8-1.4x10^8 on corn, 2.1-5.0x10^8 on millet (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)x10^8; M. anisopliae, strain 1080, 1.40x10^4 from (0.6 to 2.6)x10^4, and V. lecanii, strain STSP-151, 0.80-2 (from 0.3 to 1.5)x10^8 per ml of medium containing water (Sikura, 1968), 7.3x10^7 on potato-peptone medium (Sikura, 1968; Sumaya et al. 1974). The second method can provide the following conidia production per one milliliter of medium: B. bassiana 0.8-1.4x10^8 on corn, 2.1-5.0x10^8 on millet (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)x10^8; M. anisopliae, strain 1080, 1.40x10^4 from (0.6 to 2.6)x10^4, and V. lecanii, strain STSP-151, 0.80-2 (from 0.3 to 1.5)x10^8 per ml of medium containing water (Sikura, 1970).

### Wednesday, August 4th, 2004

**Symposium (Division of Bacteria)**

**Genomics and pathogenesis of invertebrate pathogens**

**Chair:** R. Aroian; D. Ellar

**13:30 IDENTIFICATION OF NOVEL BACILLUS CEREUS VULINARIS CLONES IN ETHIOPIA THROUGH EXPRESSION TECHNOLOGY IN AN INSECT INFECTION MODEL**

Sinda Fedhila, Didier Lereclus, Unité Génétique Microbiologie 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 is highly virulent when co-infected with Ctx 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 recombinase as a reporter of transient gene activation. The genetic recombinase is promoted by the site-specific recombinase, TnpI. The insertion of an active promoter upstream of tnpI results in the

and 20 to 70.91% in the second trial Mortality was proved to be due to synergism 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 significant mortality to grasshopper population below which could cause economic injury level when applied under field conditions. Discussion will attempt 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 BEAUVIERIA BASSIANA (HYPHOMYCETES)**

Justin Hatting, ARC-Small Grain Institute, SOUTH AFRICA; Stephen P. Wright, 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 cervids for species specific and commercial potential. Preliminary data on the 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 using direct contact inoculation 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. 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 confidence intervals below 4%. A second series 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 triotrophic 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.
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 positively select conditionally expressed genes. In order to identify bacterial genes that are specifically induced during host infection, a B. cereus genomic DH plasmid 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. The expression of these genes was confirmed in vivo and in vitro to quantify their activity. A gene encoding an orthologue of Listeria monocytogenes InlA, known to promote the internalization of the bacterium by the mammalian host, was identified as specifically and highly 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 ENTO-MOPATHOGENIC NEMATODES

Eric Duchaud, Atelier de Bioinformatique, 12 rue Cuvier, 75252 Paris Cedex 05, FRANCE; Alain Givaudan, Noël Boe- mare, Laboratoire Pathogènes Pathètes Pp, 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 Enterobacte- riaceae that comprises intestinal bacteria living in symbiosis with ento- mopathogenic nematodes (EPNs) of the genera Heterorhabditis and Steinernema. Hosts of these bacterial species are orally toxic or pathogenic for insect larvae when injected into the hemocoel. While most of the insect symbionts are endo- cytobionts and not culturable, Photorhabdus has the advantage to grow on standard growth media. Symbiotic individuals of photorhabdial nematodes are thus important since Photorhabdus genes encoding entomotoxicins 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 proteins, virulence factors and genes involved in the interaction with the host (insect and nematode). The conservation of a large number of gene families is the most surprising result of the analysis of this genome. The virulence factors and genes involved in the interaction with the host (insect and nematode). The conservation of a large number of gene families is the most surprising result of the analysis of this genome. The virulence factors and genes involved in the interaction with the host (insect and nematode). The conservation of a large number of gene families is the most surprising result of the analysis of this genome.

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

Stephen A. Kaba, Laboratory of Virology, Wageningen Univer- sity, THE NETHERLANDS; Anthony J. Musoke, Interna- tional Livestock Research Institute, Nairobi, KENYA; Dick Schaap, Intervet International BV, THE NETHERLANDS; Vith Nene, The Institute for Genome Research, USA; USA; M. M. Vlak, 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 avian coronavirus 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:p67ASS) was the best immunogen followed by the C-terminus of p67 fused to GFP64 (GFP64:p67C). These two immunogens also provided a high level of serum conversion 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 Georgievskà, 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 incorporating baculoviruses in feed supplements. 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 virulence of a recombinant Helioverpa 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-glycoayltransferase (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.georgievskà@wur.nl.

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

Regina G. Kleespies, Federal Biological Research Centre for Agriculture and Forestry, 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 virus, and results of histo- and cytopathological studies, as well as on the morphology of the virus will be presented.

V-4 USING PHYLGENIES TO DELIMIT SPECIES

Elisabeth Herniou, Imperial College London, UK; Jenny Cory, Wageningen University, THE NETHERLANDS; Julie Ovezski, Shippensburg University, USA; David O’Reilly, Syngenta, UK; Tim Barracough, 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 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 seen blurred. The taxonomy of baculoviruses provides an excellent framework to test a new phylogenetic method that delimitates 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 thousands of problems related to naming and classification. In this study 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 rate 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 a similar rate, as shared evolutionary histories. Comparisons of distribution of rates of isolates can be interpreted as species groups. This method provides an objective way to delimit species without a priori assumptions of host use.

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

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 in CoGV, another granulovirus CriGV 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 another granulovirus ChelGV. ChelGV and CpGV (114aa) motifs were found (cogv17, cogv18, cogv19) along with three copies of fgf (thromboid 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 Sp1NPV with an overall 25.7% amino acid identity. Five homologous regions were found (without palindromes) which were not similar to NPV homologous regions in CoGV. Two ORFs 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 3180bp. Within this region, there are several 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 POLYDVIRUS

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Abstract: The baculovirus Lymantria dispar nucleopolyhedrovirus (Ld-NPV) 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. To test this hypothesis, we found that 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 48). A dose that kills 80% of 40 larva 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 Glycyphagus scalaris on persistence of Ld-NPV. 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 parasitized 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. flavicorns PDV. All larvae that died were autopsied to confirm the presence of NPV inclusions. PDV significantly increased NPV-induced mortality in
V-7 NEODIPRION SERTITER AND NEODIPRION LECONTEI NUCLEOPOLYHEDROVIRUSES: COMPARATIVE GENOMICS AND EVOLUTION

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Abstract: The recent sequencing of the hymenopteran baculoviruses, Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) and Neodiprion sertiter NPV (NeseNPV) has provided further insight into the evolution and comparative genomics of baculoviruses. Both genomes are relatively small at 81,775 and 86,462 bp, respectively, have a low GC content and contain 89 (NeleNPV) and 90 ORFs (NeseNPV). They share 67 ORFs (average amino acid identity = 52.7%) of which only 43 were identified baculovirus homologs. The number of conserved baculovirus genes is now 29 as both NeleNPV and NeseNPV have an Ld12 on their 24 conserved genes 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 carboxy-locator-like protein, 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 imaginal. 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. Phylogenetic trees of baculovirus genomes that have shown that the dipteran Culex nigripalpus NPV is a large evolutionary distance from the lepidoptera baculoviruses. Phylogenetic analysis of NeseNPV using DNA polymerase and NeleNPV using containers of 29 conserved baculovirus genes from 24 generations 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 concatenators 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

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Abstract: An outbreak of widespread pest species cause important losses 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., Oporophthora brumata L, Orgia antiqua L., Yponomeuta malinellus Zell., the forest pests Bupalus piniarius L., Eriogaster lanestris L., Gilpinia pallida Kl., Lymantria monacha L., Neodiprion sertiter (Geoffr.), Yponomeuta evonymella L. These baculovirus isolates 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 of molecular characterization 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 virus was 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 BACULOVIRUS

