50th Annual Meeting

and

Golden Jubilee

Celebration

of the

Society for Invertebrate Pathology

13 – 17 August 2017

UC San Diego

La Jolla, California

MEETING AT A GLANCE
2017 SIP Annual Meeting and Golden Jubilee Celebration

NOTE: The abstracts included in this book should not publications and should not be cited in print without the author's permission.

POSTERS should be put up after 8:00 am on Monday in the West Ballroom.
They must be removed by noon on Thursday or they will be discarded.

STU indicates papers being judged for graduate student presentation awards.

Presenting authors for oral talks and posters are indicated in ***bold/italics*** type.

SUNDAY – 13 August

8:45 – 5:00 Council Meeting (*Village Tower West, Room 15C*)

2:00 – 6:00 Registration (*Village Conference Center Check-in Desk*)

6:00 – 8:00 Mixer / Welcome Reception (*Institute of the Americas, Plaza*)

MONDAY – 14 August

7:30 – 5:00 Registration (*Price Center*)

8:00 – 12:30 Opening Ceremony (*Theater*)
Awards Presentations
Golden Jubilee Founders Symposium

10:00 – 10:30 *Coffee break*
Golden Jubilee Founders Symposium **(continued)**

12:30 – 1:30 *Lunch*

1:30 – 3:30 ***Bacteria/Microbial Control X-Division Symposium*: Challenges for Biocontrol in Latin America** (*Theater*)
Virus Division #1 (*The Forum*)
Microsporidia Division #1 (*Roosevelt Room*)
Nematodes Division #1 (*Marshall Room*)

3:30 – 4:00 *Refreshment break*
***Virus Division Symposium*: Insect virology: Historical achievements and recent advances** (*Theater*)
Bacteria Division #1 (*The Forum*)
Fungi Division #1 (*Roosevelt Room*)
DBI #1 (*Marshall Room*)

8:00 – 10:00 **Bacteria Division** business meeting (*Village Tower West, 15th floor, Rm. 15B*)
Diseases of Beneficial Invertebrates Division business meeting (*Village West, Bldg. 2, Rm. 2C*)
Microbial Control Division business meeting (*Village Tower West, 15th fl., Rm. 15C*)
Nematodes Division workshop (*Village West Bldg. 2, Rms. 2º/B*)

TUESDAY – 15 August

6:30 – 7:30 5K Run/Walk

8:00 – 10:00 ***Virus/DBI X-Division Symposium*: Honoring Just Vlak** (*Theater*)
Bacteria Division #2 (*The Forum*)
***Fungus Division Symposium*: Insect defense against fungal pathogens: mechanisms, variations and efficiency** (*Roosevelt Room*)
Microbial Control Division #1 (*Marshall Room*)

10:00 – 10:30 *Coffee break*

10:30 – 12:30 Virus Division #2 (*Theater*)
***Nematodes Division Symposium*: Nematode Omics** (*The Forum*)
Fungi Division #2 (*Roosevelt Room*)
DBI #2 (*Marshall Room*)

12:30 – 5:00 EXCURSIONS

6:00 – 10:00 Barbeque (*Birch Aquarium*)

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13–17 August 2017

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SIP OFFICERS

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SIP COMMITTEES

Archivist	Betty Davidson
Awards & Student Contest	Monique van Oers (Chair), Patricia Stock (Vice-Chair), Andreas Line Hyun-Woo Park, Kelly Bateman
Endowment & Financial Support.	Roma Gwynn (Chair), Michael Brownbridge, Michael Dimock James Harper, Dirk Ave, Stefan Jaronski (ex officio)
Founders Lecture	James Becnel (Chair), Neil Crickmore, Mark Goettel
Golden Jubilee	Peter Krell (Chair), Julie Hopper, Surendra Data, Betty Davidson Johannes Jehle, Nina Jenkins, Kelli Hoover, Leellen Solter, Grant Stentiford Monique van Oers
History	Elizabeth Davidson (Chair), James Harper, Donald Roberts, Harry Kaya, Fernando Vega, Juerg Huber, Mark Goettel, Just Vlak
Meetings	Mark Goettel (Chair), Nina Jenkins, Elisabeth Herniou, Jörg Wennmann
Membership	Surendra Dara (Chair) Peter Krell (Vice Chair) Ambassador Program, Stefan Jaronski (ex officio)
Nominating	Peter Krell (Chair), Jørgen Eilenberg, Leellen Solter, Mark Goettel, Madoka Nakai
Publications	David Shapiro-Ilan (Chair), Selcuk Hazir, Albrecht Kopperhöfer, Bryony Bonning, Johannes Jehle (ex officio), Jean-Louis Schwartz (Newsletter Editor; ex officio), Cecilia Schmitt (website, ex officio), Lee Solter (JIP, ex officio)
Student Affairs	Julie Hopper (Chair), Louise-Marie Roux (Co-Chair), Patricia Stock (Faculty Advisor), Satomi Adegawa (Bacteria Div.), Katharina Saar and Carina Ehrich (Fungi Div.), Andreas Larem (Microbial Control Div.); Rousel Orozco (Nematodes Div.), Yue Han and Carina Bannach (Virus Div.)

SIP DIVISION OFFICERS:

Bacteria	Kenneth Narva (Chair); Marianne Carey (Chair Elect); Sun Ming (Secretary/Treasurer); Shuyuan Guo, Juan Ferré (Members as Large); Baltasar Escriche (Past Chair); Satomi Adegawa (Student Representative)
Diseases of Beneficial Invertebrates	Helen Hesketh (Chair); Mark Freeman (Chair Elect); Kelly Bateman (Secretary/Treasurer); David Bass, Annette Bruun Jensen (Members at Large); Kelly Bateman (Past Chair); Georgia Ward (Student Representative)
Fungi	Nicolai Meyling (Chair); Stefan Jaronski (Chair Elect); Ann Hajek (Secretary/Treasurer); Dietrich Stephan, Chad Keyser (Members at Large); Katharina Saar, Carina Ehrich (Student Representatives)
M**icrobial Control**	Dietrich Stephan (Chair); vacant (Chair Elect); Michael Brownbridge (Secretary/Treasurer); Mary Barbercheck, vacant (Members at Large); Andreas Larem (Student Representative)
Microsporidia	Jyliya Sokolova (Chair); George Kyei-Poku (Chair Elect); Julie Hopper (Secretary/Treasurer); Naomi Fast, Bryony Williams (Members-at-Large); Susan Björnson (Past Chair); Sarah Biganski (Student Representative)
Nematodes	Glen Stevens (Chair): Raquel Campos-Herrera (Chair Elect); Patricia Stock (Secretary/Treasurer); Ivan Hiltpold, Barton Slatko (Members-at-Large); David Shapiro-Ilan (Past Chair); Rousel Orozco (Student Representative); Sylvia Libro (Facebook Representative)
Viruses	Madoka Nakai (Chair); Elisabeth Herniou (Chair Elect); Vera Ros (Secretary/Treasurer); Holly Popham, Jørg Wennmann (Members-at-Large); Martin Erlandson (Past Chair); Yue Han, Carina Bannach (Student Representatives)

2017 MEETING ORGANIZING COMMITTEE

Chair	Surendra Dara
Scientific Program	Richard Humber (Chair), Louela Castrillo (Co-Chair), Ed Lewis, Lerry Lacey (ex officio)
Program Book	Richard Humber
Local Arrangements	Kelli Hoover, Dennis Bideshi, Wendy Gelernter, Hyun-Woo Park, Patricia Stock
Excursions	Raffi Aroian
5K Race/Walk	Nina Jenkins, Ken Narva

FIFTY YEARS OF SIP MEETINGS

1968 **Columbus, Ohio, USA**
Organizer: John D. Briggs

1969 **Burlington, Vermont, USA**
Organizer Thomas Cheng
Excursion: Lake Champlain and Green Mountains

1970 **College Park, Maryland, USA**
with American Institute of Biological Sciences
Organizers: James Vaughn, Art Heimpel

1971 **Montpellier, France**
1st International Colloquium
Organizer: Max Bergoin
Excursion: Pont de Garde, Camargue, San Christol

1972 **Minneapolis, Minnesota, USA**
Organizer: Marian Brooks
Excursion: Water quality laboratory

1973 **Oxford, England, UK**
2nd International Colloquium
Organizer: Thomas Tinsley
Excursion: Warwick Castle; Stratford
Amherst, Massachusetts, USA
with American Institute of Biological Sciences

1974 **Tempe, Arizona, USA**
with American Institute of Biological Sciences
Organizers: Elizabeth Davidson, Patrick Vail
Excursion: Wave pool, desert hike

1975 **Corvallis, Oregon, USA**
Organizers: Mauro Martignoni, Michael Mix,
Chris Bayne

1976 **Kingston, Ontario, Canada**
3rd International Colloquium
Organizer: Tom Angus, Aaron Rosenfield,
Peter Faulkner
Excursion: Fort Henry

1977 **East Lansing, Michigan, USA**
with American Institute of Biological Sciences
Organizers: John Bresnak, T. Rizki
Excursion: Pont de Garde, Camargue, San Christol

1978 **Prague, Czechoslovakia**
Organizer: Jaroslav Weiser
Excursion: Pont de Garde, Camargue, San Christol

1979 **Gainesville, Florida, USA**
Organizer: John Harshbarger

1980 **Seattle, Washington, USA**
Organizer: Albert Sparks

1981 **Bozeman, Montana, USA**
Organizer: John Henry
Excursion: Madison River float trip

1982 **Brighton, England, UK**
4th International Colloquium
Founder's Lecture Honoree/Speaker:
K.M. Smith / C.F. Rivers
Organizers: Denis Burges, Chris Payne
Excursion: Brighton Pavilion

1983 **Ithaca, New York, USA**
Founder's Lecture Honoree/Speaker:
J. Mackin / A.H. Sparks
Organizers: Donald Roberts, Robert Granados

1984 **Davis, California, USA**
Founder's Lecture Honoree/Speaker:
E.A. Steinhaus / D.W. Roberts
Organizers: Harry Kaya, Donald Roberts
Excursions: City of Sacramento; Bodega Bay
Marine Laboratoy

1985 **Sault Ste. Marie, Ontario, Canada**
Founder's Lecture Honoree/Speaker:
G.H. Bergold / T.A. Angus Organizers:
Terry Ennis, John Cunningham,
Sardar Sohi

1986 **Veldhoven, Netherlands**
5th International Colloquium
Founder's Lecture Honoree/Speaker:
E. Metschnikoff / J. Weiser Organizers:
Just Vlak, Rob Samson, Dick Peters
Excursion: Philips EM facility; Heinekin Brewery

1987 **Gainesville, Florida, USA**
Founder's Lecture Honoree/Speaker:
L. Pasteur / C. Vago Organizers: Drion
Boucias, James Maruniak,
Al Undeen, Clayton McCoy,
John Couch, Patrick Greany

1988 **La Jolla, California, USA**
Founder's Lecture Honoree/Speaker:
"State of the Art" / H. Whiteley
Organizer: Brian Federici

1989 **College Park, Maryland, USA**
Organizers: James Vaughn, Charles Reichelderfter, Edward Daugherty
Founder's Lecture Honoree/Speaker:
R.R. Kudo / V. Sprague

1990 **Adelaide, Australia**
6th International Colloquium
Founder's Lecture Honoree/Speaker:
T.D.C. Grace / K. Maramorosch
Organizer: Dudley Pinnock
Excursion: Winery tour

1991 **Flagstaff, Arizona, USA**
5th International Colloquium
Founder's Lecture Honoree/Speaker:
R.W. Glaser / G.O. Poinar Jr.
Organizer: Elizabeth Davidson
Excursion: Philips EM facility; Heinekin Brewery

1992 **Heidelberg, Germany**
Founder's Lecture Honoree/Speaker:
E. Müller-Kögler / A. Vey
Organizers: Jurg Huber, Gisbert Zimmermann, Wolfgang Schnetter, Norbert Becker
Excursion: River boat tour

1993 **Asheville, North Carolina, USA**
Founder's Lecture Honoree/Speaker:
J.N. Couch / H.C. Whisler
Organizers: James Harper, Wayne Brooks
Excursion: Biltmore Estate

1994 **Montpellier, France**
7th International Colloquium
Founder's Lecture Honoree/Speaker:
C. Vago / L.K. Miller
Organizer: Max Bergoin
Excursion: Aigues-Morte tour

1995 **Ithaca, New York, USA**
Founder's Lecture Honoree/Speaker:
H.T. Dulmage / D.J. Ellar
Organizers: John Vandenberg, Alan Wood
Excursion: Taughannock Falls State Park

1996 **Cordoba, Spain**
Founder's Lecture Honoree/Speaker:
A. Bassi / D.W. Roberts
Organizers: Candido Santiago-Alvarez, Enrique Vargas-Osuna
Excursion: Alcazar de los Reyes Cristianos

1997 **Banff, Alberta, Canada**
Founder's Lecture Honorees/Speaker:
T.A. Angus, A.M. Heimpel / K. van Frankenhuyzen
Organizers: Mark Goettel
Excursions: Columbia Icefields and Banff Springs Hotel; Nordic Center; Lake Louise

1998 **Sapporo, Hokkaido, Japan**
Founder's Lecture Honoree/Speaker:
K. Maramorosch / J. Mitsuhashi
Organizer: Toshihiko Iizuka
Excursions: Mt. Tarumae and Shikotsu Lake; Noboribetsu Hotspring

1999 **Irvine, California, USA**
Founder's Lecture Honoree/Speaker:
Y. Tanada / J.K. Kaya
Organizers: Brian Federici
Excursions: Wine tasting; Mission San Juan Capistrano

2000 **Guanajuato, Mexico**
Founder's Lecture Honoree/Speaker:
H.D. Burges / B.A. Federici
Organizers: Jorge Ibarra, Alejandra Bravo
Excursion: San Miguel de Allende, Teatro Juarez

2001 **Noordwijkerhout Netherlands**
(relocated from scheduled site in Israel)
Founder's Lecture Honoree/Speaker:
J. Weiser / W. Brooks
Organizers: Just Vlak, Meir Broza
Excursion: Van Gogh Museum, canal boat tour

2002 **Foz de Iguassu, Brazil**
8th International Colloquium
Founder's Lecture Honoree/Speaker:
H. de Barjac / P. Lüthy
Organizers: Flávio Moscardi, Sérgio Batista Alves
Excursion: Iguassu Falls

2003 **Burlington, Vermont, USA**
Founder's Lecture Honoree/Speaker:
L. Miller / R.R. Granados Organizers: John Burand, Ann Hajek
Excursion: Lake Champlain boat cruise

2004 **Helsinki, Finland**
Founder's Lecture Honoree/Speaker:
H.G. Boman / K. Söderhall
Organizers: Heikki Hokkanen, Jørgen Eilenberg
Excursion: Marimekko factory

2005 **Anchorage, Alaska, USA**
Founder's Lecture Honoree/Speaker:
E. Canning / J. Becnel
Organizers: Kelli Hoover, Diana Cox-Foster, Bryony Bonning
Excursion: Train ride to top of Mt. Alyeska

2006 **Wuhan, Hubei, China**
9th International Colloquium
Founder's Lecture Honoree/Speaker:
Gao S.Y. / J. Vlak
Organizers: Yu Ziniu, Sun Ming, Hu (Rose) Zhihong
Excursion: Yellow Crane Tower; Three Gorges; Xi'an

2007 **Québec City, Québec, Canada**
1st Internat. Forum on Entomopathogenic Nematodes and Symbiotic Bacteria
Founder's Lecture Honoree/Speaker:
A. Sparks / F. Morado
Organizers: Conrad Cloutier, Jean Louis Schwartz, Basil Arif, Roland Brousseau
Excursion: St. Lawrence river cruise

2008 **Warwick, England, UK**
Founder's Lecture Honoree/Speaker:
A. Paillot / J. Jehle
Organizers: David Chandler, Bryonny Bonning, Doreen Winstanley
Excursion: Warwick Castle; Stratford-upon-Avon

2009 **Park City, Utah, USA**
Founder's Lecture Honoree/Speaker:
D.W. Roberts / R.J. St. Leger
Organizers: Donald Roberts, Rosalind James
Excursion: Olympic Park, ski jumpers

2010 **Trabzon, Turkey**
Founder's Lecture Honoree/Speaker:
M. Martignoni / B. Arif
Organizers: Zihni Demirbag, Basil Arif
Excursion: Sumela Monastery

2011 **Halifax, Nova Scotia, Canada**
Organizers: Susan Bjornson, Christina Noronha, Michael Agbeti, Thomas Steele
Founder's Lecture Honoree/Speaker:
J.D. Briggs / E. Davidson
Excursion: Peggy's Cove; Citadel, Pier 21

2012 **Buenos Aires, Argentina**
1st International Congress on Invertebrate Pathology and Microbial Control
Founder's Lecture Honorees/Speaker:
S.B. Alves, F. Moscardi* / I. Delalibera Jr
(* to be the Founder's Lecturer but died shortly before this meeting)
Organizers: Alicia Sciocco-Cap, Victor Romanowski, Juan Ferré
Excursion: Tigre-Delta river tour

2013 **Pittsburgh, Pennsylvania, USA**
Founder's Lecture Honoree/Speaker:
R. Granados / G. Blissard
Organizers: Nina Jenkins, Kelli Hoover
Excursion: Carnegie Museum; Three Rivers boat tour

2014 **Mainz, Germany**
Founder's Lecture Honoree/Speaker:
A. Huger / T. Jackson
Organizers: Johannes Jehle
Excursion: Rhine River cruise, Schloss Rheinfels

2015 **Vancouver, British Columbia, Canada**
Founder's Lecture Honoree/Speaker:
P. Johnson / G. Stentiford
Organizers: Mark Goettel, Todd Kabaluk
Excursion: Cheakamus Center

2016 **Tours, France**
Founder's Lecture Honoree/Speaker:
D. Ellar / N. Crickmore
Organizers: Elisabeth Herniou, Christine Nielsen-LeRoux, Jean-Michel Drezen
Excursion: Domaine de Cande

Registration:

Sunday	2– 6 pm, *Village Conference Desk (adjacent to check-in desk for housing)*
Monday	7 am –5 pm, *Price Center*
Tuesday	7: am – 1 pm, *Price Center*
Wednesday	8 am – 5 pm, *Price Center*
Thursday,	8 am – 12 noon, *Price Center*

Posters: *Price Center, West Ballroom*

Hang on Monday, after 8:30 am
Posters will be displayed throughout meeting
Remove on Thursday, by 12:00 noon

Speaker Ready Room, Slide Loading:

Sunday	Adjacent to mixer site
All other days	8:00 am – 5:00 pm, *Revelle Room, Price Center*

On-Campus Transportation

A campus shuttle–from 7 am until 6 pm–stops near the Price Center and campus housing at the Village (North campus). It It is a 1/2 mile walk from the Village to the Price Center and for those driving there is parking at Gilman Parking Deck. More information is available at *http://transportation.ucsd.edu/shuttles/campus-loop.html #Academic-Break-and-Summer-Sched*

Other useful information

A large food court is located inside the Price Center.
The Village Market, at the residence halls, is open 7 am to 8 pm with snacks, beverages, and grab and go food.

There is also an Expresso place in Building 4 on the East side of the Village. Restaurants close by: Ocean View in the Village with a mini food court. One block down and across the street is the restaurant Bella Vista.

Uber and Lyft services are available for transportation into La Jolla or elsewhere.

SUNDAY

8:45 – 5:00 Council Meeting,
Village Tower West, 15th Floor, 15C

6:00 – 9:00 **MIXER**,
Institute of the Americas – Plaza

MONDAY, 8:00 am – 12:30 pm

Theater

OPENING CEREMONY

8:00 Surendra Dara, Chair, Organizing Committee
Johannes Jehle, SIP President
Glenda Humiston, Vice President of Agriculture and Natural Resources Division, University of California

8:20 Awards Presentations
Monique van Oers, Chair, Awards and Student Committee

SIP Founders' Golden Jubilee Celebration: Historical Perspectives of the Society and Invertebrate Pathology

Organizers: Peter Krell, Jimmy Becnel

Introduction – Jimmy Becnel, Chair, Founder's Lecture Committee

Looking Back: 50 Years of SIP – *Elizabeth W. Davidson* •1•

School of Life Sciences, Arizona State University, Tempe, AZ, USA
Corresponding author: e.davidson@asu.edu

The concept of a society for the study of diseases of invertebrates began more than 10 years before the founding of SIP. The International Conference on Insect Pathology and Biological Control, hosted by Jaroslav Weiser in Prague in 1958, brought scientists together from around the world to share findings and ideas. Edward Steinhaus, professor at University of California, Berkeley, mentored many of the early scientists in our field, and was founding editor of the Journal of Insect (now Invertebrate) Pathology. In 1967 Steinhaus brought together 8 scientists to discuss the concept of establishing a Society. The first SIP meeting was held at Ohio State University in 1968. SIP has remained international, diverse, and collaborative ever since. We have met in 16 countries, and welcome members from around the world. We are looking forward to many more wonderful SIP meetings.

Personal reflections of a founding SIP member – *Donald W. Roberts* •2•

Department of Biology, Utah State University, Logan, Utah, USA.
Corresponding author: donald.roberts@usu.edu

It will be a pleasure to join you, my fellow Society for Invertebrate Pathology (SIP) members, in celebrating the jubilee (50th) anniversary of our annual meetings. It will be extremely gratifying to note how well our society has survived the vicissitudes of the years, and that it continues to serve its members in very productive and caring ways. My personal SIP history began due to my association with a key member of SIP's organizing committee, Dr. Edward Steinhaus. I joined his laboratory as a student the same year (1959) he initiated publication of the Journal of Invertebrate Pathology, so I was very aware of his desire to increase the visibility of this branch of science. When he and others initiated SIP in 1967, I immediately joined as a Founding Member in the hope that I could, in some way, help this group become established. It grew, became visible, and, in fact, was a key element in distinguishing Invertebrate Pathology as an identifiably separate discipline. Over the years, SIP became very important to my professional development. In fact, I now wonder if I would have fared nearly as well as I have professionally, as limited as that might be, had SIP never come into being. How would ***you*** answer that question concerning the existence of SIP in ***your*** professional development? We must be very thankful to the small group that 50 years ago expended the time and effort to initiate our unifying organization, SIP. One positive characteristic of our society is our exceptionally high level of friendship between our members. We care about and frequently help each other in both professional and personal advancement, and this provides the climate for developing very useful long-term research connections. SIP has a long history of providing awards for exceptional scientific performance, and these awards were instituted as a way to help our members advance both academically and professionally. Our society welcomes new members and encourages them to volunteer for activities that improve its effectiveness for its members. My advice and invitation to any of you who currently feel you are not fully involved with our SIP movement is that, if you have good ideas to improve SIP, please make them known, and also offer to help implement those ideas, as well as proposals of others that you support. In summary, my experience with SIP has been almost universally positive. Thank you, my fellow members, for making it so.

SIP Divisions, the Beginnings – *Peter J. Krell*[1], *James J. Becnel*[2] •3•

[1]Department of Molecular and Cellular Biology, University of Guelph, Canada ; [2]USDA, ARS, CMAVE, Gainesville, Florida, USA
Corresponding author: pkrell@uoguelph.ca

In 1969 Mauro Martignoni was appointed Chair of the **Divisions Committee** by then President Albert K Sparks with members Wayne Brooks, Horace Denis Burges, Phyllis T Johnson, Carl Sindermann and Marenes R Tripp. The **first Division** was spear headed by Victor Sprague in August 1969. The now **Division of Microsporidia** was approved in 1970 electing Chairman Jiří Vávra, Vice Chairman Roy McLaughlin, Secretary Ann Cali with Council Members John Henry and Edwin Hazard. A decade later, the **second Division, Division of Microbial Control** was conceived in 1979 under Chairman Jaroslav Weiser and later James D Harper as ad hoc Chairman. An executive consisting of Chairperson James D Harper, Vice Chairperson H Denis Burges, Secretary-Treasurer James Fuxa and Members-at-Large Don Roberts and John Cunningham were elected. The **third and fourth Divisions** were formalized at the 1995 meeting in Ithaca, NY, USA. The **Division of Bacteria** elected Chair Andre Klier, Vice Chair Barbara Knowles, Secretary Treasurer Bill Moar and Members-at-Large Sue Macintosh and Elizabeth (Betty) Davidson. The **Division of Viruses** elected Chair Norman Crook, Vice Chair Suzanne Thiem, Secretary Treasurer Just Vlak and Member-at-Large Bryony Bonning. **The fifth Division, Division of Fungi,** was approved in 1998 and in 2000 elected Chair Stephen Wraight, Chair-Elect Judith Pell, Secretary Treasurer Michael Brownbridge and Members-at-Large Travis Glare and Paresh Shah. The **sixth Division**, **Division of Nematodes,** was approved in 1999 and at its 2000 meeting elected Chair Itamar Glazer, Chair-Elect Noel Boemare, Secretary Treasurer Albrecht Koppenhöffer with Members at Large Bryon Adams and Patricia Stock. The **seventh Division** was the **Division of Beneficial Invertebrates** and in 2009 elected Chair Grant Stentiford, Chair-Elect Elke Genersch, Secretary Treasurer Kate Aronstein with Trustees Regina Kleespies and Huang Shaokang. **The first Division Student Representatives** were Christina Campbell (2004) for Microsoporidia, Todd Ugine (2005) for Microbial Control, Claudia Perez (2005) for Bacteria, Ernst Jan Scholte (2005) for Fungi, Heather Smith (2005) for Nematodes, Jondavid de Jong (2006) for Viruses and Kelly Bateman and Eva Forsgren (2009) for Beneficial Insects. The SIP Divisions continue to unify members and provide a unique forum for each discipline of Invertebrate Pathology.

Microsporidia – *Ann Cali* •4•

[See Addendum]

Microbial Control of Insects 1967-2017: How far we have come (and how far we still have to go) – *Stefan T. Jaronski* •5•

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Northern Plains Agricultural Research Laboratory
Sidney MT USA 59270
Corresponding author: stefan.jaronski@ars.usda.gov

In 1967, the year Ed Steinhaus and colleagues created the Society for Invertebrate Pathology, the biopesticide industry was still, more or less, in its infancy esp. in the U.S. Greater strides had been made in eastern and central Europe, but there were very few commercial products anywhere. The industry struggled through the 1970s, 1980s and even the 1990s, with momentum generally in the hands of small-medium enterprises. In the past two decades, however, the pace of commercial development of microbials has quickened all over the world. Now, even the multinationals have begun to stick their toes in microbial waters. We still have a long way to go. Despite increasing acceptance (e.g., 38% of California growers polled say they use microbial agents to manage pests and pathogens), chemical pesticides still hold sway in most regions. One problem is that many times microbial agents have been cast into a chemical paradigm, in which the microbial is expected to perform like a chemical, as good as chemical, to simply replace a chemical. What is often forgotten is that microbials should be one tool in an integrated system. (Remember the original concept of Integrated Pest Management?) Recently, efforts have been made to integrate microbials with other measures, from cultural practices to other biologicals, to create successful systems to manage insect pests. More research and *implementation* is needed, however. This, in my opinion, is the path for greater acceptance of microbials, as well as biologicals in general. This is the direction we need to pursue. Harder? Yes. More complicated? Yes. But necessary.

10:00–10:45 am Coffee Break

Insecticidal Bacteria: A remarkable success for invertebrate pathology – *Brian A. Federici*[1], *Jean-Luis Schwartz*[2] •6•

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The modern era of research on insecticidal bacteria, especially *Bacillus thuringiensis* (Bt), began after WWII when Edward Steinhaus at the University of California and international collaborators such as H. Dulmage, D. Burges, H. de Barjac developed standardized bioassays and serological identification for Bt strains. These advances yielded the first insecticides for lepidopteran pests based on *B. t.* subsp. *kurstaki* (HD1). Later, *B. t.* subsp. *israelensis*, active against mosquito and black fly larvae, was discovered, commercialized in the 1980's, and used successfully in WHO's Onchocerciasis Control Program in west African countries, and for controlling mosquitoes. Advances in molecular biology led to Schnepf and Whiteley's discovery in 1981 that HD1's bipyramidal crystal contained three different Cry proteins, Cry1Aa, Cry1Ab, and Cry1Ac. This quickly advanced basic and applied Bt research, resulting in new bacterial insecticides and Cry-based genetically engineered crops in the 1990's. Bt cotton and Bt corn were rapidly adopted by farmers in the U.S. and other countries, and current seed sales exceed $US 10 billion per year, with annual revenues for these crops now more than $300 billion, which includes engineered soybeans. This success was paralleled by basic research in the 1980's on Cry protein mechanism of action, showing insect gut damage was due to colloid-osmotic lysis, followed in the 1990's by demonstrating Cry proteins formed pores in target cell membranes and triggered cellular signaling. Simultaneously, APN, cadherin and ALP proteins were identified as Bt receptors, and 3-D atomic structures of Cry and Cyt toxins became available, opening the way in the 1990's and 2000's to structure-function analyses of molecular determinants for receptor binding and pore formation. The current millennium has witnessed discovery of new Bt toxins (acting as single or binary toxins against new targets including mammalian tumor cells, displaying completely new 3-D structures); a new class of Cry receptors was identified (ABC transporters); and cellular defense mechanisms induced by these proteins have been discovered. This progress is a remarkable success for insecticidal bacteria, but the future is even brighter made possible by synthetic biology techniques including rapid sequencing of new toxins and retargeting others for improved pest control.