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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 EGFr 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 [797]. Following inoculation, larvae were placed in screened plastic coke bottles and larval heights were determined at 24 hour intervals. Inoculation with LdNPV did not induce climbing behavior whereas infected larvae died at normal altitudes. Inoculation with LdNPV-hsp70/lacZ/EGT(-) did not induce climbing behavior whereas infected larvae died at normal altitudes. Inoculation with LdNPV-hsp70/lacZ containing the EGT gene [797] did not induce climbing behavior whereas infected larvae died at normal altitudes.
in the midgut of a non-permissive insect, Homona magnanima. Ayako Hirao, Shohei Okuno, Jun Takatsuka, Takayoshi Ishii. Madoka Naka, Ryosuke Watanabe, Yasuhisa Kunimi, Tokyo University of Agriculture and Technology, Fuchu-shi, Tokyo 183-8509, Japan

Abstract Adoxophyes honmai nucleopolyhedrovirus (AdhoNPV) is a host-specific entomopoxvirus isolated from A. honmai larvae. The virus infects six species of tea and orchard pests, including H. magnanima, and has potential for biological control of these insects. We focused on primary infection in the midgut: viral gene transcription and protein expression. Early (<48 h) and late (>72 h) gene expression were studied in midgut cells of U- and S-strain A. honmai larvae at 0, 3, 6, 12, 24, 48 and 72 h post inoculation (hpi). Transcription was detected by RT-PCR, and viral DNA replication by quantitative PCR. In the non-permissive H. magnanima, transcription of the early genes ie-1 and ie-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 hemocele 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 HOMMAI ENTO- MOPOXVIRUS FIELD ISOLATES FROM JAPAN

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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 entomopoxovirus (AhEPV) and other invertebrate EPVs, the AhEPV spheroidin and fusolin genes were sequenced. The spheroidin gene has an open reading frame (ORF) of 1056 bp, encoding a predicted 351 amino acid protein with a molecular mass of 38 kDa. The fusolin gene has an ORF of 3039 bp, encoding a predicted 1013 amino acid protein with a molecular mass of 116 kDa polypeptide which shows high amino acid sequence similarity to the spheroidins of other lepidopteran EPVs (e.g. 73% identity with Amsacta mori 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, Yamanashi, Gunma, Ibaraki, Mie and Fukuoka), we identified 12 diverse AhEPV isolates by comparing the restriction endonuclease profiles of DNA extracted from individual infected insects. Isolates from Tokyo, Ibaraki and Kyushu were chosen for further phenotypic and bioassay 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 BRASSIC- CAE (LEPIDOPTERA: NOCTUIDAE) BY GRAN- ULOVIRUS PROTEINS AND A FLUORESCENT BRIGHTENER

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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 larvae. We also examined the effect of Tinopal UNPA-GX in Mamestra brassicae and Mythimna separata infected with entomopoxovirus (MyseEPV). M. brassicaceae larvae were infected with MyseEPV using the diet contamination method and slightly reduced the lethal time, in fifth-instar larvae of M. brassicaceae inoculated with MyseEPV. These additives allow efficient low dose infection and higher virus yields. These additives also reduced the LC50 of MyseEPV against 5th-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 METEREUS PUL- CHRICORNIS IN MYTHIMNA SEPARATA INFECT- INATED WITH ENTOMPOXVIRUS

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Abstract: Growth and survival of Meteorus pulchricornis in Mythimna separata infected with entomopoxovirus. Aki FujimotoShohei OkunoTakayoshi IshiiJun TakatsukaKazuko Nakani- shiMadoka NakaiYasuhisa KunimiDepartment of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan

Abstract: We have established a colony of Adoxophyes honmai (Lepidoptera, Tortricidae) that is resistant to a nucleopolyhedrovirus (AdhoNPV). Baculoviruses infect the host insects via oral ingestion or injection (inoculation). Both (i) primary infection and (ii) secondary infection are important for the mechanism of acquired resistance in neonate larvae. We infected 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 larvae resistance to AdhoNPV. Baculoviruses infect the host insects via oral ingestion or injection (inoculation). Both primary infection and midgut cells, and secondary infection of tissues in the hemocoel (e.g. fat body). In 5th-instar larvae, the degree of acquired resistance was similar between S- and U-strain larvae. 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 activity 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 heads; phenoloxidase activity in the hemolymph). Fifth-instar larvae that were infected with entomopoxovirus 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 from high virus load during the primary infection. GV proteins and Tinopal UNPA-GX were added to the midgut content of larvae that were inoculated with MyseEPV, and the enhancement of infectivity was important not only for field application but also for mass production of virus insecticides.
poli was first detected at 6 and 24 hours post-inoculation (hpi), respec-

tively, 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

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Abstract: It was reported before that the fp25k gene of baculovirus is responsible for the generation of few polyhedra (FP) phenotype due to baculovirus infection. Here we characterized the fp25k gene of Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus (HaSNPV). Next, 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 fold lower level of budded virus. Fp25k protein was first detected at 6 and 24 hours post-inoculation (hpi), respectively. A Western-blot 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?

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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 formation or function. The pif gene of Autographa californica NPV (AcMNPV) is also essential for oral infection in Trichoplusia ni larvae (R.D. Possee, personal 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; PANOS, 100, 9797- 802). This result suggest that PIF is not conserved among the Baculovirus. This result and the one of PIF in SpInPV 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-Delta19) lacking 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 was also recog-
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 and TnSNPV-1, 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 TnSNPV-RJ and the vast majority of differences consist of single nucleotide changes, largely transitions. A few differences to six nucleotide insertions/deletions also were noted. These differences in REN profiles of TnSNPV-RJ compared to the FV isolates where sequence information is available result from single nucleotide substitutions and there appears to be no major genomic rearrangements 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 SPODOPERA LITTORALIS STRAINS TO SLNPV AND IN VIRULENCE VARIABILITY OF FOUR EGYPTIAN S. LITTORALIS NUCLEOPOLYHEDROVIRUS ISOLATES

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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, 5.6x104, 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 variant was used to compare the larval susceptibility of three S. littoralis strains collected from Baharia Oasis, Fayoum Oasis and Giza regions. The 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 SPODOPERA SPP. NUCLEOPOLYHEDROVIRUS ISOLATES TO VIRUS ENTRY OR THE PRIMARY INFECTION CYCLE

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Abstract: The multicaspid nucleopolyhedrovirus (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 intrahemocoelic injection with occlusion derived virions (ODVs). RT-PCR analysis on total RNA from inoculated insects, targeted at immediate early (ie-0), early (eg, DNA polymerase), late (chitinase) and very late genes (polyhedrin), indicated that each of the NPVs initiated an infection in all three host species tested. SpiliNPV produced a fatal NPV disease in both heterologous hosts, S. frugiperda and S. exigua, by oral inoculation or injection. SINic 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 mixes with SpliNPV or SpliNPV 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 replication cycle are not determinative factors for SeMNPV infection of heterologous Spodoptera species.

V-23 QUANTITATIVE GENETIC DIVERSITY OF SPODOPERA EXIGUA MEASUREMENTS IN SOIL RESERVOIRS IN SOUTHERN SPAIN

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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 multicaspid 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 larvae. Four strains 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 assessed by restriction 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 the viral OBs isolated from different soil samples were mixed, with genotypes purified from SeMNPV-SP2A, whereas others displayed novel genotypes. Phenotypic characteristics of the most abundant isolates are currently being determined.