Fifty years of Invertebrate Virology: a stellar course – *Just Vlak*[1, 2], *Zhihong Hu*[2] •7•

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Invertebrate virology over the last fifty years has the Society for Invertebrate Pathology (SIP) a lot to thank. SIP brought together interested (and interesting!) scientists and promoted invertebrate pathology as an integrative discipline. After establishment of the Society (1967) the focus was primarily on virus discovery and use as biocontrol agents. The focus soon shifted to molecular and cell biological approaches. The Society willingly took in and facilitated these emerging disciplines by providing a platform for a new breed of young and committed virologists. Invertebrate virologists also brought their research to the forefront of science and the scientific community at large, with publications in high ranking journals and major fundamental advances like discovery of the anti-apoptotic response and practical applications such as production of pharmaceuticals, diagnostic reagents and even effective vaccines. Although molecular virology was dominant for some time, increased interest in environmental issues in the late 1990s promoted the resurgence of invertebrate virus ecology, also stimulated by major diseases in commercial invertebrates, and re-appreci-

ated field oriented science. The molecular and ecological disciplines, and associated techniques, mutually benefitted and promoted the molecular ecology of invertebrate viruses. The prominence of Invertebrate Virology was institutionalized by the formation of the SIP Virus Division in 1995. While initially analysis of viruses relied on SDS-PAGE for proteins and DNA restriction enzyme profiles, the 2000's that gave way to sequencing of first viral DNA/RNA fragments then genes, genomes and metagenomes. Molecular engineering came to its full potential providing detailed insights in the functioning of virus genes. Molecular detection/identification techniques became indispensable for invertebrate virus research and virus registrations. Understanding the interaction between virus and invertebrate host, at levels ranging from cell to organism to populations to ecosystem, now became feasible. This is paramount to understanding the response of organisms to virus infection and essential to control or mitigate invertebrate virus diseases. It also allows tailored application of viruses for controlling insect populations. The lesson learned here is that fundamental research of societal relevance necessarily precedes a rational exploitation of this knowledge. The SIP can take pride in having fostered this development in Invertebrate Virology over the last fifty years.

Fifty years of Fungi in fewer than fifteen minutes?! – ***Richard A. Humber***[1]*, Jørgen Eilenberg*[2] •8•

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This presentation attempts to condense a half century of very active progress and significant transitions in our knowledge of all aspects of entomopathogenic fungi (especially EPFs from the most studied taxa fromAscomycota and Entomophthoromycota) and their fungus-like allies (biflagellate, oomycetous pathogens). Subjects to be covered include our expanding knowledge of the biodiversity and ecology of EPFs; new understandings about the life histories and pathobiology of EPFs; their interaction with insects, plants and environment; access to formal training in basic and applied aspects of insect mycology; and the astonishing (and, too often, bewildering) changes in our knowledge of the systematics and classifications of these fungi. The status of the practical uses of EPFs as biological control agents and as sources of biologically active compounds will also be noted.

Symbiosis between entomopathogenic nematodes and bacteria: from taxonomy to ecology and genomics – ***Harry K. Kaya*** •9•

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Different groups of nematodes including mermithids, tylenchids, diplogasterids, and rhabditids infect insects. Among the rhabditids, two families, Steinernematidae and Heterorhabditidae, referred to as entomopathogenic nematodes (EPNs), have received the most attention over the past four decades. These EPNs are associated with bacteria in the genus *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis,* which form a nematode-bacterium complex that work mutualistically to kill their insect host within 48 hours. Since the first description of *Steinernema* (*Aplectana*) *kraussei* by Gotthold Steiner in 1923, more than 100 species of both nematode genera have been described. In 1936, Rudolf Glaser was the first to produce *S. glaseri in vitro* and apply it for microbial control of the Japanese beetle, *Popillia japonica*. Although the application of *S. glaseri* was partially successful, Glaser considered that the contamination by microorganisms affected nematode production and efficacy. In 1959, S. R. Dutky demonstrated that a symbiotic bacterium, later to be described as *Xenorhabdus nematophila*, is retained and required for successful production and use of *S. carpocapsae*. In the 1980s, commercial companies mass produced several EPN species *in vitro* and made them available to researchers for efficacy trials against a number of soil insect pests. With some successes and many failures, more fundamental research was required to define what environmental and biotic factors affected efficacy.

Understanding the response of EPNs to abiotic and biotic factors is essential for increasing the success of EPNs against an insect pest. Abiotic factors such as soil type, temperature, UV light, and humidity and biotic factors such as nematode foraging behavior and persistence, host susceptibility and resistance, and host density affected nematode efficacy. Recent studies show that EPNs not only respond to host cues but also to nematode pheromones and plant signals. Moreover, genomics and related studies, as well as the in-depth knowledge of how the bacteria maintain their mutualistic relationship with EPNs and how bacterial virulence affects efficacy will further advance EPN use in microbial control programs.

Diseases of Beneficial Invertebrates – ***Kelly Bateman***[1]*, Elke Genersch*[2]*, Grant Stentiford*[1]*, Helen Hesketh*[3] •10•

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The Diseases of Beneficial Invertebrate (DBI) division was established in 2009 at the Park City meeting of the Society for Invertebrate Pathology (SIP). The division addresses diseases of invertebrates that are generally considered as non-pest species, covering aquatic and terrestrial invertebrates which are ecologically or economically beneficial. The division focuses on the pathology and host-pathogen interface within various host groups (crustaceans, molluscs, insects and arachnids) and addresses significant bigger picture issues that can be studied via these relationships (e.g. climate change, ocean acidification, epizootiology, pollination, insects for food and feed, disease mitigation). When first established the Society for Invertebrate Pathology possessed a strong interest in aquatic pathology, in particular mollusc and crustacean disease. Over the years this area of the society had diminished, a particular aim of the Division was to attract pathologists and disease experts with an interest in pathogens and pathology of aquatic animals in order to improve the standing of this discipline in the wider society. Alongside this, the significantly increased interest in bee diseases and diseases of other beneficial terrestrial invertebrates co-stimulated the formation of a host directed division. The division has held some key symposia in recent meetings, in 2011 an OECD funded symposia was held to discuss Disease in Aquatic Crustaceans: Problems and Solutions for Global Food Security and in 2015 an additional OECD symposia was funded to examine Microsporidia in the Animal to Human Food Chain: An International Symposium to Address Chronic Epizootic Disease. In 2015, the Founders Lecture honoree was Dr Phyllis Johnson, a pioneer of aquatic invertebrate pathology and a key figure in establishment of the SIP as we know it today. This presentation will provide an overview of the division from conception to now.

The SIP–JIP connection through the years: Supporting invertebrate pathology research – ***Leellen Solter***[1]*, Dale Seaton*[2]*, Brian Federici*[3] •11•

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The Journal of Invertebrate Pathology and the Society for Invertebrate Pathology have been partners in promoting and supporting invertebrate pathology research since the inception of the Society in 1967. The journal was inaugurated first, in 1959, as Journal of Insect Pathology. The name was changed to Journal of Invertebrate Pathology in 1965, at which time Edward Steinhaus wrote to Albert Sparks that he had proposed a society of the same name. A subsequent global survey of invertebrate pathologists showed overwhelming support for the establishment of the Society and inclusion of the Journal of Invertebrate Pathology as its official organ. In 1968, the Journal of Invertebrate Pathology was first Published under the auspices of the Society for Invertebrate Pathology. Since 1965, over 6,200 papers have been published with Society members well represented as authors. We highlight the history of the JIP-SIP connection as well as researchers and

papers published that garnered significant attention from the scientific community. As well, we celebrate 50 years of enthusiastic encouragement and advancement of invertebrate pathology!

12:15 Presentation of Plaques – Jimmy Becnel

MONDAY, 12:30 – 1:30 pm
LUNCH BREAK

JIP Editorial Board Meeting and Luncheon
Price Center West, Bear Room

MONDAY, 1:30 – 3:30 pm

Theater

BACTERIA AND MICROBIAL CONTROL CROSS-DIVISIONAL SYMPOSIUM

Challenges for Biocontrol in Latin America

Organizers and Moderators: Travis Glare and Ken Narva

The use of *Bacillus thuringiensis* and Baculovirus based biopesticides in Brazil **1:30** **•12•**

Fernando H. Valicente

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The search for Baculovirus and *Bacillus thuringiensis* (*Bt*) based biopesticides has increased since 2012/13, when *Helicoverpa armigera* was detected in Brazil. Embrapa Maize and Sorghum has a *Bt* and a baculovirus collection, with more than 4,600 Bt strains and more than 100 baculovirus isolates, collected from different regions of Brazil. Most of these insect pathogens have been tested to insect pests, such as: fall armyworm *Spodoptera frugiperda*, black armyworm *S. cosmioides*, southern armyworm *S. eridania*, corn earworm *Helicoverpa zea*, cotton bollworm *H. armigera*, soybean looper *Chrysodeixis includens, the* green belly stink bug *Dichelops sp* and sugar cane borer *Diatraea saccharalis*. Some baculovirus, all of them NPV, have been developed as biopesticides (wettable powder-WP) to control fall armyworm, soybean looper and cotton bollworm. *Baculovirus spodoptera* (isolate 6) is very efficient and doesn´t cause the disruption of the integument of the caterpillar, and this factor is essential in a large-scale production system. Isolates for cotton bollworm where identified as HearNPV and showed a CL_{50} of 4.1 x 10^4, and is formulated as WP. *B. thuringiensis* isolates have been tested for many *cry (1, 2, 3, 4, 5, 6, 8, 9, 12, 13, 14*, 22 and 51) and *Vip (1, 2, vip3Aa1, vip3Ah1, vip3Ae1, vip3Ba1, vip3Aa2, vip3Af1)* genes in order to control from caterpillars to sucking bugs. Some Bt isolates have been developed as biopesticides to control fall armyworm, soybean looper and cotton bollworm. Brazilian companies, with high production capacity, have signed contracts with Embrapa in order to put these products in the market in the near future. Baculovirus (I6) and Bt have been registered. Baculovirus for fall armyworm is already in the market.

Cry1F resistance among lepidopteran pests: A model for improved reistance management? **2:00** **•13•**

Ana María Vélez

University of Nebraska, Lincoln

The Cry1Fa protein from *Bacillus thuringiensis* (Bt) is known for its potential to control lepidopteran pests, especially through transgenic expression in maize and cotton. The maize event TC1507 expressing the cry1Fa toxin gene became commercially available in the United States in 2003 for the management of key lepidopteran pests including the European corn borer, *Ostrinia nubilalis*, and the fall armyworm, *Spodoptera frugiperda*. A high-dose/refuge strategy has been widely adopted to delay the evolution of resistance to event TC1507 and other transgenic Bt crops. Efficacy of this strategy depends on the crops expressing a high dose of the Bt toxin to targeted pests and adjacent refuges of non-Bt host plants serving as a source of abundant susceptible insects. While this strategy has proved effective in delaying *O. nubilalis* resistance, field-evolved resistance to event TC1507 has been reported in *S. frugiperda* populations in Puerto Rico, Brazil, and the southeastern United States. This talk will examine available information on resistance to Cry1Fa in *O. nubilalis* and *S. frugiperda* and discuss how this information identifies opportunities to refine resistance management recommendations for Bt maize with an emphasis in Latin America.

Microbial products for agriculture in Uruguay **2:30** **•14•**

Nora Altier[1], Elena Beyhaut[1], Federico Rivas[1,2], Eduardo Abreo[1], ***Trevor A. Jackson[3]***

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Biotechnologies based on microbial resources have made possible the development of biological products to improve plant protection and plant nutrition, without compromising the environment. These technologies also contribute to the achievement of high end market requirements for low agrochemical use. The Platform of Microbial Products for Agriculture at INIA-Uruguay (National Institute for Agricultural Research) has emphasized the role of microorganisms in biocontrol of plant pests and diseases, and in nitrogen and phosphorus plant nutrition. The long term strategy lies on strengthening research facilities and team work, recruitment and continuous training of human resources, research and technology ventures with the public and private sectors, fostering a legal framework for registration and intellectual property of products, and promoting education and extension for farmer adoption. Research areas include: (1) biological control, (2) biological nitrogen fixation, (3) microbial mediated phosphorus availability to plants, and (4) bioproduction and formulation. Current biocontrol projects focus on identifying strains of microbe for product development to be incorporated in Integrated Pest Management (IPM) for targeted crops and pests. Biological nitrogen fixation is being efficiently exploited by inoculating legumes with suitable rhizobial inoculants, supported by appropriate legislation, which sets requirements for product registration, mandatory strain recommendation, and quality surveillance of commercial inoculants. Supporting research addresses inoculation and seed coating technologies, fungal endophytes, as well as metagenomics for assessment of microbial diversity and soil health.

Fungal-based biopesticides in Brazil: Challenges and Opportunities **3:00** **•15•**

Italo Delalibera Júnior

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Brazil is the world's largest consumer of chemical pesticides, but adoption of biological control has grown at a rapid pace in the country. The future of biological control will depend on scientific research guidelines and regulatory mechanisms to ensure the quality of products available on the market. A very limited number of isolates of few species of microorganisms is marketed today in the country in the form of biopesticides. Motivated for facilities and speed in the registration of phytosanitary products for organic agriculture, many companies commercialize the same microorganisms. The same happens with sugarcane mills that use entomopathogenic fungi for their own consumption. The micopesticides in the country are based on aerial conidia produced by solid fermentation almost exclusively using rice, with production system without substantial innovations since the 70's. More efficient production systems must be developed, either by automating stages in production by solid fermentation or by producing other structures such as blastospores and microsclerotia by liquid fermentation to meet the growing demand. Many microbial products in the Brazilian market are of questionable quality, unformulated and with short shelf life. To improve these products it is necessary to develop formulations taking into account the pest and target crop and adapted to the Brazilian climate conditions. Fungi are used for the control of few pests only through aqueous sprays. The

development of biological control strategies in Brazil is a challenge, considering the large continuous areas of the country's agricultural land, growing crops successively over a long period of the year and the various pest cycles per year. It is in this context that scientific research must play a fundamental role in the direction and future of biological control in the country.

The Forum

Virus Division #1

Moderators: John P. Burand, Robert L. Harrison

AcMNPV adaptive evolution in varying environment **1:30** **•16•**

Aurélien Chateigner, Yannis Moreau, Cindy Pontlevé, Carole Labrousse, ***Elisabeth A Herniou***

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Genetic variation underpins the evolutionary process of adaptation. As populations become adapted to different environments, they diverge from one another. Baculoviruses infecting different host species thus usually belong to different species. Yet some viruses, such as *Autographa californica multiple Nucleopoly-hedrovirus* (AcMNPV), have retained the capacity of infecting many host species. To understand how AcMNPV could draw on standing genetic variation to adapt to different host species we undertook an experimental evolution protocol. A highly polymorphic AcMNPV population was taken through 10 in vivo infection cycles in 4 host species of various susceptibilities, to create 50 evolutionary lines in which the virus was allowed to evolve either as specialist on separate species (4x10 lines) or as generalist on all the hosts (10 lines), whereby the viral population resulted from the infection of all hosts at each generations. We then characterized the genetic make up of the original and evolved baculovirus populations by ultra-deep Illumina sequencing. Using a population genomics approach, we then estimated the global fitness and genetic diversity of each of the evolved populations and their divergence from one another. We found that the specialist lines that evolved on the same host species were more similar to one another than to other lines, especially in the most susceptible hosts, showing experimental evolution could lead to specific adaptation. Furthermore, we found a general diminution of genetic diversity in the specialist lines compared to the ancestral population. The viral lines that could adapt to more resistant host retained higher genomic diversity than those that did not, showing genetic variation is an important component of baculovirus adaptation. Evolution in all the hosts led to high genetic diversity in the generalist lines, which could foster ecological resilience in variable environments such as those encountered by the virus in natural landscapes.

Role of Plutella xylostella nucleopolyhedrovirus-CL3 *ie2* in host range adaptation **1:45** **•17•**

Rahul P. Hepat[1]*, Martin A. Erlandson*[2]*, Robert L. Harrison*[3]*, Leslie G. Willis*[1]*, David A. Theilmann*[1]

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The diamondback moth (*Plutella xylostella*) is one of the most important destructive insect pests of cruciferous crops worldwide. Previous studies identified both alpha- and betabaculoviruses that can infect and kill this important lepidopteran pest. The genome of the alphabaculovirus isolate Plutella xylostella nucleopolyhedrovirus-CL3 (PlxyNPV-CL3) was recently sequenced and was found to be nearly identical (98.5%) to the genome of Autographa californica multiple nucleopolyhedrovirus (AcMNPV). Interestingly PlxyNPV-CL3 is highly lethal for *P. xylostella,* whereas AcMNPV is not. Therefore, any differences in the genome of these two viruses may identify genes that are required for host adaptation. The dominant difference between the PlxyNPV-CL3 and AcMNPV-C6 genome is the open reading frame of the *ie2* gene. The predicted amino acid sequences of the two IE2 proteins exhibit only 37.5% similarity. To determine if PlxyNPV-CL3 IE2 is responsible for adaptation of AcMNPV to *P. xylostella*, recombinant viruses were generated using bacmids. These viruses were an *ie2* knockout (v*ie2*KO), and the *ie2* knockout repaired with AcMNPV or PlxyNPV-CL3 *ie2* tagged with HA epitope (v*ie2*KO-acie2HA and v*ie2*KO-pxie2HA respectively). Each of these viruses was tested for replication in various cell lines which including Sf9, Tn5b1-4, and PxE-Po#583. Previous studies have not analyzed the impact of a complete AcMNPV *ie2* knockout. Surprisingly, our results showed that deletion of *ie2* led to a greater than 90% reduction in budded virus (BV) production compared to wildtype virus. In addition, occlusion body (OB) production was also greatly reduced. Initial results with v*ie2*KO-pxie2HA indicate that PlxyNPV-CL3 IE2 can rescue v*ie2*KO producing BV titers nearly as high as AcMNPV IE2. Further studies analyzing virus infection in *P. xylotella* larvae will be reported.

Tracing the origin of nucleopolyhedrovirus from an invasive species, winter moth *Operophtera brumata* L. (Lepidoptera: Geometridae) **STU** **2:00** **•18•**

Hannah J. Broadley[1]*, Woo Jin Kim*[2]*, John P. Burand*[3]*, Joseph S. Elkinton*[1]

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The exchange of pathogens between populations or related species can mediate insect invasions in complex ways, either suppressing or stimulating population growth. We evaluated sources of nucleopoly-hedroviruses (NPV) in winter moth *Operophtera brumata* L. (Lepidoptera: Geometridae). Invasive populations of this destructive insect from Europe may be infected with NPV from its source population in Europe or from native, sympatric geometrids. Winter moth may also introduce novel isolates of NPV to native species when infesting new regions. We sequenced two NPV loci (polyhedrin and p74) from winter moth and its congeners across winter moths' introduced and native range and ran phylogenetic analyses to (1) evaluate differences between NPV isolates of the three populations of invasive winter moth in the United States and (2) compare the NPV from these invasive populations to potential native winter moth source populations and to sympatric geometrids. We found that the virus from winter moth and Bruce spanworm are closely related and constitute a clade within the Alphabaculovirus Group 2 NPVs but are distinct, suggesting that both strains are highly host specific. However, evidence indicates that winter moth NPV may cross-infect related geometrids in Europe. Further, we suggest that the winter moth virus found in the northeastern U.S. may originate from northern Europe.

Genome features of Troides aeacus nucleopolyhedrovirus (TraeNPV) from golden birdwing larvae (*Troides aeacus*) **2:15** **•19•**

Yu-Feng Huang[1]*, Tzu-Han Chen*[1]*, Zi-Ting Chang*[2]*, Se Jin Lee*[3]*, Jong Cheol Kim*[3]*, Jae Su Kim*[3]*, Chung-Hsiung Wang*[4]*, Kuo-Ping Chiua*[1]*, Yu-Shin Nai*[2]

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Golden birdwing butterfly (*Troides aeacus*) is rarely observed species in Taiwan, but recently we found a population, which showed a classic symptom of nucleopolyhedrovirus infection. Primary PCR was performed to confirm NPV infection in *T. aeacus* larvae. To better understand this NPV, the nucleotide sequence of the NPV DNA genome was determined by a high throughput genome sequencing (NGS) from infected larvae. The genome of TraeNPV consisted of 125,523 bases, and had a G+C content of 40.35% and contained 142 putative open reading frames (ORFs). The gene content and gene order of TraeMNPV were similar to those of AcMNPV, with 90 ORFs identified as homologous to those reported in the AcMNPV genome. Eight homologous regions (hrs) were identified containing 29 repeated sequences

composed of 30bp imperfect palindromes. Analyses of the gene parity, gene homologues and a phylogenetic analysis suggested that TraeMNPV is a Group I NPV that is closely related to AcMNPV, BmNPV and PlxyMNPV. From the preliminary result of range test, it showed that TraeNPV expanded host range to Silkworm (*Bomyx mori*). More biological data and the further genome-wide analysis would clarify the identity of TraeNPV.

The Operophtera brumata nucleopolyhedrovirus (OpbuNPV) represents a new and distinct lineage within genus *Alphabaculovirus* **2:30 •20•**

Robert L. Harrison[1]*, Daniel L. Rowley*[1]*, Joseph Mowery*[2]*, Gary R. Bauchan*[2]*, John P. Burand*[3]

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Operophtera brumata nucleopolyhedrovirus infects larvae of the winter moth, *Operophtera brumata*. Recently, an isolate of this virus, OpbuNPV-MA, was described from a population of winter moth in Massachusetts, USA. This isolate was characterized by electron microscopy (EM) of OpbuNPV occlusion bodies and by sequencing of the viral genome. The occlusion bodies of OpbuNPV-MA were polyhedral in shape and measured approximately 1 – 1.5 µm in diameter. Virions within the occlusion bodies consisted of a single nucleocapsid within each unit envelope. The OpbuNPV-MA genome was found to be 119,054 bp in length, with a 39.83% G+C nucleotide distribution. Five homologous repeat regions consisting of 40 – 44 nt imperfect inverted repeats were identified in the sequence. A total of 130 ORFs were identified and annotated, including the 38 core genes found in all viruses of family Baculoviridae. ORFs encoding homologues of DNA photolyase, DNA ligase III, and LdMNPV host range factor-1 were found, as well as two separate ORFs encoding homologues of nicotinamide riboside kinase 1. In addition, 22 ORFs with no homologues in baculovirus genomes were identified, including an ORF with sequence similarity to an uncharacterized winter moth transcript. OpbuNPV-MA homologues of ORF1629/*ac9*, ribonucleoside-diphosphate reductase small and large subunits, dUTPase, chaB/*ac58/59/60,* and ubiquitin/*ac35* were identified that exhibited little to no sequence similarity with other baculovirus homologues and did not group with baculovirus taxa in phylogenetic trees. Phylogenetic inference with concatenated alignments of core gene amino acid sequences placed OpbuNPV-MA on a branch lying outside the group I and group II clades of genus *Alphabaculovirus*. These results collectively indicate that OpbuNPV-MA represents an alphabaculovirus lineage that appeared early during the divergence of *Alphabaculovirus*.

Crossing kingdoms: Providence virus (Family *Carmotetraviridae*) infects and replicates in plants, insects and mammalian cell lines **2:45 •21•**

Rosemary A Dorrington[1]*, Meesbah Jiwaji*[1]*, Janet Awino Awando*[1]*, Ritah Nakayinga*[1,2]*, Mart-Mari de Bruyn*[1]

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Providence virus (PrV) is a positive-sense single-stranded RNA virus belonging to the Family *Carmotetraviridae*. It was first isolated from a persistently infected *Helicoverpa zea* midgut cell line and until recently was the only tetravirus able to replicate in cell culture. While the PrV capsid displays the *T*=4 icosahedral architecture that is typical of the insect-infecting tetraviruses, its Carmovirus-like replicase is related to those of the plant-infecting *Tombusviridae* and umbraviruses. This has led us to hypothesize that PrV originated by re-assortment between a plant and an insect virus. Here we present evidence that PrV has indeed arisen via horizontal virus transfer between plants and animals. In addition to infecting lepidopteran insects, PrV readily infects and replicates in human tissue culture cells, establishing persistent infections in cervical (HeLa) and breast (MCF7) cancer cell lines. We show that functional conservation between the PrV and Tombusvirus replicases extends to the ability of PrV to replicate in plants. We also present data demonstrating that the spread of the virus from the point of infection and the production of infectious PrV particles in *Vigna unguiculata* (cowpea) plants. These and other data lead us to conclude that PrV is an extant example of horizontal virus transfer between plants and animals which, remarkably, has retained its roots in the plant world, while expanding its host range from insects to mammals.

No longer a simple virus: The alphatetravirus, Helicoverpa armigera stunt virus, expresses three small proteins that co-localize with the viral replicase **3:00 •22•**

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Tetraviruses are small insect RNA viruses with capsids that exhibit T=4 icosahedral symmetry. They are classified into three families (*Alphatetraviridae*, *Permutotetraviridae* and *Carmotetraviridae*) based on the divergent characteristics of their viral replicase (REP). The Tetraviruses are considered to be amongst the simplest viruses known, encoding only the viral REP and capsid protein (CP) precursor, but recent studies show that this is no longer true. The genome of the alphatetravirus Helicoverpa armigera stunt virus (HaSV) comprises two RNAs: RNA2 encodes the CP and p17, a non-structural protein that is involved in RNA packaging. In addition to REP, RNA1 encodes three small ORFs, which overlap with the REP ORF at its 3' terminus, potentially producing p11, p15 and p8, all proteins of unknown function. These ORFs are arranged in tandem, separated by UGA (between p11 and p15) and UAA GGG (between p15 and p8). We investigated whether p11, p15 and p8 play a role in the viral life cycle. We report that the three ORFs are expressed in the midgut cells of HaSV-infected *Helicoverpa armigera* larvae as p11, p8 as well as a 34 kDa polyprotein comprising p11-p15-p8. Analysis of the sequences surrounding the stop codons suggests that the polyprotein is likely expressed via a novel translation mechanism involving two read-through stop codons. EGFP-tagged p11 and p15 localize to punctate structures that co-localize with the viral REP in the cytoplasm of *Spodoptera frugiperda* (Sf9) cells. These punctate structures are associated with the Golgi apparatus and the vesicles of the secretory pathway. These data lead us to propose that p11, p15 and p8 play a role in the infectious viral lifecycle and likely in the initiation of viral replication in HaSV-infected cells.

Characterization of a Group I alphabaculovirus specific gene, *ac5,* from Autographa californica nucleopolyhedrovirus **STU 3:15**

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Autographa californica nucleopolyhedrovirus (AcMNPV) *orf5* (*ac5*) is a Group I alphabaculovirus-specific gene with unknown function, although AC5 was reported previously to be associated with *per os* infectivity factor (PIF) complex. The purpose of this study is to characterize AC5 during AcMNPV infection. Transcription and expression analyses suggested that *ac5* was a viral late gene. Western blot and immunoelectron microscopy showed that AC5 was an occlusion body (OB) structural protein, but was not detected as a component of budded virus (BV) or occlusion-derived virus (ODV). An *ac5*-deleted recombinant AcMNPV was generated by homologous recombination to investigate the role of *ac5* in virus life cycle. One step growth curve assay indicated that *ac5* was not required for BV production and infection in Sf9 cells. Scanning electron microscopy and transmission electron microscopy demonstrated that the deletion of *ac5* didn't affect OB morphogenesis and embedding of ODV into OB. Bioassay showed that the *ac5*-deleted virus was

still orally infectious to host larvae and the LC_{50} value was not significantly different from that of the *ac5*-repaired virus. Partial denatured SDS-PAGE and co-immunoprecipitation assay suggested that AC5 was not associated with PIF complex. In conclusion, AC5 is a structural protein of OB instead of a component of PIF complex, and is not essential for either BV- or ODV infection.

Roosevelt Room

Microsporidia Division #1

Moderator: TBD

Genome size in Microsporidia: An evolutionary hypothesis **1:30** **•23•**

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It has long been suspected that the Microsporidia may, as obligate intracellular parasites, have small genomes because, for example, they could use Krebs cycle intermediates generated by the host. Genomic analysis has borne out the fact that microsporidia have small genomes. However the idea that an organism might have a small genome because it doesn't need a large genome is not an evolutionary explanation. In fact may organisms have genomes 10 to 100 times larger than the proteome. In addition studies comparing genome size versus phylogeny have shown that microsporidia genomes grow and shrink over evolutionary time. While we are aware that genetic drift could be responsible for the changing of microsporidial genome size we hypothesize that genome size may be a limiting factor in the rate of reproduction in the microsporidial genome. As such there would be a tradeoff between having a small genome and reproducing rapidly versus having a larger genome and giving the parasite more genetic options with which to challenge a host.

Genome, virulence factors, and specific molecular diagnosis of the microsporidian *Enterocytozoon hepatopenaei* (EHP) **STU** **1:45** **•24•**

***Pattana Jaroenlak**[1,2], Piyachat Sanguanrut[2,3], Bryony A. P. Williams[4], Dominic Wiredu-Boakye[4], Grant D. Stentiford[5], Timothy W. Flegel[2,6], Kallaya Sritunyalucksana[3,6], Ornchuma Itsathitphaisarn[1,2]*

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Hepatopancreatic microsporidiosis (HPM), a new emerging disease caused by *Enterocytozoon hepatopenaei* (EHP), has become a major issue in the Asian shrimp industry. EHP has been reported to cause slow growth in both black tiger shrimp (*Penaeus monodon*) and white-legged shrimp (*P. vannamei*) resulting in significant economic loss. Unfortunately, treatment against EHP infection has not been developed due to the lack of the insight into the parasite infection mechanism. Therefore, a genomic study of EHP was carried out to identify potential pathogenic genes of EHP. Among the virulent factors identified are spore wall proteins (SWP) and nucleotide transporters. The former has already been used to as a marker to improve the specificity of EHP diagnosis. To further characterize subcellular localization and function of SWP in spore attachment, antibody against SWP has been produced for immunofluorescent analysis. If the SWP is located in the exospore layer, it may provide a new marker for the development of a pond-side EHP diagnostic kit. In addition to SWP, this study will investigate the role of the nucleotide transporters of EHP in energy parasitism. Taken together, the insight from the functional characterization of the aforementioned proteins may lead to a pathway to control EHP infection.