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

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Abstract: Best 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 multicaspid nucleopolyhedrovirus (SeMNPV) as a biological insecticide has proved to be effective in glasshouses in northern Europe. The incorporation of stibine 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 + 15% Leucophor AP, and (iv) 108 OB/m2 SeMNPV + 15% Leucophor AP with the optical brightener. 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 bioassy 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 plants. OB persistence was not significantly improved when formulated with the optical brightener.
A NEW ASCOVIRUS (SEAV6a) ISOLATED FROM SPODOPTERA EXIGUA LARVAE IN CALIFORNIA

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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 is a major variant of SfAV1a, and possibly gene, for which SeAV6a and SfAV1a were 91% identical, compared to 75% sequences encoding the major capsid protein (mcp) and DNA polymerase fragments. Comparison of virion protein profiles revealed that SeAV6a and SfAV1a were more similar to each other than to HvAV3a. In addition, most of the SfAV1a restriction fragments hybridized with a SeAV6a genomic probe, whereas only a few HvAV3a restriction fragments were compared. Results showed that the SeAV6a and SfAV1a mcp genes were 92% identical, which was significantly higher when the genes of the new isolate were compared with the corresponding genes of HvAV3a or TnAV2a (63%). Similar results were observed with the dnaP gene, from which SeAV6a and SfAV1a were 75% identical, compared to 72% 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.

IDENTIFICATION OF A NOVEL SHRIMP PROTEIN PHOSPHATASE AS THE INTERACTING PARTNER FOR LATENESS-ASSOCIATED PROTEIN ORF427 OF WHITE SPOT SYNDROME VIRUS

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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 encoding late expression factor 7. These genes are thought to undergo positive selection in alternate hosts and may modulate the fitness of the respective viruses: ctl-2 encoding a conotoxin-like protein (disulfide-rich ion channel antagonist) and present only in a few baculoviruses 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 LaMNPV and OpMNPV and the consequences for their taxonomy and nomenclature will be discussed. Finally, we will address the issue of how these viruses evolved over time into diverse ecological niches.

RESISTANCE MANAGEMENT FOR BACILLUS THURINGIENSIS SPRAYS AND TOXINS: IS IT COMPATIBLE WITH THE USE OF BACULOVIRUSES AS ADDITIONAL BIOCONTROL AGENTS?

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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 sprays and toxins? The interaction of baculoviruses and Bt toxins in the diamondback moth, Plutella xylostella, did not produce any change in resistance to baculoviruses, PxGV and AcNPV. Coinfection bioassays using AcNPV and Cry1A revealed that low doses of NPV and toxin inhibited each other as mortality agents for Cry1A resistant insects, while no such inhibition was found for Cry1A resistant insects. Although the strength of this interaction and the dosages at which inhibition was found indicate that this interaction is relevant to other insect pests, the interaction in the field, the predicted direction of such an inhibition would be to maintain an increased fitness of Cry1A susceptible relative to resistant insects in the presence of virus. Subsequent experiments using insects maintained on whole plants in the laboratory have confirmed that Cry1A susceptible insects are significantly more fit than Cry1A resistant insects in the presence and absence of virus. More significantly of all, in the presence of low doses of toxin and high doses of NPV the fitness advantage of Cry1A sus-
ceptible insects is maintained relative to Cry1Ac resistant insects. While further experiments 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.

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 reported for the Ac-resistance 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
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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 nites and slugs; plant diseases; mammals; weeds and endoparasites.

The centre is part of the core groups in Denmark who have a broad established net-work co-operation with other national and international research groups 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 biological control on human health and the environment.


MC-5 EFFECTS OF SEVERAL ABIOTIC FACTORS ON THE VIRAL ENHANCING ABILITY OF THE VIRUS LECANII, BACILLUS THURINGIENSIS
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Abstract: Proteinaceous structure called the spindles of an entomopoxvirus (EPV) from Anoma cuprea (Coleoptera: Scarabaeidae) is known to enhance the infectivity of some nucleopolyhedroviruses (NPVs) and the Anomala cuprea EPV (AcEPV) itself. The enhancing degree was higher than that of any other virus tested. The enhancement was 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 in bio-control. The stability of the activity in several chemicals, including B. thuringiensis in three products was confirmed by 16S-23S ribosomal spacer DNA analysis and formation of toxin crystals visualised by phase contrast microcopy. These results suggest that the spindles are a strong synergist of bio-control agents such as NPVs or EPVs. Stability in the preparation of the synergist of microbial insects from A. cuprea larvae. In the present study, we examined effects of different abiotic factors on the viral enhancing ability of the AcEPV spindles. Spindles heated at 75, 85 or 95°C 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 spindles is a relatively stable material. In addition, we present effects of UV ray, formaldehyde, ethyl alcohol, etc. on the ability of the spindles.

MC-6 EFFECT OF BEAUVIERA BASSIANA, VERTICILLIUM LECANII, BACILLUS THURINGIENSIS SUBSP. TENEBRIONIS AND AZADIRACIN COMPOUNDS ON SITOPHILUS ORYZAE (L.) AND TRI-BOLIUM CONFUSUM DU VAL IN STORED RYE
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Abstract: The insecticidal effect of Naturalis [Beauveria bassiana (Bal-sano) Vuillemim at 7.16% w/v, c. 2.3x107 conidia/ml], Mycotals [Verticillium lecanii] Zimmerg at 16% w/v, i.e. c. 5x106 spores/g], Novodor (Bacillus thuringiensis subsp. tenereis) 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. 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, 14 and 21 days of exposure. The 21-day count, at the highest application rates of Naturals, 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 Naturals, 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. However, offspring production was significantly lower 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: A PLANT AREA OR FAVORED BY SELECTIVE FUNGICIDES?

Daniel R. Sosa-Gómez, Jose J. Da Silva, Embrapa Soja, BRAZIL; Francilene Angelotti, Universidade Estadual de Maringá, BRAZIL; Ivan T.V. Licurci, Fundação Dalmo Gometti, BRAZIL; Eduaro Polotto, Universidade Estadual de Maringá, BRAZIL.

Abstract: Nomuraea rileyi is one of the most important natural control agents of the corn borer (Ostrinia furnacalis) and soybean loopers (Pseudoplusia includens and Rachiphusia nu) populations. In Brazil, their epizootics, usually initiate at the end of December, declining in March. Epizootic initiation and intensity can be delayed if cultures of this fungus are not sprayed on the main crop. However, if the epizootic is done early, at the beginning of the epizotic phase. In order to know if the epizotic process is triggered by the microenvironmental conditions favored by soybean planting or if selective fungicides favored it, 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, 1999/2000 and 2001/2002 where the populations were 100%, 71% and 66% respectively. In addition, the application of the four formulations tested significantly reduced progeny production in the treated substrate, in comparison with the untreated rye. The potential of using some of these formulations/dose rate combinations, as reliable alternatives to conventional pesticides is also discussed.

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

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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 ribosome-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 characterized (pH optimum, temperature optimum) and used in bioassays. Additionally, the expression of CHI and GLU was tested in the isolated DNA 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 was investigated whether the expression of CHI and GLU has a possible synergistic effect on the activity of M. brassicae nucopolyhedrovirus and the Bacillus thuringiensis preformation Xentari on larvae of M. brassicae. The results will be presented and discussed.