First finding of a microsporidium of the family Tubulinosematidae infecting *Drosophila suzukii* **STU** **2:00** **•25•**

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The demand of biological control agents against *Drosophila suzukii* (spotted wing drosophila, SWD) has become urgent around five years ago since the pest has been noticed as a major thread to commercially grown fruits in Europe. Microbial antagonists isolated from these invasive fly might be qualified as biological control agents to SWD. To find potential microbial antagonists for biological control we focused on specimens from countries of origin and early introduction like the USA, where natural control factors may be already established. In wild populations from different origins, microsporidia were detected. With one species infections of lab populations of SWD were successful. Molecular studies revealed a nearest relatedness with the family Tubulinosematidae. Blast results showed about 98% identity to *Tubulinosema loxostegi* with only 28 bp difference compared to the sequenced SSU-ITS-LSU region and phylogenetic reconstruction implies an assignment to the clade III microsporidia (Aquasporidia). Further gene regions will be sequenced. Morphological analyses by light and electron microscopy are conducted and will be compared to the molecular data. Bioassays in the lab with this microsporidium to investigate possible reduction of life span, fertility and fecundity of SWD are ongoing.

The new species, *Nosema maddoxi* is a widespread pathogen of the green stink bug *Chinavia hilaris* and the brown marmorated stink bug *Halyomorpha halys* **2:15** **•26•**

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A new microsporidian species infects the green stink bug, *Chinavia hilaris*, the brown marmorated stink bug, Halyomorpha halys, the brown stink bug, *Euschistus servus*, and the dusky stink bug, *Euschistus tristigmus*. All life stages are unikaryotic, but analysis of the consensus small subunit region ofthe ribosomal gene places this microsporidium in the genus *Nosema*, which historically has been characterized by diplokaryotic life stages. It is also characterized by having the reversed arrangement of the ribosomal gene (LSU –ITS- SSU) found in species within the true *Nosema* clade. This microsporidium is apparently Holarctic in distribution. It is present in H. halys both where it is native in Asia and where it is invasive in North America, as well as in samples of North American native *C. hilaris* collected prior to the introduction of *H. halys* from Asia. Prevalence in *H. halys* from mid-Atlantic, North America in 2015-2016 ranged from 0.0-28.3%, while prevalence in *C. hilaris* collected in Illinois in 1970-1972 ranged from 14.3-58.8%. Oral infectivity and pathogenicity were confirmed in *H. halys* and *C. hilaris*. Morphological, ultrastructural and ecological features of the microsporidium, together with a molecular phylogeny, establish a new species named *Nosema maddoxi* sp. nov.

Experimental infection of caged honey bees and bumblebees with *Nosema ceranae* **2:30** **•27•**

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Managed colonies of *A. mellifera* and *Bombus* sp. are both widely used as commercial pollinators in agriculture, thus, they considerably contribute to human food security. Therefore, diseases of bees (honey bees and bumblebees) are of concern not only for scientists but also for beekeepers, farmers, and consumers alike. Among the parasites and pathogens that cause disease in and death of bee colonies are microsporidia, which are fungal-related, obligate intracellular parasites. Three microsporidian species are known to infect bees: *Nosema apis*, *N. bombi* and *N. ceranae*. While *N. apis* is a specific parasite of *A. mellifera*, *N. bombi* is specific for *Bombus* sp.. In contrast, *N. ceranae* is a rather promiscuous parasite. It was considered for long to be specific for the Eastern honey bee (*A. cerana*), but extended its host range to *A. mellifera* probably in the last century. Recently, it was suggested that *N. ceranae* also switched from *A. mellifera* to *Bombus* sp.. Infected *A. mellifera* colonies are the most likely indirect source of infection via spore-contaminated fecal spots left on flowers by defecating forager bees. The reported host-parasite range expansion of *N. ceranae* may pose a serious threat to wild bees in general; however, productive infection of *Bombus* sp. by *N. ceranae* has not been proven so far. We here present our results obtained from experimental infection of caged honey bees and bumblebees with *N. ceranae* spores. Productive *N. ceranae* infection was analyzed via PCR analysis of midgut extracts as well as via *N. ceranae*-specific fluorescence *in situ* hybridization analysis of midgut sections. We show that oral infection of caged naïve honey bees with *N. ceranae* spores resulted in 100% infected animals, whereas oral infection of caged naïve bumblebees did not lead to a detectable *N. ceranae* infection of the midgut.

The microsporida *Nosema ceranae* has a unique Heat Shock Response system and is highly sensitive to proteotoxic stress **2:45**

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Nosema ceranae is a microsporidian parasite that is pathogenic to honey bees and can cause disease at the individual and colony level. While *N. ceranae* infection can currently be controlled by treatment with the drug Fumagillin, high doses of this drug are toxic to host cells and the potential for development of resistance suggests the need for alternative treatment strategies. Fumagillin, as a methionine aminopeptidase 2 inhibitor, works by interfering with protein synthesis and disrupting proteostasis. In addition to a pronounced sensitivity to Fumagillin, *Nosema* spp. exhibit an increased vulnerability to heat shock, a canonical trigger of proteotoxic stress. Thus, we hypothesized that a more complete understanding of proteostasis in this species, focusing on the Heat Shock Response (HSR), could help identify novel treatment strategies for microsporidia infection in honey bees. We confirmed that N. ceranae demonstrates increased sensitivity to heat stress relative to its host, the honey bee. In characterizing the HSR in *N. ceranae*, we found that this and other microsporidian species have lost the canonical heat shock transcriptional regulator HSF and possess a reduced core HSR pathway. Ongoing work aims to understand the apparently novel mechanism of HSR regulation in this species. We also found that N. ceranae cells display a striking sensitivity to pharmacological disruption of cytosolic proteostasis, suggesting the possibility of exploring such stress-inducing compounds as novel anti-*Nosema* chemical agents. Such discoveries, if applicable to microsporidian infections more broadly, may have widespread impact on microsporidia infection in food production and human health.

Marshall Room

Nematodes Division #1

Moderator: Gwen Stevens

Formulation of three species of entomopathogenic nematodes in alginate beads and diatomaceous earth **STU 1:30 •28•**

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Costly and scarce formulation and application techniques are hindering the use of entomopathogenic nematodes (EPNs) as biological control agents in integrated pest management programs. For plausible solutions, three local South African species of entomopathogenic nematodes including; *Steinernema yirgalemense*, *S. jeffreyense* and *Heterorhabditis bacteriophora* using different formulation techniques were studied. Encapsulation of the infective juveniles (IJs) in alginate beads, in addition to the use of diatomaceous earth (DE) with reduced water activity (a_w -value at 0.97) to induce quiescence; and to reduce the metabolism of the IJs were attempted. Survival of the IJs in the formulations was determined at 6^0C, 14^0C and 25^0C for 4 weeks. It was observed that 10-20% of the IJs could escape from the beads, depending on temperature, and hence, readily survived the encapsulation process. Accordingly, desiccation effect of DE, on the survival of EPNs on the was pronounced at 6^0C. Hence, in both formulations, survival significantly differed at 6^0C, as compared to at 14^0C and 25^0C, with a drastic decrease over time for all species. The species exhibited poor survival at low temperature in either formulations. It should be noted that EPNs require low metabolism during storage and transportation. Thus, future research should investigate survival of these species between 8^0C and 10^0C for better results. The beads successfully retained most of the IJs, and, thus, can be stored for a longer time. Of the two methods studied, formulating EPNs in DE should be further investigated, due to ease of optimisation.

Influence of *Xenorhabdus* symbionts on ascaroside production and development of first-generation adults of their *Steinernema* hosts **STU 1:45 •29•**

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Entomopathogenic *Steinernema* nematodes have a mutualistic relationship with *Xenorhabdus* bacteria. The two partners form an insecticidal alliance that is successful in killing a wide range of insects. A few studies have shown that *Steinernema* IJs have an enhanced virulence and reproductive fitness when they associate with their cognate symbionts. However, there are unanswered questions regarding the physiological interactions that govern and perpetuate the interactions between different nematode developmental stages and their bacterial partners. Hence, in this study, we evaluated gonad development and maturation time of 1^{st}-generation *S. carpocapsae* adults when reared under four bacterial scenarios: a) cognate-symbiotic bacteria, b) closely related non-cognate-symbiotic bacteria, c) distantly related non-cognate-symbiotic bacteria and d) non-symbiotic bacteria. Furthermore, we also measured production of signaling pheromones (ascarosides) that are known to play a key role in mating and reproduction. For this purpose, we considered *in vitro* rearing methods (with/without *X. nematophila* symbiont) to quali- and quantitatively characterize signaling pheromone molecules produced by first generation adults. Our data showed that gonad development and adult maturation time are tightly dependent on the presence of the nematodes' cognate symbiont. Ascaroside analysis also revealed quantitative diversity in the ascarosides produced by *S. carpocapsae* adults in relation to the different bacteria culture conditions.

Activated entomopathogenic nematode infective juveniles release lethal venom proteins **2:00** **•30•**

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Entomopathogenic nematodes (EPNs) are unique parasites due to their symbiosis with entomopathogenic bacteria and their ability to kill insect hosts quickly after infection. It is widely believed that EPNs rely on their bacterial partners for killing hosts. Here we disproved this theory by demonstrating that the *in vitro* activated infective juveniles (IJs) of *Steinernema carpocapsae* (a well-studied EPN species) release venom proteins that are lethal to several insects including *Drosophila melanogaster*. We confirmed that the *in vitro* activation is a good approximation of the *in vivo* process by comparing the transcriptomes of individual *in vitro* and *in vivo* activated IJs. We further analyzed the transcriptomes of non-activated and activated IJs and revealed a dramatic shift in gene expression during IJ activation. We also analyzed the venom proteome using mass spectrometry. Among the 472 venom proteins, proteases and protease inhibitors are especially abundant; and toxin-related proteins such as Shk domain-containing proteins and fatty acid- and retinol-binding proteins are also detected, which are potential candidates for suppressing the host immune system. Many of the venom proteins have conserved orthologs in vertebrate-parasitic nematodes and are differentially expressed during IJ activation, suggesting conserved functions in nematode parasitism. In summary, our findings strongly support a new model that *S. carpocapsae* and likely other *Steinernema* EPNs have a more active role in contributing to the pathogenicity of the nematode-bacterium complex than simply relying on their symbiotic bacteria. Furthermore, we propose that EPNs are a good model system for investigating vertebrate- and human-parasitic nematodes, especially regarding the function of excretory/secretory products.

Cross-talk between nitric oxide and eicosanoid: a specific inhibition of *Xenorhabdus hominickii* metabolite **2:15** **•31•**

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Upon nonself recognition, biochemical mediators act in signaling insect immune functions. These include biogenic amines, insect cytokines, eicosanoids and nitric oxide (NO). Treating insects or isolated hemocyte populations with different mediators often leads to similar results. Separate treatments with an insect cytokine, two biogenic amines and an eicosanoid lead to a single result, hemocyte spreading, understood in terms of intracellular cross-talk among these signaling systems. This study focuses on cross-talk between NO and eicosanoid signaling in our model insect, *Spodoptera exigua*. Bacterial injection increased NO concentrations in larval hemocytes and fat body and RNA interference (RNAi) of SeNOS suppressed NO concentrations. RNAi-treatment also led to a significant reduction in hemocyte nodulation following bacterial injection. Similar RNAi treatments led to significantly reduced PLA_2 activities in hemocytes and fat body compared to control larvae. Injection of L-NAME also prevented the induction of PLA_2 activity following bacterial challenge. An injected NO donor, S-nitroso-N-acetyl-DL-penicillamine, increased PLA_2 activity in a dose-dependent manner. An entomopathogenic bacterium, *Xenorhabdus hominickii*, inhibited PLA_2 activity, but did not suppress NO production. We infer that NO and eicosanoid signaling operate via cross-talk mechanisms in which the elevated NO concentrations activate PLA_2 and eicosanoid biosynthesis, which finally mediates various immune responses.

Root-feeding insects adapt their behavior to bypass plant inducible defenses and attraction of entomopathogenic nematodes **2:30** **•32•**

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Living belowground is tough; soil-dwelling insect pest constantly face rough dense soil material, wearing their cuticles out, rendering any movement a challenge, and putting them in constant contact with microorganisms which cannot be always considered allies. If herbivores are lucky enough to find roots to sustain them, they then have to face an arsenal of plant defenses in order to turn chewy roots into biomass. Plant defenses can be characterized into two broad categories: constitutive and induced. Constitutive defenses are mechanical barriers or metabolites always present on or in the plant tissues whereas induced defenses are triggered only in presence of the threatening herbivore. Despite plants investing in root defenses, it seems common for root herbivores to thrive and eventually overcome their host plants. Is it possible that root herbivorous insects circumvent inducible root defenses? Here we show for the first time that root herbivores may clip part of the roots, disconnecting them from the main root system before feeding on the detached root material. We hypothesize that this behavior prevents the build-up of inducible defenses (emission of volatiles to attract entomopathogenic nematodes) which relies on primary metabolites and energy from the main root system. In addition, clipped roots could be left vulnerable to detritivores microbiota which could assist in the detoxification of constitutive defenses.

Susceptibility of invasive gastropods and non-targets to different *Phasmarhabditis* species in the US **2:45** **•33•**

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Three *Phasmarhabditis* spp including *P. hermaphrodita* were recently discovered in the US, and tests were conducted to evaluate their lethal effects on invasive slugs, snails and non-targets. Invasive gastropods showed different levels of susceptibility to the three *Phasmarhabditis* species. *P. hermaphrodita* caused significant to highly significant mortality on *Lehmannia valentiana* (Valencia slug), *Deroceras reticulatum* (grey field slug), *Cornu aspersum* (European brown garden snail) and *Lissachatina fulica* (giant African land snail) neonates at rates equivalent to or higher than Nemaslug®'s recommendation of 30 IJs/sq cm (1x). It also infects *Limacus flavus* (Tawny garden slug) but does not cause mortality and is safe to three earthworm species *Amynthas gracilis* (Alabama jumper), *Eisenia fetida* (Red wigglers), and *Eisenia hortensis* (European night crawler). *L. valentiana* is more susceptible to *P. papillosa* than *P. hermaphrodita* and *P. californica*. However, a month after exposure, all three killed more than 50% of the pest slugs with *P. papillosa* and *P. californica* showing more virulence than *P. hermaphrodita*. Exposure to the three *Phasmarhabditis* species at 1x or 5x recommended rate is safe to non- target species *Rumina decollata. L. valentiana, L. flavus,* and *L. fulica* are new host records for *Phasmarhabditis*.