MC-9 ISOLATION OF ENTOMOPATHOGENIC FUNGI FROM SOUTH AFRICAN SOILS USING THE GALLERIA MELLONELLA-BAIT TECHNIQUE

Justin Hatting, ARC-Small Grain Institute, SOUTH AFRICA; Selcuk Hazir, Hacettepe University, TURKEY; Gloria Macuera, Hanetje Jooste, Astrid Junkerschijn, 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 Bletheim, 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 boilworm (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

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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 period 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 the field were kept in the captivity. In this way we 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 isolate of this fungus is 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 of Nepal, M. anisopliae is common and was present in about 50% of the samples irrespective of their origin. How-ever, 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 possibility to develop the laboratory strain of this fungus and to integrate them into existing pest management systems is considered as very promising.
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 microbial loads of about 60 bacterial species and 12 fungal species per termite after treatment and although these loads were variable, they were broadly similar to those observed for dampwood termites in the US. The median survival time for untreated termites 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 spore) treatments kept separately or together 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 HYMENOPTERES (DEUTEROMYCOTA, HYMENOPTERES) TO ORTHOPTERAN INSECTS

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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 started activities to test selected fungi for use in the 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. migratoria. All larval stages were susceptible when treated with conidial suspensions as a 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 xerophile, that favors their field application. In field trials, fungi were tested on IV-V instar larvae of L. m. migratoria in Astrakhan 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 Beauveria strains and started only 14 hrs after moisturizing. As a result, in a trial when a shower rain occurred 12 hrs p.i., M. anisopliae efficacy was reduced two fold comparing to rainless trial, while activity of Beauveria strains was not decreased.
nymphal stages before they are capable of population increase. We con-
says because, although adult aphids are highly susceptible to most fungal
against aphid pests, most have targeted the adult stage of a single pest
Although numerous studies have screened fungal isolates
SELECTION OF ENTOMOPATHOGENIC FUNGI
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Furthermore, quality of field-collected material started to decrease in the
increasing demand for the biological insecticide in the last four seasons.
production reached a plateau and the companies could not cope with the
2.0 million ha of soybean in Brazil. Up to the 2003 season all virus pro-
carssia gemmatalis Hübner (AgMNPV) is being employed annually on nearly
Abstract: The nucleopolyhedrovirus of the velvetbean caterpillar, Anti-
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 as
says 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 con-
ducted a series of laboratory bioassays assessing the efficacy of isolates of
extomopathogenic 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 (A. gossypii) were exposed to spray applications of a single
(5.16 mg spore-crystal/l). The positive control B. thuringiensis subsp.
 incons 405s containing a LCB3 gene of a spore-crystal/1. The donor
and wild bean respectively, resulting in 106 recombined genes. For that
recombination (DNA shuffling) of alpha-AI1 and alpha-AI2 from common
alpha-amylases, and, in this context, this research focused on the screening
of these crops in area and in importance to the honeybee is oilseed rape
diseases. There are many important crop plants grown in the Nordic coun-
tries, frequently visited by the honeybees. Most of these crops have key
pests, the cotton boll weevil secretes a high level of alpha-amylases and
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AMYLASES
MC-19
TARGETED DISSEMINATION OF BIOCONTROL AGENTS BY USING THE HONEY BEE:
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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 flowering stage during its blooming period. Moreover, the main charac-
teristics of biocontrol agents such as a high level of specificity and toxicity
towards pests, the cotton boll weevil secretes a high level of alpha-amylases and
screening and training, the honeybee is used as a mobile delivery agent for the
insecticide thiacloprid (10 mg per colony). We found that honeybees are
highly attracted to thiacloprid, and that they readily transfer this compound to
other colonies when returning to their hives. These results are in line with
studies indicating that in a field setting, thiacloprid can be transferred and
metabolized by individual honeybees, and that the compound may remain in
the body of the honeybees for up to 2 months. Since the honeybees used
in this experiment were fed 0.5% thiacloprid, we can conclude that the
biocontrol agents are transferred to the honeybees in an effective and
persistent manner. As thiacloprid is highly toxic to aspen aphids, we also
found that the treated colonies were able to control this pest, which
reduced the number of aphids in the colonies by about 93%. Thus, our results
confirm previous findings that honeybees can be used as a mobile delivery
agent for the insecticide thiacloprid and that thiacloprid can be used for the
biocontrol of aspen aphids.
and Botrytis on raspberry, Zophodia and Pachynematus on currants, De-}
N-2 EVALUATING EFFICACY OF APPLICATION OF ENTOMOPATHOGENIC NEMATODES VIA A DRIP LINE IRRIGATION SYSTEM

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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 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 hermaphroditica 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

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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 strains in the presence or absence of the wax moth Galleria mellonella 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 days post application. The first treatment resulted in 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 explored in future studies.

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

Yulia Sokolova, Institute of Cytology Russian Academy of Sciences, St. Petersburg, RUSSIA; Iryna Isi, Yury Tokarev, Hidetoshi Iwano, Hideki Tanaka, Tetsufumi Yazu, Kouji Iyama, Elena Issi, Irma Issi, Yuriy Tokarev, Yulia Sokolova, College London, UK; Simon Piggott, Andrew Brown, Simon Piggott, College London, UK.

Abstract: An entomogenous microsporidium, Microsporidium sp. Sd-NIU-45920, was isolated from the lawn grass cutworm, Spodoptera depraevata, caused systemic infection in larvae of the silkworm, Bombyx mori. Spores were purified from this larva stage or when the larvae were grown with 0.2M KCl. Spores were cultured at 27 in IPL-41 insect medium enriched with 10% FBS. Sporulation and meronts were observed in the cultured cells within the first 48 hr post-inoculation. Sporogonial stages were seen at 60 hr p.i. Many-coiled type spores developed in infected cells at 120 hr p.i. The change in the morphology of infected cells became conspicuous after 24 hr p.i. Their cytoplasm was hypertrophied and their nuclei increased in number up to 10 per cell. Ultrastructural studies revealed that the multinucleate cells were still syctial but giant mononuclear cells. Non-infected cells did not exhibit any of these changes. The proportion of infected cells did not change during 48-168 hr p.i. 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.

M-2 HYPERTROPHY OF SPODOPtera FRUGIPErDA CELLS INDUCED BY MICROSPORIDIAN INFECITION

Hidetoshi Iwano, Hideki Tanaka, Tetsufumi Yazu, Kouji Iyama, Tsuishoku Hukuhara, Ni hors University, JAPAN

Abstract: An entomogenous microsporidium, Microsporidium sp. Sd-NIU-45920, was isolated from the lawn grass cutworm, Spodoptera depraevata, caused systemic infection in larvae of the silkworm, Bombyx mori. Spores were purified from this larva stage or when the larvae were grown with 0.2M KCl. Spores were cultured at 27 in IPL-41 insect medium enriched with 10% FBS. Sporulation and meronts were observed in the cultured cells within the first 48 hr post-inoculation. Sporogonial stages were seen at 60 hr p.i. Many-coiled type spores developed in infected cells at 120 hr p.i. The change in the morphology of infected cells became conspicuous after 24 hr p.i. Their cytoplasm was hypertrophied and their nuclei increased in number up to 10 per cell. Ultrastructural studies revealed that the multinucleate cells were still syctial but giant mononuclear cells. Non-infected cells did not exhibit any of these changes. The proportion of infected cells did not change during 48-168 hr p.i. 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-isotherm 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 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
Contributed Papers (Division of Bacteria) 

bacteria / contributed paper session 2

16:00 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, Microbiological Methodology Department of the Instituto Biotecnología, UNAM, MEXICO; Lorenzo Segovia, Cellular Biology and Biosafety Department of the Instituto de Biología, UNAM, MEXICO; Oswaldo Lopez, Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO; Sarjeet Gill, Cell Biology @ Neuroscience of California, University of California, USA; Jaelane Bravo, Mario Soberón, Molecular Microbiology Department of the Instituto Biotecnología, UNAM, MEXICO