Improved RNAi for *Brugia malayi* parasitic nematodes in *Aedes aegypti* **STU** **3:00** **•34•**

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The parasitic nematode, *Brugia malayi*, exacts a significant burden on human health but remains difficult to study using RNA interference (RNAi) due to limited viability of larval stages in culture and low RNAi sensitivity. In *Caenorhabditis elegans*, suppression of anti-RNAi eri-1 and rrf-3 genes dramatically increases RNAi knockdown success; however the function of *B.*

malayi homologs is not known. In RNAi hypersensitive backgrounds study of *C. elegans* essential genes have been highlighted as potential drug targets for *B. malayi*. To validate 'essential' gene knockdown impact in *B. malayi* larvae we must first improve RNAi efficacy during larval development. To address this we utilize a previously pioneered 'in squito' approach that exposes *B. malayi* infected *Aedes aegypti* to RNAi triggers via direct haemolymph injection. Using fluorescence microscopy and tailored Northern blotting techniques we track the bio-distribution and persistence of RNAi triggers through larval life-stages of *B. malayi*. Additionally, we show life-stage expression profiles of *Bma-eri-1, Bma-rrf-3,* and select essential *B. malayi*. Finally, through co-suppression of Bma-eri-1 or Bma-rrf-3 and essential genes we assess the possibility for RNAi to induce desirable phenotypes that can be utilized in controlling filarial worms as well as optimizing RNAi practices

An enterotoxin-like binary protein from *Pseudomonas protegens* with potent nematicidal activity **3:15** **•35•**

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Soil microbes are a major food source for free-living soil nematodes. It is known that certain soil bacteria have evolved systems to combat predation. We identified the nematode-antagonistic *Pseudomonas protegens* strain 15G2 from screening of microbes. Through protein purification we identified a binary protein, designated as Pp-ANP, that is responsible for the nematicidal activity. This binary protein inhibits *Caenorhabditis elegans* growth and development by arresting larvae at L1 stage and killing older staged worms. The two subunits, Pp-ANP1a and Pp-ANP2a are active when reconstituted from separate expression in *Escherichia coli*. The binary toxin also shows strong activity on three other free-living nematodes, *Pristionchus pacificus*, *Panagrellus redivivus*, and *Acrobeloides* sp., but no activity on insects and fungi under test conditions, indicating specificity for nematodes. Pp-ANP1a has no significant homology to any known proteins, while Pp-ANP2a shows 30% homology to *E. coli* heat-labile enterotoxin subunit A (LT-A) and cholera enterotoxin subunit A (CT-A). Despite sequence similarity, Pp-ANP2a shows several characteristics distinct from enterotoxins. Namely, the A-like subunit has no C-terminal ER-retention signal or cleavage site and the two conserved cysteines are in different positions. Our results indicate that Pp-ANP is a potent toxin, specific to nematodes, that appears to be analogous to yet distinct from previously known AB5 enterotoxins. The potency and specificity of Pp-ANP suggests applications in controlling parasitic nematodes and opens an avenue for further research on its mechanism of action and role in bacterium-nematode interaction.

3:30–4:00 pm Refreshments Break

MONDAY, 4:00 – 6:00 pm

Theater

VIRUS DIVISION SYMPOSIUM

Insect virology: Historical achievements and recent advances

Organizer and Moderator: Elisabeth Herniou

The development and impact (or impacts) of the baculovirus expression system **4:00** **•36•**

Robert D Possee[1,2]

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Baculovirus expression vectors have developed as an important tool for the production of recombinant proteins over the past 30+ years. From uncertain beginnings where non-specialists struggled with an arcane polyhedrin-negative virus selection method, they have progressed to a point where very little knowledge of virology is required to use them successfully. In parallel with the improvements in recombinant virus selection systems insect cell culture methods have also advanced. Cloned cell lines are available that can be propagated to high density in serum-free media to provide high concentrations of virus-infected cells for recombinant protein extraction. The number of examples of different recombinant proteins made using the baculovirus expression system continues to grow. The vectors are extremely flexible and can be used to make both simple and complicated recombinant products alike. This is demonstrated admirably by their use to produce multi-component virus-like particles or enzyme complexes. Frequently, baculoviruses will save the day when all other vector systems have failed. Therefore, much is owed to those pioneers of insect virology who performed the early biological and molecular studies on baculoviruses that resulted in the expression system we have today. However, perhaps is now the time to take a pragmatic look at the impact that baculovirus vectors have made to society in general. Have they had a positive benefit to the health and well being of the wider population? As scientists, we may rejoice in the way they have bolstered our publication lists and *curriculum vitae*, but beyond our narrow discipline do baculovirus vectors really matter? The introduction of clinical products in healthcare suggests that we are on the right track but they are few in number. We must ask what the future for baculovirus expression vectors is in the next 30 years. What would a similar talk at SIP2047 report?

Taking the next step: from bugs to cells **4:24** **•37•**

Taro Ohkawa[1]*, Loy E. Volkman*[2] *, Matthew D. Welch*[1]

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The study of molecular insect virology has largely focused on the virus particle. Structural studies on virus morphology moving into molecular studies of viral genes and gene expression. Effects on the host have been studied in the pathology of disease in insects, virus spread and host killing. But only fairly recently have insect host-pathogen interactions focused more finely on the level of virus-cell interactions: the crossroads of virology and cell biology. In the late 1980s a link between baculovirus infection and the host cytoskeleton was discovered. And further examination revealed the great extent to which a virus can appropriate and manipulate the cytoskeleton of the cell. These findings were significant in that we can gain important knowledge about both how a virus can hijack host systems for their own ends, as well as how cells regulate their own cytoskeletal operations. This wedding of cell biology and insect virology has led to the discovery of many unexpected phenomena that occur during virus infection, at the cellular level. My studies have focused on the baculovirus AcMNPV, which has been shown to manipulate host actin at numerous steps during the viral life cycle. AcMNPV uses actin-based motility, polymerizing actin

'comet' tails for propulsion through the cell cytoplasm following entry into the cells. Furthermore, AcMNPV has been demonstrated to mediate the translocation of actin into the nucleus, where it accumulates and polymerizes during the late stage of infection. After virus nucleocapsids are assembled, they initiate intranuclear actin-based motility, which is critical for egress from the nucleus, involving the formation of protrusions of the nuclear envelope. Nucleocapsids escape from the nucleus following a disruption of nuclear envelope integrity, and utilize cytoplasmic actin-based motility to transit to the plasma membrane. Studying the mechanisms of this viral manipulation of cellular actin will shed light both on viral appropriation of cellular processes, as well as how these processes may be regulated in non-infected cells.

Bee viruses and honey bee health **4:48** **•38•**

*Laura Brutscher[1,2,3], Alex McMenamin[1,2,3], Katie Daughenbaugh[1,3], **Michelle Flenniken**[1,3]*

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Bees are important pollinators of numerous crops and plant species that enhance the biodiversity of both agricultural and non-agricultural landscapes. Unfortunately, bee populations are declining in many regions of the world. Many factors negatively impact bee health including reduced habitat, lack of quality forage, climate change, and pesticide-exposure, and pathogens. Bees are infected by a variety of pathogens (i.e., viruses, bacteria, microsporidia, trypanosomatids, and the Varroa destructor) and honey bee colony losses in North American and Europe are associated with elevated pathogen incidence and abundance. The largest group of bee pathogens is made up of positive sense, single-stranded RNA viruses, thus understanding virus pathogenicity, inter-species transmission, and bee antiviral defense mechanisms are important areas of investigation that may result in the development of strategies that limit bee losses associated with virus infections. Michelle Flenniken will summarize the current understanding of the impact of viruses on bee health, interspecies transmission, and bee antiviral defense mechanisms with a particular focus on recent results in the field.

Antiviral immunity in insects: the impact of RNA interference and *Wolbachia*. **5:12** **•39•**

Karyn N. Johnson

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The interaction between a virus and its insect host can have important consequences for disease transmission. Understanding these interactions may facilitate development of approaches to control insect vectored viral diseases of both plants and animals. *Drosophila* has emerged as an important model to understand the host-virus interaction. In insects, the outcome of virus infection is greatly impacted by the insect innate immune response, which includes both inducible responses and RNAi. Arguably the most robust immune response in insects is the RNAi response. The presence of double-stranded RNA generated during virus replication stimulates the siRNA pathway leading to the degradation of virus RNA. In response, many insect viruses encode proteins that suppress the RNAi machinery, indicating co-evolution of the host and virus. In addition to the host antiviral mechanisms, tripartite interactions between viruses, bacteria and hosts can have significant implications for the outcome of virus infection. The endosymbiotic bacterium *Wolbachia,* which is present in an estimated 40% of all insect species, has the ability to alter viral dynamics in both *Drosophila* and mosquitoes, a feature that in mosquitoes may be utilised to limit the spread of important arboviruses.

What dawns at the horizon for invertebrate pathology? **5:36** **•40•**

***Monique M. van Oers**, Vera I.D. Ros*

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In recent years, we see a shift in invertebrate virology from functional viral genomics to an integrative analysis of virus-host interactions. Current applications are extending from biotechnology and biocontrol of pest insects to prevention of viral scourges in pollinators, aquatic invertebrate farming, and insect rearing for food and feed; all areas in which virus outbreaks can be devastating. Additional virological risks arise from invading invertebrates through global trade and travel, and through climate change. Next generation sequencing approaches are revealing a wealth of new viral sequences, underscoring the large diversity of invertebrate viruses and opening up untapped potential of viruses awaiting to be discovered. The challenge is to understand the biological meaning of all the 'omics' data being generated, starting by asking the relevant biological questions. Considering the large number of apparently orphan viruses, for which accompanying diseases are not known, virus latency may be more a rule than an exception, forcing us to adjust our perspective of viruses. Instead of being merely pathogenic entities they will turn out to be important ecological players. Only by combining disciplines, such as genetics, cell biology, synthetic biology, physiology and ecology, we will be able to fully comprehend how viral infections affect invertebrate hosts as well as other biological entities that rely on these hosts, e.g., co-infecting pathogens or mutualist organisms, or other organisms that are part of various multitrophic interactions. Some of the complex processes still to be understood, such as virus latency and persistency, might be exploited to control pathogenic viruses in the future.

The Forum

Bacteria Division #1

Moderators: Baltasar Escriche, Juan Luis Jurat-Fuentes

Understanding the basis of Cry2A toxin specificity towards *Aedes aegypti* **STU** **4:00** **•41•**

***Lazarus Joseph,** Elmi Dahiru, Thomas Courty, Neil Crickmore.*

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High specificity is one of the important attributes of *Bacillus thuringiensis* insecticidal Cry proteins. Therefore, understanding the basis of specificity through determining regions of specificity and molecular interactions of the toxins, is essential not only for the development of novel insecticides but also for the assessment of their risk to non-target organisms. This study involved determining the activity of various Cry2A toxins, and in vitro created hybrids, towards the mosquito *A. aegypti*, combined with bioinformatic analyses to define regions that determine or influence activity towards this pest. Previous studies have identified a D-block within domain II that is believed to influence activity towards *A. aegypti*. We have identified an alternative region within domain I and found that substituting just four amino acids within this region can convert Cry2Ab, which is inactive against

Importance of Domain III in Cry1Ac for toxicity against the soybean looper (*Chrysodeixis includens*) **4:15** **•42•**

*Rubina Mushtaq[1], Abdul Rauf Shakoori[1], **Juan Luis Jurat-Fuentes**[2]*

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The Cry1Ac insecticidal protein from the bacterium *Bacillus thuringiensis* (Bt) is produced by transgenic soybeans to control larvae of the soybean looper (*Chrysodeixis includens*). In this three-domain protein, domain III is critical for binding to protein receptors in the insect midgut. In this study, we performed substitution of domain III of Cry1Ac with the same domain in Cry2Ac, a toxin also active against *C. includens* larvae. Bioassays comparing parental and hybrid proteins showed lack of activity against *C. includens*. Binding assays with radiolabeled toxins demonstrated lack of hybrid toxin binding to brush border membrane vesicles from *C. includens*. In contrast, significant toxicity and binding were detected for the hybrid toxin against larvae of another soybean pest, the velvetbean caterpillar (*Anticarsia gemmatalis*). Taken together, these results support that domain III is critical for Cry1Ac receptor binding in *C. includens* and suggest different critical regions for toxicity against *A. gemmatalis*.

Study of *Bacillus thuringiensis* Cry1Ab and Cry1Ac protoxins interaction with cadherin-like receptor and GPI-anchored proteins extract from *Manduca sexta* **4:30** **•43•**

***Arlen Peña-Cardeña**, Alejandra Bravo, Mario Soberón, Isabel Gómez*

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Bacillus thuringiensis (Bt) produces insecticidal Cry toxins. To induce the activity, the crystals have to be ingested by susceptible larva, solubilized in the gut and activated by midgut proteases to yield an activated toxin. In the case of Cry1A, toxins undergo a sequential binding mechanism with glycosylphosphatidylinositol (GPI) anchored proteins such as alkaline phosphatase (ALP) or aminopeptidase-N (APN), and cadherin-like protein resulting in the formation of a prepore oligomeric structure and its insertion in membrane. The currently accepted paradigm asserts that only activated toxins can bind to larval midgut receptors and exert toxic effects. However, previously we reported that Cry1Ab protoxin or activated toxin bind cadherin-like receptor with similar affinities and two different prepores are produced. Here we test the interaction of Cry1Ab and Cry1Ac protoxins with a extract of GPI-anchored proteins obtained from midgut of *M. sexta.* Our results showed that Cry1Ab protoxin binds to the GPI-anchored proteins extract with the same affinity than activated toxin suggesting that, *in vivo*, both would be able to bind to these receptors. In contrast, Cry1Ac protoxin has no interaction with cadherin-like receptor neither GPI-anchored extract, suggesting that another protein may act as receptor for Cry1Ac protoxin that is important to identify.

Toxicity of Cry1A toxins to CF1 cells does not involve activation of adenylate cyclase/PKA signaling pathway **4:45** **•44•**

*Leivi Portugal, Mario Soberón, **Alejandra Bravo***

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Here we analyzed the mechanism of action of Cry1Ab and Cry1Ac toxins and a collection of mutants from these toxins in the insect cell line CF-1, from the lepidopteran *Choristoneura fumiferana,* that is naturally sensitive to these toxins. We show that different non-toxic mutant toxins affected in receptor binding, oligomerization or pore formation lost also toxicity against CF1 cells. Our results show that both Cry1Ab and Cry1Ac toxins induced permeability of K^+ ions into the cells. The initial response involves the activation of a defense response that involves the phosphorylation of MAPK p38. Analysis of activation of PKA and AC activities indicated that the signal transduction involving PKA, AC and cAMP was not activated during Cry1Ab or Cry1Ac intoxication. In contrast we show that Cry1Ab and Cry1Ac activated apoptosis. These data indicate that Cry1A toxins in CF1 cells can induce an apoptotic death response triggered by pore-formation not related with AC/PKA activation. Our results also show that defense responses triggered by Cry1A toxin activating phosphorilation of MAPK p38 is conserved in different insect species and in CF1 cell line, in contrast to PKA death response that has only been observed in another cell line, *Trichoplusia ni* H5, transfected with cadherin receptor from *Manduca sexta*.