Abstract: The mosquitoicidal bacterium Bacillus thuringiensis subs. israelensis (Bti) produces four major endotoxins proteins: Cry11Aa, Cry4A, Cry4B and Cry1A, that show toxicity in the range of many synthetic chemical insecticides. Due to the resistance of some lepidopteran pests to chemical insecticides Bti formulations are being used worldwide for mosquito control. Bti produce endotoxins as crystalliferous 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 epithelium 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 imbalance and larval death. High specificity of the Bti toxins is determinate by the interaction between toxins and specific receptors on midgut epithelial cells of the target insects. The Cry11Aa lepidopteran toxins, two receptors have been characterized, a GPI-anchored aminopeptidase (APN) and a cadherin-like protein (Bt-R1). In the case of the mosquitoicidal toxons Cry11Aa and Cry4Aa, the identity of the receptor molecules remains unknown. Cry11A toxin is the most active Bti toxin against Ae. aegypti and the regions of the toxins 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 inhibit 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 ANALYSIS OF THE INTERACTION BETWEEN CRY11A AND CYTIA OF BACILLUS THURINGIEN-ESIS SUBSP. ISRAELIENESIS: BIOLOGICAL ROLE IN SYNERGISM

Claudia Pérez, Luisa Fernández, IBT-UNAM, MEXICO; Sarjeet Gill, University of Riverside, California, UNITED STATES; Mario Soberón, Jaelane 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 proposed by bioassays against Culex spp., Aedes spp. and Anopheles sp. larvae, that these toxins increased the 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 Cyt toxins, gives a new and interesting vision about the potentiality of synergism for coping 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 Cry 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 Cyti1A 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 Cyti1A proteins to identify the regions of interaction. With these results, we demonstrated that these toxins interact in their native and denatured form. Finally, the interaction of specific fragments of Cyti1A 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 Cyti1A in nitrocellulose membranes. Our results suggest that the first study in the class of Cry toxins that show mosquitoicidal activity. This is 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 BI-NARY TOXIN IN AN EPITHELIAL CELL LINE.

Yannick Pauchet, INRA, UMR 1112 "Réponses des Organismes aux Stress Environnementaux", FRANCE; Frédéric Luisset, 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 gènes bactériens, FRANCE; David Pouar, 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 larvicide 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 from transfected MDCK cells. These data demonstrate 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 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 in the mosquito midgut. These results suggest that the receptor is a GPI-anchored aminopeptidase (APN) and a cadherin-like protein (Bt-R1). In the case of the mosquitoicidal toxons Cry11Aa and Cry4Aa, the identity of the receptor molecules remains unknown. Cry11A toxin is the most active Bti toxin against Ae. aegypti and the regions of the toxins involved in the interaction with the receptor are still unknown. Previous works in our laboratory demonstrated that phage display could be a pow-
16:45 UNFOLDING EVENTS IN THE MONOMERIC CRY1AB TOxin TRANSITION TO MEMBRANE INSERTED OLIGOMERIC PORE: DOMAIN I IS THE ONLY INTEGRAL MEMBRANE DOMAIN

Carolina Rausell, Instituto de Biotecnología, Universidad Nacional Autónoma de México, MEXICO; Jorge Sanchez, Carlos Munoz-Garay, Claudia Morera, Mario Soberón, Alejandro Bravo, Instituto de Biotecnología, Universidad Nacional Autónoma de México, 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 favoring 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 Cry1Ab toxin in 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 emission. The Cry1Ab 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 Cry 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 analyzing the energy transfer of Try 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.

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

Salvador Herrero, Laboratory of Virology, Wageningen University and 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, AL, USA; Monique Mac 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 widespread 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 Cry1Ac toxin. A similar approach was employed to study the expression of the Bt toxin superfamily 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 Cry1Ac 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 by heterologous expression in S. cerevisiae. These 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 Cry1Ac toxin and another selected for resistance to a Bt-based product like XenTari.

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

Christina Nielsen-Le Roux, Unité Génétique Mycobien et Environnement, INRA, la Minière, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE; Myriam Beller, Unité d’Histotechnologie et Pathologie, Institut Pasteur, FRANCE; Patricia Nel, Elisabeth Guillet, Unité Génétique Mycobien et Environnement, INRA, la Minière, Groupe Génétique et Physiologie des Bacillus pathogènes, Institut Pasteur, FRANCE; Didier Lereclus, Unité Génétique Mycobien 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 larva is used as a model to study the effect of Bt virulence factors different from Cry toxins. The importance of the Bt pleiotropic PIIr regulator was demonstrated by reduced mortality in Galleria mellonella of spores from a Bt 407 cry- pii-r mutant, infected orally by feeding 5 instars with spores or vegetative cells combined with a sub-lethal dose of Cry1C toxin. (Salamitou et al., 2000). PIIr governs many putative virulence factors (phospholipases, enotoxins, 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 al. 2003 and abstract by Hajaj M. this volume). Among other possible PIIr regulated genes with putative virulence impact, we describe here the cloning of a new gene (mpb, metalloprotease bacillus enhancin) encoding a protein with homology to Enhancin of T.ni GV baculovirus, having a peritrophic membrane protein activity. 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 septicaemia 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 toxins, pilA, pilB or pilC promoters. The expression of the alpha-X toxin is a fireapporter protein, has cytolytic activity on insect hemocytes (=immunocytes), has been purified from X. nematophila 24h-old nutritive broth-growth. The X. nematophila alpha-X (alpha-X) is a toxin with cytolytic and necrotic 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
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 infection 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 elicited in vivo. This work gives new insights in the understanding of the larval infestation with X. nematophila, apoptosis of granulocytes was also

Judith Pell; Ingeborg Klingen

Chair:

fungi / contributed paper session 3

Contributed Papers (Division of Fungi)

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 (EBF)-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 stimulated 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 stimulated 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 PARASITOID SPECIES 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, Ulensvang 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 in cuticle composition, especially lipids, of each stage (no data). Molting of spore germination on the surface of 1st instar nymphs were lower than approximately one half of that on 3rd instar nymphs and adults. Also, rates of spore germination on the surface of 1st instar nymphs was lower than on the surface of other stages of the aphid. The difference in spore germination on the different stages of the aphid is due 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 ecdisces remove conidia from the body. More the early nymphal stages, more the conidia on surface of nymphs in 1st instar nymphs was due to three factors: low numbers of attached conidia, low germination rates and rapid ecdisce. In addition, spore sprays influenced the biology of cotton aphid. Both fecundity and longevity were significantly reduced for cotton aphid in response to fungal sprays. Therefore, for P. neoaphidis and A. ervi to be effective multi-species biocontrol agents, the obligate, spe- a dramatic increase in alarm production just prior to conidiation. These results were further supported by observations of the response of settled uninfected and B. bassiana-infected aphids to the stimulated 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 stimulated 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.

16:30

INTRAGUILD INTERACTIONS BETWEEN THE APHID PATHOGEN PANDORA NEOAPHIDIS AND THE PARASITOID APHIDUS 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. C. Alder- son, Division of Agricultural Sciences, The University of Notting- ham, UNITED KINGDOM; Judith K. Pell, Plant and In- vertebrate 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 bio- control agents they must co-exist spatially. However, to result in intraguild competition and therefore a net-decrease in the ability of the natural enemy species to control the herbivorous pest. Here we inves- tigate whether A. ervi enters and forages in aphid colonies infected with 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-spurulating cadav- ers 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 the development of the fungal infection prevented them. These results 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 parasitoid population at several generations of the parasitoid and fungus are currently in progress.

16:45

THE RELATIONSHIP OF NUMBER OF CONI- DIA, MOLTING AND INSECT DEVELOPMENTAL STAGE TO SUSCEPTIBILITY OF COTTON APHID, APHIS GOSSYPII, TO THE FUNGUS VERTICIL- LIUM 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 En- tomology, NIAST, RDA, KOREA; Donald Roberts, Dept. Bi- ology, Utah State University, USA

Abstract: Aphids are some of the most serious pests of greenhouse veg- etables in the world. Verticillium lecanii has high virulence to aphids and whitewings and is under consideration as a microbial control agent. An iso- late from Korea, V. lecanii CS625, is being evaluated as a mycoparasite 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 ap- proximately 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 germi- nation on the different stages of the aphid is due 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 es- cape fungal disease by molting quickly and the ecdisces remove conidia from the body. More the early nymphal stages, more the conidia on surface of nymphs in 1st instar nymphs was due to three factors: low numbers of attached conidia, low germination rates and rapid ecdisce. In addition, spore sprays influenced the biology of cotton aphid. Both fecundity and longevity were significantly reduced for cotton aphid in response to fungal sprays.
Abstract: First cases of mite mycoses caused by Hirustella modulosa and Verticillium erythopis on Dendroaegas 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 Poznań, 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, Hirustella, Paecilomyces, prostrate forms of Verticillium sensu W. Gams, Conidiobolus and Pandorà), about 20 different resting spore forms were found and identified or described under the generic-form name Taricium, counted into the order Entomophthorales. More extensive studies were undertaken in 1990s by the University of Podlasek in cooperation with RCAFE on mycoses of plant parasitic mites of the families Aphididae and Tetranychidae. Among the myco-pathogens of Abacarus hystrix on grasses Hirustella thompsoni 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 reached 29-82%, with typical cases from 10 to 40%. Th. thompsonii var. symnematosi occurs regularly in Aphidomatae on pear leaves and H. nodulosa together with H. kirchneri on Pytthenum pallidus spp. frariae in strawberry plantations. High autumnal mortality of Tetranychus urticae is demonstrated. In our studies we therefore aim to clarify the effect of different strawberry growing systems and pesticides on the reduction of T. urticae in strawberry. In one study, the mortality caused by different strawberry growing systems and pesticides on 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 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 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 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 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 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 reduction of T. urticae in strawberry.
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 differed in infectivity to the entomopathogenic nematode, Heterorhabditis bacteriophora. We created 22 inbred lines from a genetically diverse foundation stock. Three inbred lines and the foundation strain were repeatedly subcultured in the laboratory. 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 38°C), 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 Thuya 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 inofchemicals in natural ecosystems.

16:30 CROP INFLUENCE ON THE ABUNDANCE OF STEINERNEMA FELTIAE

Holgerophilpsein, Department of Ecology, The Royal 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 farm. 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. Soil moisture and temperature were recorded and the different 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 herbaceous plants, such as rape, peas and clover. That effect can probably be explained by the relative higher number of insect host availability in those crops.

16:45 ESTABLISHMENT AND PERSISTENCE OF ENTOPATHOGENIC 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 was 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 per month were collected. EPN were trapped with 2 Galleria mellonella larvae at 25 degrees Celsius for 3 days. This procedure was repeated twice. During the application petri dishes were put on the soil (5 meters 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% by day 95 (Helianthus annuus). 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 inofchemicals in natural ecosystems.

17:00 INTERACTIONS BETWEEN FUSARIUM OXYSPOROTUM 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; Benjamin Rivera-Orduñó, División de Ciencias Administrativas, Contabla y Agrarias, Universidad de Sonora, Santa Ana, Sonora, MEXICO; 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 the fungus Diceroprotora orna (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 the population of H. bacteriophora in the soil, may lead to a more effective management strategy. However, approximately 70% of the cicadas collected from these fields have been infected with Fusarium oxysporum f. sp. asparagi, a soil-borne fungus highly pathogenic to asparagus. We hypothesize that F. oxysporum might affect the success of Heterorhabditis in the control of cicada populations in this system. To address this question, labeling experiments were conducted to determine the fate of F. oxysporum in the cicada populations and the success of the native Heterorhabditis sp in the control of the cicada population. However other biotic and abiotic factors might also influence the dispersion 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 EN TOMOPATHOGENIC NEMATODES ON THE FOLIAGE


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 formulations. Several studies on the effect of EPN with and without formulation adjuvants 70% of the EPN applied to cabbage foliage with or without PA or SF. However, Ten hours after application the decrease of EPN infectivity is 50% using the polymer-surfactant-formulation, with or without PA or SF. 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 EN TOMOPATHOGENIC NEMATODE PRODUCTS

Arne Peters, E-nema GmbH, GERMANY; Ursula Koelker, GAB Biotechnologie GmbH, GERMANY; Klaus Iwahn, Ore Bio-Protec GmbH, GERMANY; Frank Stepper, Sautter & Stepper GmbH, GERMANY

Abstract: In todays commercial world controlling product quality is of ever increasing 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 Nutzlingsanbieter 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 4th, 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.

16:15 A RECIPE FOR DEATH: THE INTERPLAY BETWEEN HONEYBEE IMMUNITY, IMMUNOSUPPRESSION BY MITES, AND PICORNA-LIKE VIRUSES

Diana Cox-Foster, Xiaolong Yang, Miaooling Shen, Liwarg 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 pathogenic organisms 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-infection and the levels of viral RNA were significantly higher in individual bees parasitized by Varroa. The KBV virus, many bees lack 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, 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.

16:25 IMMUNE SYSTEMS (I. FAYE)

I. Faye,  , -

Abstract: Abstract is not available at the time of printing.

16:50 LECTIN-INDUCTED HEMOCYTE INACTIVATION: A PARADIGM FOR PARASITOID-MEDIATED IMMUNE-SUPPRESSION

Richard Glatz, University of Adelaide, AUSTRALIA; Sas san Asgari, University of Queensland, AUSTRALIA; Otto Schmidt, University of Adelaide, AUSTRALIA

Abstract: Many insect parasites that deposit their eggs inside immu-nature 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 suffi-cient to cause immune suppression when injected into the host hemocoel, where CrV1 is taken up by hemocytes. Uptake is dependent on dimer for-mation, which is also required 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 CrV1-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 pos-sible, cellular immune inactivation in insect parasites may involve mas-sive 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.
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 10^9 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: Paenibacillius 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. thiamolyticus 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, Cry8), and Diptera (Cry9) toxins. The presence of this 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. aphanius and indicates that these genes are not unique to Paenibacillus str. IDAS1529.

11:00

NOVEL SERRATIA ENТОМОМОРPHA ANTI-FEEDING GENES CONTAIN A PUTATIVE DETECTIVE PHAGIE ACTIVE AGAINST THE GRASS GRUB COSTELLYTRA 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, larval coloration, and eventual death. A 155-kb plasmid, termed pADAP for amber disease associated plasmid encoding 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 overlap and it will be of interest to determine the roles of each of the 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 complexes, the insecticidal complex of Serratia entomophila and the oral toxic Toxin Complex (tc) proteins. The Mtxc toxins are large single polypeptide molecules of approximately 3000 amino acids (aa), which show a domain structure similar to the large Clostridial toxins. Mfc 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 TcAD (1473 aa), TccB (1044 aa) and TccC (1144 aa), heterodimer toxins encoded by 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 bacteriocins, such as enterolactocin of Y. enterocolitica, or xenorhabdixin from X. nematophilus.

10:30

PHOTORHABDUS: A NATURAL BORN KILLER.

Nick Waterfield, Andrea Dowling, Michelle Hares, Phil Dobson, 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 entomopathogenic nematodes expel 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 overlap and it will be of interest to determine the roles of each of the 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 complexes, the insecticidal complex of Serratia entomophila and the oral toxic Toxin Complex (tc) proteins. The Mtxc toxins are large single polypeptide molecules of approximately 3000 amino acids (aa), which show a domain structure similar to the large Clostridial toxins. Mfc 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 TcAD (1473 aa), TccB (1044 aa) and TccC (1144 aa), heterodimer toxins encoded by 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 bacteriocins, such as enterolactocin of Y. enterocolitica, or xenorhabdixin from X. nematophilus.

10:15

NONTARGET EFFECTS OF ENТОМОПАТОГИЧЕСКИЕ Fungi: ARE WE FINALLY ABLE TO GENERALIZE?