Utility of Cry1Ja for transgenic insect control: Competitive binding and in planta assays in three lepidopteran species **5:00** **•45•**

***John Mathis**, Catherine Finke, Gilda Rauscher, Mark Nelson, Gusui Wu*

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Insect control traits are a key component to improving efficiency and maximizing crop yields for growers. Insect traits based on proteins expressed by the bacteria *Bacillus thuringiensis* (Bt) have proven to be very effective tools in achieving this goal. The adaptability of insects has led to resistance to certain proteins in current commercial products. Therefore, new insecticidal traits that represent a different mode of action (MoA) than those currently in use are needed. Cry1Ja has good insecticidal activity against various lepidopteran species and we find that it provides robust protection against insect feeding with in planta expression. For Bt, different MoAs are determined by their binding sites in the insect midgut. We performed competitive binding assays using brush border membrane vesicles from *Helicoverpa zea, Spodoptera frugiperda* and *Chrysodeixis includens* to evaluate the MoA of Cry1Ja relative to the classes of Bt proteins that are expressed in current commercial products. These data illustrate the potential for Cry1Ja for new insect trait development.

Structures of *Bombyx mori* ABCC transporters responsible for mediating Cry1A toxin intoxication **STU** **5:15** **•46•**

***Haruka Endo**, Shiho Tanaka, Ryoichi Sato*

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The ABC transporter C2 (ABCC2) in lepidopteran insects is a powerful receptor for *Bacillus thuringiensis* Cry1A toxins due to the outstanding activity that directly mediates pore formation. In the present study, we screened regions of the two *Bombyx mori* ABCC transporters BmABCC2 and its paralog BmABCC3 that affect receptor function for Cry1A toxins. Analysis using BmABCC2 variants having a mutation in either of six extracellular loops (ECLs) revealed that the structure comprised of ECL 1 and 4 is the putative interaction site with Cry1A toxins. Next, we generated BmABCC2 variants lacking transport activity based on the hypothesis that the powerful receptor function is derived from transport activity or following conformational change. Results indicated that the transport activity is not essential for receptor function. Finally, we sought regions generating different Cry1A receptor activities between BmABCC2 and BmABCC3. BmABCC3 mutants, whose ECL 1 or 3 are partially substituted with those of BmABCC2 increased and gained receptor activities for Cry1Aa and Cry1Ab, respectively. These results indicate that ABCC2 is a just binding target of Cry1A toxins as well as other Cry1A receptors and that structure of ECLs influences Cry toxin specificity and ABCC-mediated toxicity levels.

Participation of different regions of Cyt1Aa toxin from *Bacillus thuringiensis* in hemolysis, synergism and toxicity to *Aedes aegypti* larvae. **STU** **5:30** **•47•**

***Mary Carmen Torres-Quintero[1]**, Jazmin A. López-Diaz[1], Sarjeet S. Gill[2], Mario Soberón[1], Alejandra Bravo[1].*

[1] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apdo. Postal 510-3, Cuernavaca 62250, Morelos (Mexico); [2] Cell Biology and Neuroscience Department, University of California, Riverside, Riverside, CA, 92521, USA.
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Cyt toxins from *Bacillus thuringiensis* display insecticidal activity principally against dipteran larvae. However, these toxins also show hemolytic activity. Cyt1Aa is pore-forming toxin, which plays an important role in mosquito control since it synergizes the activity of Cry toxins resulting in a potent activity against mosquitoes. In addition, it was shown that Cyt toxin is able to overcome the resistance developed by *Culex quinquefaciatus* to Cry toxins. Cyt toxins do not interact with protein-receptors but bind to specific lipids found in apical membrane of the midgut cells leading to oligomerization, membrane insertion and pore formation. Helices α-A and α-C from Cyt1Aa had been proposed to be involved in initial membrane binding and oligomerization. To determine the role of Cyt1Aa α-helices different residues were mutated and characterized regarging to their effects on oligomerization, hemolysis and synergism with Cry11A and toxicity. Some helix α-C mutations severely affacted oligomerization, hemolytic activity and toxicity to *Aedes aegypti* larvae. However these mutants were still able to synergize Cry11Aa toxicity indicating that toxicity of Cyt1Aa and synergism of Cry11Aa are independent activities. Some mutants in helix α-A are affected hemolysis but not in toxicity to *A. aegypti* larvae indicating that insecticidal and hemolytic activities of Cyt1Aa are also independent events.

A Cry1B protein showing high activity against *Helicoverpa zea* **5:45** **•48•**

Michi Izumi Willcoxon, ***Kishore Kakani****, Ruth Cong, Sabina Lau, Yi Zheng, Jingtong Hou, Zhenglin Hou, Takashi Yamamoto*

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Cry1B is a family of *Bacillus thuringiensis* (Bt) three-domain type insecticidal proteins having diversified Domain III. For example, Cry1Bd has a Cry1Ac/h-type Domain III while Cry1Be has a Cry1Cb-type Domain III. Previously, we reported the discovery of a *cry* gene fragment consisting of sequences encoding a Cry1Ac-type Domain III and the protoxin region in a Bt isolate containing *cry1B*-type genes (Yamamoto, *et al.* Pac. Rim Conference on the Biotechnol. of *B. thuringiensis*, 2005). This indicates that Bt is naturally capable of swapping Domain III by homologous recombination. Since Cry1Ac has high specificity against the *Heliothis/Helicoverpa* complex, it seems Bt is trying to acquire a new specificity to this insect complex by combining the Cry1B Domain II with a Cry1Ac-type Domain III. However, *H. zea* activity of a Cry1B protein, Cry1Bd, which has a Cry1Ac-type Domain III, was not as high as Cry1Ac. Recently, Abad *et al*. (KT952325) reported a new Cry1B protein, Cry1Bj, from a naturally occurring Bt. Cry1Bj has a Cry1Ac-type Domain III similar to Cry1Bd, but *H. zea* activity of Cry1Bj was exceptionally high. Certain chimeric proteins made between Cry1Bd and Cry1Bj showed that the unique Domain I of Cry1Bj is important for high *H. zea* activity of that protein. This finding was confirmed by single amino acid swapping between Cry1Bd and Cry1Bj. The activity of Cry1Bj was further improved by saturation mutagenesis followed by high throughput insect screening.

Engineering of an insecticidal protein specific to corn rootworm **6:00** **•49•**

Ruth Cong, Jingtong Hou, Ericka Bermudez, Hana Ali, Erica Corson, Katie Harding, Zhenglin Hou, Phil Patten, Gusui Wu, Mike Lassner, ***Takashi Yamamoto***

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A perforin-like protein containing a MACPF domain and a DUF946 domain from *Chromobacterium piscinae* was found to be active against western corn rootworm (*Diabrotica virgifera virgifera*) (Sampson *et al*.,2016, J. Invertebr. Path., 142, 34-43). We improved the activity of a native perforin-like protein by saturation mutagenesis. We focused primarily on making certain mutations to hydrophilic amino acid residues. The site-specific, limited mutagenesis identified a number of sites which appeared to be important for the insecticidal activity of the protein. We subjected these sites to full saturation mutagenesis to identify additional mutations for further activity improvements. Single amino acid mutations with substantial activity increase were subsequently combined to further improve activity. Over 300 different combinations were found to have at least 5-fold activity improvement over the original wild type protein. Several of these combinations showed levels of activity increase of more than 30 folds.

Roosevelt Room

Fungi Division #1

Moderators: Louela Castrillo, Jørgen Eilenberg

A new insect pathogenic fungus from Entomophthorales with potential for psyllid control **4:00** **•50•**

Annette Hjorthøj Jensen[1,2], Louisa Görg[1], Jürgen Gross[1], Anant Patel[3], Annette Bruun Jensen[2], ***Jørgen Eilenberg***[2]

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Psyllid pests in fruit orchards can have major economic consequences as they often cause direct and indirect damage on the host trees. Obligate entomopathogenic fungal species from the order Entomophthorales normally have a narrow host range and can cause natural epidemics in insect populations. We investigated the potential of entomophthoralean fungi for biological control of psyllids in fruit orchards. During late summer in 2016, live psyllids were collected from both apple and pear trees in North Zealand, Denmark, in order to discover natural infections by entomopathogenic fungi. The collected psyllids (approximate 4000 individuals) were incubated alive in the laboratory, and observed daily for Entomophthorales infection. Infection was found and documented for the first time in *Cacopsylla* spp. collected from pear trees. The fungus was successfully isolated and cultivated *in vitro* from four sporulating cadavers on Sabouraud dextrose agar-medium (SDA) supplemented with egg yolk and milk using the 'ascending conidia' showering method. Based on morphological characters of the conidia and DNA sequences of the ITS-region, the fungus proved to be a species from genus *Pandora* (Entomophthorales: Entomophthoraceae). The pathogenicity of the fungus was examined in the laboratory of Julius Kühn-Institut (Dossenheim, Germany) on three different psyllid species (*Cacopsylla pyri*, *C. pyricola* and *C. mali*). The results showed that the isolated *Pandora* sp. was able to infect all species of psyllids. Our preliminary results indicate the potential of *Pandora* sp. as a biological control agent of psyllid pests in fruit orchards.

Interaction of entomopathogenic fungus *Beauveria bassiana* and predatory mite *Amblyseius swirskii* on their shared prey, western flower thrips *Frankliniella occidentalis* **STU** **4:15** **•51•**

Marjan Heidarian Dehkordi[1]*, Hossein Allahyari*[1]*, Reza Talaei Hassanlouei*[1]*, Bruce L. Parker*[2]

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For successful biological control programs, knowing the interaction between biocontrol agents, such as competition for the shared prey in an intraguild predation system, is necessary. Towards this end, bioagents including a commercial *Beauveria bassiana* (Bals.) Vuill. (Ascomycota, Cordycipitaceae) (strain GHA) and an experimental isolate, JEF-007 and the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) were evaluated in single and combined applications for controlling western flower thrips (WFT), *Frankliniella occidentalis* (Thysanoptera: Thripidae), on marigold**.** As WFT generally pupate in the soil, we also assessed efficacy of *B. bassiana* in a granular formulation, in single and combined use with the predatory mite against pupating thrips. Based on our studies, control efficacy of thrips was not significantly improved when the predator and fungus were combined. The number of thrips per plant was higher in those treatments that used *A. swirskii* and fungus simultaneously than in treatments that used fungus and predatory mite alone. We observed a self-grooming behavior for mites exposed to the fungus conidia in which the mites spent a considerable amount of time that reduced effective searching and predation. Our results suggest that the simultaneous application of these two control agents (*A. swirskii* and *B. bassiana*) will not result in an additive or synergistic effect on the thrips population, but it seems separating of each other with regard to timing in an IPM program is important: One for preventative control and another as a curative treatment.

Entomopathogenic activity of *Aspergillus flavus* against tomato leaf miner, *Tuta absoluta* (Meyrick; 1917) in Tanzania **STU** **4:30** **•52•**

Never Zekeya*, Musa Chacha, Patrick A. Ndakidemi, Ernest R Mbega*

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Microbial controls of insect pest are of great focus toward developing an integrated management strategy and reduce development resistance among pest populations. Tomato leaf miner, *Tuta absoluta* (Meyrick) has become a major threat for tomato production and market in Tanzania. The pest is reported to cause up to 100% loss of tomato yield in the field. Management of *T. absoluta* by chemical pesticides is economically and environmentally unaffordable. However, management of *T. absoluta* by biocontrols from fungal sources is of economic interest due to the fact that insect cannot

develop resistance against biocontrols. Screening for entomopathogic activity was conducted by using two local isolates of *Aspergillus flavus*. The efficacy of *A. flavus* was evaluated against larvae and adult *T. absoluta*. In larval bioassay, leaves were used as experimental units whereas in adult bioassay plastic vials were treated as experimental unit. In both larval and adult bioassays units were treated with $1x10^6$, $1x10^7$, $1x10^{8,}$ spores/ml of fungal solution. Mortality was recorded at interval of 24 hours. Results showed that *A. flavus* caused up to 70% larval mortality within 3 days whereas in untreated on 23.3% larval mortality was recorded in 16.6 days. *A.flavus* significantly reduced survival period of adult *T. absoluta* in which treated insects survived for 8 days only while untreated (control) survived for 25 days. This study reported the novel native biocontrol against *T. absoluta* in Tanzania. However further studies to isolate and characterize active compound on progress and will tested to evaluate its effectiveness under field conditions and might be potential biocontrol to other areas where *T. absoluta* is intensifying in Sub Saharan African.

Beauveria bassiana, _Isaria fumosorosea_, and _Metarhizium brunneum_ antagonizing _Fusarium oxysporum_ f.sp. _vasinfectum_ in cotton STU 4:45 •53•

Suchitra S. Dara[1]*, Sumanth S. R. Dara*[1]*, Surendra K. Dara*[2]*, Tim Anderson*[3]

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In an effort to explore the potential of entomopathogenic fungi (EPF) in antagonizing plant pathogens, a greenhouse study was conducted using commercial formulations of three EPF against *Fusarium oxysporum* f.sp. *vasinfectum* Race 4 (FOV Race 4) . Pima cotton seeds susceptible to the pathogen were planted in potting medium infested with FOV Race 4 and *Beauveria bassiana* (BotaniGard ES), *Isaria fumosorosea* (Pfr-97), and *Metarhizium brunneum* (Met 52) formulations were applied in three treatment regimens. Three biofungicide formulations based on *Streptomyces lydicus* (Actinovate AG), extract of *Reynoutria sachalinensis* (Regalia), and *Bacillus amyloliquefaciens* (MBI 110) were also used to compare the efficacy of the EPF. The three treatment regimens were: A) soil application rate immediately after planting, B) soil application rate immediately and 1 and 2 weeks after planting, and C) foliar application rate immediately after planting. Plant health and growth were monitored 3, 4, and 5 weeks after planting. EPF offered varying degrees of protection against FOV Race 4 depending on the regimen and time after treatment. *Isaria fumosorosea*-treated plants looked relatively healthier 3 weeks after planting in regimens A and B while *B. bassiana*-treated plants looked healthier 3-5 weeks after planting in regimen C. This study helps expand the understanding of the alternative uses of EPF in agriculture.

Epizootiological studies of _Beauveria bassiana_ infection in coffee berry borers in Hawaii 5:00 •54•

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As part of a project aimed at area wide control of the coffee berry borer (CBB) in Hawaii, we conducted a survey of indigenous *Beauveria* associated with the beetle and assessed their prevalence in fields where a commercial strain of *Beauveria bassiana*, GHA, has been applied. Surveys of infected CBB collected from study farms in South Kona and at survey sites in various districts in Hawaii and in Oahu from 2014 to 2017 resulted in > 1800 isolates of the fungus. Isolates were characterized initially using colony morphology to differentiate GHA from indigenous conspecifics. This facilitated rapid assessment of the prevalence of strain GHA in sprayed commercial farms. Molecular characterization of indigenous isolates using eight microsatellite markers revealed at least 10 haplotypes. Sequencing of the intergenic Bloc identified all strains as *B. bassiana* s. s., with six Bloc haplotypes present, one of which has been isolated from other insects collected in national parks and prior to CBB detection in the island. Prevalence data showed that observed mycoses in coffee farms at the beginning of the new crop cycle are predominantly due to the indigenous strains, with GHA being reestablished following resumption of spray applications. In most farms the prevalence of fungal infection due to native *B. bassiana* strains rarely exceeded 5%. But one high-elevation farm, with no history of GHA applications and surrounded by old-growth ohia forest, had 44% infection in CBB, suggesting that under highly favorable environmental conditions and with compatible cultural practices indigenous strains could play a significant role in controlling CBB populations.