Jørgen Eilenberg, Department of Ecology, The Royal Veterinary and Agricultural University, Thorsveldevej 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 only a few species of Beauveria, Metarhizium and Paecilomyces. Our presents will review data from a wealth of information about the nontarget effects of these releases. The following elements will be considered:

Economical versus physiological host range of a fungus Other non-target effects against invertebrates Potential replacement of, or recombination with, naturally occurring fungal strains Nontarget effects over time including dissipation 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 brongnari-
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 RAF-BCA (QLK1-CT2001-01891, http://www.rafbcacom). 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 5th, 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 VERUS NOVELTY

Gang Long, Xiaoyu Pan, The Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, CHINA; Zhihong Hu, 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 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), HR2 (241C-276H) 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 spectroscopy 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 link using EGS (ethylene glyco bis), this peptide presented as a stable dimer.

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

Taryn Stewart, Ilse Huijksens, 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 ie gene have been identified in multiple nucleopolyhedroviruses. Studies on the baculovirus 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 ie6. The ie6 transcript is composed of 162 bp from the exons 0 ORF spliced to the 5' end of ie1. Two translation products are made from the ie6 transcript, ie1 and ie6, a 52 amino acid N-terminally elongated form of ie1 called IE6. Our previous studies have shown that IE6 is expressed at higher levels than IE1 at early times and therefore may be more important as early viral infection than IE1. In this study we have investigated the function of IE6 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 IE6 and IE1. This AcMNPV-ie6/ie1 knock out (KO) bacmid does not infect Spodoptera frugiperda (SF 9) cells showing that IE6/IE1 is essential for viral infection. Repair viruses of the AcMNPV-ie6/ie1 KO were constructed that express only IE6 or IE1 and IE6/ie1 from the KO was isolated. Both of these viruses clearly indicate that IE6 does not function equivalently to IE1. AcMNPV viruses expressing only IE6 or IE6/IE1 produced significantly fewer cells with polyhedra than their IE1 counterpar. These results suggest that IE6 does not activate late stage infection events as efficiently as IE1 and that the quantitative ratios of IE6 to IE1 seem to be integral to proper viral infection.
INVolVEMENT OF THE RING FINGER MOTIf OF ACMNPV EXON0 IN BUDDED VIRUS PRODUCTION
Xiaojing 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 S9 cells transfected with an exon0 knockout virus is reduced at least three orders of magnitude in comparison to wild-type virus. The C-terminal of exon0 harbors a RING finger which is modulated by an amino acid sequence that is conserved in other baculoviruses. The sequence analysis identified a single transcriptional start site and one transcriptional termination site. We were also able to successfully knockout Cf103 through 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; Knirr Krell, Department of Microbiology, University of Guelph, CANADA

Abstract: The Choristoneura fumiferana multicapsid nucleopolyhedrovirus (CMNPV) is an ideal candidate as a biopesticide to control the eastern spruce budworm (C. fumiferana) due to its narrow host-range. The baculovirus (CfMNPV) is an ideal candidate as a bioinsecticide to control the R-Illusia lattoralis SL2 cells with the Auto- grapha californica multiple nucleopolyhedrovirus (AcMNPV) induces apoptosis in contrast to infection with the Spodoptera littoralis nucleopolyhedrovirus (SINV). Induction of apoptosis of SL2 cells by AcMNPV-infection or UV-irradiation involved the activation of a caspase cascade with a highly conserved, and designed Si-caspase-1. Si-caspase-1 encoded a polypeptide of about 37 kDa. Comparison of Si-caspase-1 amino acid sequence to those of Spodoptera frugiperda, Trichoplusia ni- and Bombyx mori-caspase revealed that these caspases display a high degree of homology, suggesting that their activation pathway is highly conserved in Lepidopterans. SINV-infected cells synthesized the apoptosis suppressor P35 and inhibited completely the maturation of Si-caspase-1, suggesting that P35 might inhibit the apoptosis cascade in SL2-cells upstream of the apoptotic suppressor P35 of AcMNPV. This indication was further supported by data obtained from studying SINV caspase-1, in SL2 cells stably transfected to express the p35 gene challenged by apoptosis stimuli.

10:40 IDENTIFICATION AND CHARACTERIZATION OF A CHITINASE-CONTAINING BACULOVIRUS FROM CHRYSODEIXIS CHALCITES
Monique M. Van Oers, Laboratory of Virology, Wageningen University, NETHERLANDS; Elisabeth Henriou, Department of Biological Sciences, Imperial College London, UNITED KINGDOM; Awaluddin Junaid, Magda Usmany, Department of Virology, Wageningen University, NETHERLANDS; Gerben J. Messelink, Applied Plant Research, Naaldwijk, 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 (ChcNPV) 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-3 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 sequence 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 cyclodolate pyrimidine dimer (CIP) 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 analysis of this ChcNPV gene was demonstrated by RT-PCR and 5′ and 3′ RACE 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
Qinghuen Liu, Nor Chejanovsky, The Volcani Center, ISRAEL

Abstract: Infection of Spodoptera lattoralis SL2 cells with the Autographa californica multiple nucleopolyhedrovirus (AcMNPV) induces apoptosis in contrast to infection with the Spodoptera littoralis nucleopolyhedrovirus (SINV). Induction of apoptosis of SL2 cells by AcMNPV-infection or UV-irradiation involved the activation of a caspase cascade with a highly conserved, and designed Si-caspase-1. Si-caspase-1 encoded a polypeptide of about 37 kDa. Comparison of Si-caspase-1 amino acid sequence to those of Spodoptera frugiperda, Trichoplusia ni- and Bombyx mori-caspase revealed that these caspases display a high degree of homology, suggesting that their activation pathway is highly conserved in Lepidopterans. SINV-infected cells synthesized the apoptosis suppressor P35 and inhibited completely the maturation of Si-caspase-1, suggesting that P35 might inhibit the apoptosis cascade in SL2-cells upstream of the apoptotic suppressor P35 of AcMNPV. This indication was further supported by data obtained from studying SINV caspase-1, in SL2 cells stably transfected 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 Vi- rology, 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 Heli- coverpa armigera single nucleocapsid nucleopolyhedrovirus HaSNPV were fused with a GFP fusion gene and transfected into HaAMI cells. Western blot using antisera against HaSNPV P10 revealed that all the P10-GST fusion proteins were expressed in HaAMI cells. When ob- served under confocal microscope, different GST-fusion proteins appeared in 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. Cells transfected with plasmid pN66-GFP, which contains N-terminal 66 aa of P10 and tagged with GFP, appeared branch-like structures. How- ever, 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.
Bacillus thuringiensis belongs to the Bacillus cereus sensu lato family of Gram-positive sporeforming bacteria. This group contains six genera: Bacillus, Lysobacter, Paenibacillus, Planobacillus, Stenotrophomonas, and Xanthomonas. B. thuringiensis sp. kurstaki, where it displayed a highly efficient ability to kill larvae of a wide range of insects. Excluding the spore-forming stage in the life cycle, the bacteria are found in the soil, where they can survive for long periods. They are also found in the gut of soil-dwelling insects and arthropods, and can be transmitted horizontally between hosts. Insecticidal properties of this bacterium have been used for many years. The spore-forming stage of the bacterium has been used in biopesticides, particularly for controlling insect pests. Effective control of insect pests is crucial for maintaining the health and productivity of ecosystems, and biopesticides provide a safer alternative to chemical pesticides.

14:00 PATHOGENICITY OF BACILLUS THURINGIENSI S SUBSP. ISRAELIENSIS AND ENTOMOPATHOGENIC NEMATODES OF THE GENUS STEINERNEMA AGAINST TIPULA PALUDOSA

Jens 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: Nemestrinae). For the L2stage the LD50 is 10.42 g rop 73 ITUs (International Toxic Units determined with Aedes aegypti); for the L3 41.21 g rop 289 ITUs and for the L4 440.94 g rop 3087 ITUs. Bt lectin sequence analyses have led to a functional map of this plasmid, yielding many applications of host life cycle on the genetic stability of non-essential genes will be discussed.

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

Zitlhal 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 Químina, 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 has a high potential as biological control agent. The objective of this work was to search for alternative to the use of chemical 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 Gramineae, Leguminosae, vegetables and ornamentals in Mexico and other countries. The larvae may feed on roots of plants causing severe damage. In Mexico, there are about 68 different species of white grubs (Phylophaga spp) reported as potential pests and there is no effective biological control agent to cope with them. The aim of this work was the search for entomopathogenic bacteria active against Phylophaga 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 showed homogenous colonies and yield was 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 Phylophaga 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 x2. 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 bioassay. Phenotypical and biochemical tests used for bacterial taxonomy and also, sequencing of 16S DNAr, showed that the selected bacteria were Serratia marcescens (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 discussed 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 Baija village, Donnen 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(0.72104 cfu/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 3hrs. 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 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.

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 consumed by Vibrio cholerae. Some eggs were found intact underneath the bottom. Vibrio cholerae was isolated and identified as the cause of this phenomenon. Haemaglutinin/ protease secreted by these 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 chironomus. Simultaneously we have found that 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 chironomus 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. THURINGIENSIS

Shmul Cohen, Department of Life Sciences, Ben-Gurion University of the Negev, 2Department of Chemical Engineering and Biotechnology, College Judith and Samara, ISRAEL; Eitan Ben-Dov, Department of Life Sciences, Ben-Gurion University of the Negev, ISRAEL; Jorgen Eilenberg; F. Vega, Department of Forestry, Anhui Agricultural University, CHINA

Abstract: Bacillus thuringiensis subsp. israelensis Cyt1Aa synergies Bt mosquitocidal proteins in vivo. Despite the proven 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, the destruction mechanism is oxidized 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 or 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. With the use of conjugation with a 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 acrylastiferous 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 5th, 2004

Time: 13:30 - 15:30, Lecture Room 6

Contribution Papers (Division of Fungi)

fungi / contributed paper session 4

Chair: Jorgen Eilenberg; F. Vega

13:30

BEAUVIERIA 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, Den- drolinus 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 an LT50 difference of a few minutes. 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 80% 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 strains of B. bassiana could be obtrusive, which is supposed to 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 Symphionemus veletus and the pine caterpillar are some keystone species which maintain infection of B. bassiana in the forest ecosystem, each accounts for 64.7 and 60.0%, respectively of coleopteran and lepidopteran cadavers and play important roles for maintaining inocula of B. bassiana in the forest. The pine caterpillar is a keystone species for maintaining stability of insect community in the pine plantation ecosystem.
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. Annun, Eleanor Green, Department of Biological Sciences, University of Maine, UNITED STATES; John D. Vandenbarg, 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 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 genetic 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 competitive compatibility groups among the most predominant native strains and GHA. The host selective (monophagous or narrowly obligophagous), obligatorily biotrophic species. In order to assess 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 bales or road sides enrich the communities of these fungi by about 10 to 15 species mostly pathogenic to insects, plant-hoppers and phytophagous arthropod species. In the last decades, biodiversity refuges such as woodlots, shelterbelts, perennial crops, swamps and rushes scattered among arable fields increase species diversity up to about 400 species. The knowledge of biodiversity status of fungal species and their habitats 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.

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 for a wide range of arthropod prey. The effect of non-host vectors on the efficiency of the fungi was measured by transferring single conidia to a substrate which had been previously exposed to living adults. In addition, the mortality of Anceps dorsalis (springtail) was measured when exposed to soil containing non-host vectors. The model fungi were Metarhizium anisopliae var. acridum, Paecilomyces farinosus, and Beauveria bassiana GHA. Results showed that uninfected specimens of the three collembolan species Folsomia candida, Hypogastrura assimilis, and Proisotoma minuta were all able to transmit sufficient inoculum of the entomopathogenic fungi. Beauveria bassiana, B. brongnari and M. anisopliae var. acridum were capable of infecting larvae of Neophilaena fimbriata after exposure to soil containing conidia. However, their ability differed significantly, with transmission by M. anisopliae var. acridum and B. brongnari significantly lower than that of B. bassiana. 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, whereas 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 sterilized freeze killed uninfected collembolans, the resulting mortalities of A. dorsalis due to M. anisopliae, P. farinosus, and M. anisopliae var. acridum were respectively 20%, 60% and 60%. 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.

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 crops. The decline in their occurrence in 2001 is attributable to 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.

SENSITIVITY OF FOLSOMIA CANDIDA (COLLEM- BOLA) 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 their ability to act as non-host vectors of entomopathogenic hyphomycete fungi for a wide range of arthropod prey. Collembola may be an important source of infection of both soil dwelling larvae and adult predators by entomopathogenic fungi.
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 pest control that can be used in the field. In small forest nurseries authors have studied 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.

USE OF STEINERNEMA CARPOCAPSAE FOR POST HARVEST CONTROL OF NAVEL ORANGE-WORM (AMYELOIS TRANSITELLA) IN FALLEN PISTACHIOS

Juel 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 injurious to NOW adults and that bioassays confirmed 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 berms (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.

Abstract: The oral toxicity of excretion products of several Photorhabdus and Xenorhabdus strains was tested on two thrips species: Frankiniella occidentalis and Thrips tabaci. Out of 46 Phototurbidus 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 killed after sucking from leaves covered with the toxic excretion products. Ingestion of thrips will be discussed. Possibilities of using P. temperata in the control of important pest of California almonds and pistachios. Previous USDA researchers demonstrated that high concentrations of Steinernema carpocapsae were injurious to NOW adults and that bioassays confirmed 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 berms (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.

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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. bacterial, 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 parasitize D. v. virgifera larvae, but adults were rarely found parasitised. At concentrations of 7.9 and 15.0 EPN/cm², S. glaseri, S. arenarium, S. abassi and H. bacteriophora caused the highest mortality of D. v. virgifera larvae, i.e. 63 % and 80 %, 63 % and 70 %, 60 % and 30 % and 27 %, respectively. 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 alleles are maintained in T. ni populations in the absence of Bt exposure. However, the repeated and rapid evolution of resistance to Bacillus thuringiensis (Bt) does appear to be costly in T. ni as there is a rapid decline of resistance alleles in T. ni populations in the laboratory without selection. Therefore, it is possible that fitness costs are not as deleterious in the field as in the laboratory.
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 production, 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.

SUSCEPTIBILITY OF VARIOUS DEVELOPMENT STAGES OF GLASSHOUSE WHITEFLY TO INFEC-
tion BY ENTOMOPATHOGENIC FUNGUS PAECI-
Lomyces fumosoroseus

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Abstract: Virulences of five Paecilomyces fumosoroseus isolates, (AR-
SEF 2658, 4400, 4406, 4408 and 4415), were investigated on egg, nymphal, pupal and adult stages of Trialeurodes vaporariorum with single dose (107 spores/ml) treatments under laboratory conditions. Two millilitres of conidial 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 whitely 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 variations in virulence of the five isolates of P.fumosoroseus on each de-
velopmental 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 life stages of the life cycle.

VARIABILITY IN RESPONSES OF DISCRETE LAB-
ATORY POPULATIONS OF WESTERN FLOWER
THIRPS, FRANKLINIella OCCIDENTALIS (PER-
gande) 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 Res-
earch Council, Vegreville, CANADA

Abstract: A collaborative research project was undertaken to evaluate en-
tomopathogenic fungi against geographically-discrete populations of west-
ern flower thrips, a major pest of greenhouse and field vegetable and orna-
mental crops. Using a standardised laboratory bioassay technique involv-
ing 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.
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 pathogen 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 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.

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.
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