Pathogenicity of _Beauveria bassiana_ native strain in tobacco budworm _Heliothis virescens_ 5:15 •55•

Cipriano García-Gutiérrez*, Cosme Bojórquez-Ramos, Cesar Marcial Bonilla.*

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Pathogenicity of a native strain of *Beauveria bassiana* Vuill. (Ascomycota: Hypocreales) was evaluated on third-and fifth-instar larvae of tobacco budworm, (TBW) *Heliothis virescens* (Fabricius). Larvae were immersed in $1x10^8$ conidia ml^{-1}. After 72 hours, the mortality rate for treating larval instar were 71 and 55%, while to nontreated check had 8 and 11%. The fungus was found across the cuticle and grew into the hemocoel 48 to 60 hours after inoculation in third-instar, and 60 to 72 hours in the fifth instar. The microscope and staining study showed that mycelia grew as filamentous hyphae on the surface and penetrated the cuticle and spiracles killed larvae. Inside the larvae, fungus grew as filamentous hyphae, but blastospores were not noticed, showed a specific infection events in TBW larvae, suggested that a native strain has potential as a bioinsecticide against *H.virescens*.

Use of generalist pathogens to control the invasive predator of bees _Vespa velutina_ in Europe. STU 5:30 •56•

Juliette Poidatz[1]*, Rodrigo J. López Plantey*[2]*, Denis Thiéry*[1]

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While they arrive in a new area, invasive species often miss their native parasites. This was the case in the invasive hornet predator of bees, *Vespa velutina*, as he invaded Europe. Finding and destroying *V. velutina* colonies is problematic for several technical reasons (low detection rate, difficult access, stings risks), and furthermore for products toxicity reasons: destruction is dangerous for the applicator and the environment. In our work, we evaluated the potential efficiency of different French isolates of generalist entomopathogenic fungi, *Metarhizium sp.* and *Beauveria sp*. as biocontrol agent against *V. velutina*. We inoculated the hornets using different methods, mimicking natural ways of contamination: by immersion, by contact with a contaminated surface, in the food, and finally by inter-individual transfers, and kept them in controlled conditions. The isolates and inoculation methods efficiency were assessed by measuring two parameters: the Mortality Index (fungi lethality) and the aggressiveness (fungi killing speed). Our results reveal that the most lethal method of inoculation was immersion, and the less lethal was the contaminated food. No lethality difference was observed between the isolates when considering each inoculation method separately, except for the infected food, where the *Metarhizium* isolate EF3.5(2) was found deadlier. There was no difference in the aggressiveness between isolates for all and for each inoculation methods. The tested isolates showed a very high efficiency level in the controlled conditions that we tested here. Their potential as biocontrol agents on *V. velutina* must now be assessed on whole colonies. The possibility of using formulation adjuvants and strains mixes to enhance their efficiency must also be evaluated. This study is the first to test the potentiality of using biocontrol agents to limit the spread of an invasive hornet and to limit its impact.

Field tests on the biological control of leaf-cutter ants, the case of *Acromyrmex lundii* **5:45** **•57•**

Patricia J. Folgarait, *Daniela Goffré, Ariel Marfetán*

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Leaf-cutter ants are considered one of the most important pests of several crops in the Neotropics. Common chemical pesticides utilized to control them present serious problems to the ecosystems and human health, reasons why these compounds are being regulated by several certification organizations. Biological control using fungal pathogens is one of the alternatives which offer promising results in laboratory assays. However, there are few reported cases of utilization of biological control agents in field assays. The aim of this work was to demon-strate that *Acromyrmex lundii* colonies treated with fungal pathogens baits would reduce their survival, in contrast with colonies treated with control baits without fungi. The granulated baits used in this assay contained either *Purpureocillium lilacinum* (treatment Pl), one entomopathogen that directly affects survival of ants or a combination (treatment BT) of another entomopathogen, *Beauveria bassiana*, and a mycopathogen, *Trichoderma lentiforme,* this latter affects the symbiotic fungus cultivated by ants as food (*Leucoagaricus* sp.). Each colony was assigned to one treatment and the activity (as the number of ants in each trail per minute) was measured weekly for 27 weeks and regularly until 51st week from the beginning, and the last measure was taken in the 58th week, this being the longest activity record known so far. At the end of the assay, 63% and 50% of colonies in treatment BT and Pl, respectively, while only 14% of the control, were dead. Furthermore, our results allowed us to determine that 7 consecutive weeks of inactivation was a good criterion of colonies' death. Our results demonstrate the negative effect of these fungal baits on *A. lundii* colonies´ survival and allow us to propose them as biological control agents against this ant species.

Marshall Room

Diseases of Beneficial Invertebrates Division #1

Moderators: Annette Bruun Jensen, Helen Hesketh

Missing sting reflex in *Nosema ceranae* infected honey bees **STU** **4:00** **•58•**

Wei-Fone Huang*, Zhi Ma*

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Nosema ceranae is the most common gut pathogen in adult honey bees. *N. ceranae* is not identical with the original *Nosema* pathogen, *N. apis*, in *Apis mellifera*. Replacement of *N. apis* by *N. ceranae* has led to redefinitions of *Nosema* disease symptoms in honey bees. *N. ceranae* is thought causing some-how different symptoms. Many symptoms for *N. apis* infection were often neglected for *N. ceranae* without investigation. Missing sting reflex of moribund bees is one symptom that is usually ignored for *N. ceranae* although it is the first identified behavior change of *Nosema* disease. Sting behavior is an altruistic suicide for bees and applied for negative feedback learning experiments. We investigated if missing sting reflex is still a symptom for *N. ceranae* infection using electric stimuli settings in sting extension response (SER) learning experiments. *N. ceranae* inoculated bees showed significantly higher threshold of electric stimuli for SER. In addition, the infection intensity was correlated with the threshold. Missing sting reflex, however, did not indicate the moribund status that described in the classical symptoms. Bees with higher threshold for SER did not have signi-ficantly higher mortality after the test. In this study, we identified that *N. ceranae* infection can significantly retard the sting reflex after the infection reaches high intensity, and such infection seems not killing the host soon. We hypothesized that this behavior change can benefit *N. ceranae* since the hosts fully loaded with *N. ceranae* spores tended not to sting and lead to altruistic death.

Analysis of C3larvin - a putative virulence factor of *Paenibacillus larvae*, the causative agent of American Foulbrood **STU** **4:15** **•59•**

Julia Ebeling[1]*, Anne Fünfhaus*[1]*, Daniel Krska*[2]*, Ravikiran Ravulapalli*[2]*, A. Rod Merrill*[2]*, Elke Genersch*[1]

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Paenibacillus larvae is a Gram-positive, spore-forming bacterium causing the devastating honey bee brood disease American Foulbrood (AFB) which is leading to colony losses worldwide. There are four genotypes of *P. larvae* identified by rep-PCR with enterobacterial repetitive intergenic consensus (ERIC) primers which are thereafter referred to as ERIC I- IV. The genotypes differ phenotypically, amongst others in virulence, and seem to possess different strategies to breach the larval gut epithelium and enter the host's haemocoel. For ERIC I and ERIC II, which are most frequently isolated from current AFB cases, several virulence factors have been identified so far and confirmed in exposure bioassays. Recently, a further putative virulence factor, the C3 exoenzyme C3larvin, has been biochemically and structurally characterized. Yet the role of C3larvin as virulence factor still needs to be examined. The aim of this study was to further analyze the *C3larvin* locus in the genome of the different *P. larvae* genotypes *in silico* and to investigate the biological role of C3larvin in exposure bioassays with knockout mutants.

Solitary bee nesting behavior in the presence of pathogen killed larval cadavers **4:30** **•60•**

Ellen Klinger*, Diana Cox-Foster*

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The alfalfa leafcutting bee, *Megachile rotundata*, is a solitary nesting bee currently managed for commercial production of alfalfa seed in the northwest United States and Canada. Larvae of this bee suffer from a disease caused by fungi in the genus *Ascosphaera*. This disease, known as chalkbrood, results in hardened larvae that block the nesting cavity and must be bypassed by healthy nestmates when those nestmates emerge as adults. It has been thought that these emerging healthy adults chew through, and may partially consume part of these larval cadavers. We conducted a three year field study to characterize how larval cadavers affected adult bee nesting behavior. This study showed that the presence of cadavers improved the number successful offspring. Using lab based experiments, we quantified this behavior to a greater degree to determine if adult bees are consuming fungal cadavers and propose what this may mean for chalkbrood dynamics in these bee populations.

Deformed Wing Virus of Honeybees – transmission, diversity and impact on honeybee development **STU** **4:45** **•61•**

Jessica Fannon[1]*, Dave Chandler*[1]*, David Evans*[2]

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The spread of the parasitic mite, *Varroa destructor* (and the RNA viruses that it transmits between honeybees) is closely linked to increases in overwintering losses and declining honeybee health. Deformed wing virus (DWV; *Iflaviridae*) a picorna-like single-stranded, positive-sense, RNA virus, is widespread in honeybees and usually present as a low level, asymptomatic infection. However, the transmission of DWV by *Varroa* to developing pupae causes highly elevated virus levels and characteristic developmental abnormalities. Our work aims to further the understanding of DWV virulence and pathogenicity and reveal how this is linked to *Varroa* parasitism. We are using molecular techniques and an *in vitro* larval rearing system to study how transmission route influences DWV accumulation and localisation in honeybee tissues. We are also investigating the impact of *Varroa* on DWV levels and diversity on both a colony and a landscape level, testing the hypothesis that a single virulent strain has a selective advantage when transmitted by *Varroa*. We have found that the route of transmission determines DWV infection and tissue location in developing honeybees, as

shown by RNA-FISH. The relative expression levels of key developmental genes from the Homeobox and Ecdysone families have been established using qPCR in an attempt to reveal more about the developmental abnormalities in symptomatic bees. In addition to this, a long-term study of viral diversity in the Ardnamurchan Peninsular, Scotland, illustrates how the viral landscape is affected by the introduction of the *Varroa* mite.

Metagenomic analysis reveals novel RNA viruses in honey bee colonies in Brazil **5:00** **•62•**

Fernando L. Melo[1]*, Diouneia L Berlitz*[2]*, Daniel Ardisson-Araujo*[3]*, Lidia Fiuza*[4]*, Bergmann Morais Ribeiro* [1]

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Bees are considered to be the most important group of pollinators. In recent years populations of these insects have declined worldwide and considerable losses have been also reported in the southern and southeastern states of Brazil. Several viruses have been described infecting honey bees, however the studies are still rare and a number of honey bees viruses are still unknown and remain to be discovered. In this study, adult insects and larvae were collected in several production regions in Rio Grande do Sul State, Brazil. The insects were macerated in SM buffer and centrifuged in a low rotation to remove the solid matter followed by an ultracentrifugation with 20% sucrose cushion to viral particles concentration. RNA extraction was performed using ZR Soil/Fecal RNA MicroPrep (Zymo Research). The sample was processed for rRNA removal using Ribo-Zero rRNA removal kit (Bacteria) (Illumina, San Diego, CA, USA) and cDNA library construction using TruSeq RNA library preparation kit (Illumina). The samples were sequenced at Macrogen (Seoul, Republic of Korea) using Illumina HiSeq 2000 paired-end method. The raw reads were quality trimmed and *de novo* assembled using CLC Genomics Workbench version 6.3. The resulting contigs were compared to ViralRefSeq complete genomes available in genbank using BLASTx implemented in Geneious version 9.1.5. This revealed several viruses already reported infecting honey bees (such as Bee Macula-like virus, Varroa destructor virus-1, Aphid lethal paralysis virus and Lake Sinai virus) and also two novel viruses, including a Rhabdoviruses and an Orthomyxovirus. All viral genomes were complete or almost complete and their ORF distribution resembles that of their most close relatives. Their host range and geographical distribution are currently under evaluation.

Creating a baseline and examining spillover with an all-taxa parasite inventory of bumble bees in the United States **5:15** **•63•**

Amber D. Tripodi*, James P. Strange*

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Bumble bees are important crop pollinators, and their commercial production has led to wide-scale movement of mass-produced colonies. There are concerns about the potential for pathogen spillover into wild populations, yet little is known about the current distribution of pathogens and parasites in wild bumble bee communities. To understand the distribution of parasites geographically and across bumble bee species, a total of 3670 bumble bee specimens, representing 31 species, were collected from 18 states across the USA in 2015. To address pathogen spillover, we included 13 site pairs in which a site with a known history of commercial bumble-bee use was paired with a wild site nearby. Both microscopy and molecular diagnostics were implemented to inventory invertebrate parasites, pathogenic microorganisms, and the presence of common honey-bee viruses in each specimen. Of the macroparasites observed, conopid flies were most common, although phorid flies, tracheal mites, and nematodes were also observed. Trypanosomatids were common, with *Crithidia bombi* more common than *C. expoeki.* Of the microsporidia, only *Nosema bombi* was observed. Black Queen Cell virus and Sacbrood virus were the most commonly seen viruses, although little is known about the pathology of these viruses in bumble bees. Parasite communities differed between commercial and wild sites, and among species and geographic regions. Our results provide a baseline of parasite pressures faced by bumble bees as well as insight into pathogen spillover across the US.

Viability of *Dicrocoelium dendriticum* metacercariae in *Formica polyctena* ants after freezing, boiling or ethanol treatments **5:30** **•64•**

Annette. B. Jensen*, Joanne Malagocka, Jørgen Eilenberg, Brian L. Fredensborg*

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Edible insects have gained increased recognition for their taste, nutritional value, and potential for commercial-scale production of foods for human consumption and animal feed. Insect pathogens and parasites are naturally occurring in insect species used for human consumption. The presence of such pathogens and parasites in insects collected in nature is, however, a concern due to the potential hazards they may cause consumers. Red wood ant *Formica polyctena*, is an intermediate host of the trematode *Dicrocoelium dendriticum* and infected ants are often observed in nature. Since *D. dendriticum* via invertebrate hosts can infect mammals, care is needed when using ants collected in nature for human consumption. In this study we developed a viability assay including trypsin treatment of *D. dendriticum* metacercariae from the abdomen of the red wood ant *Formica polyctena*. Then we tested the viability of *D. dendriticum* metacer-cariae in relation to: 1) freezing (- 20 and −80 °C), 2) boiling (100 °C), and 3) ethanol (50 %). The metacercariae in all control ants were alive and displayed movements either in the cyst, or after excystation, whereas all metacercariae from all treatment groups were recorded as dead. Freezing at -20 °C or −80°C for 30 min, boiling for 1 minute or storage in 50 % ethanol for 24 h proved effective in killing the lancet liver fluke *D. dendriticum* metacercariae. We therefore recommend treating ants collected in wild by one of the treatments before consumption.

MONDAY, 8:00 – 10:00 pm

Bacteria Division – Business Meeting
(*Village Tower West, 15th fl., Rm. 15C)*
Diseases of Beneficial Invertebrates – Business Meeting,
(Village West, Building 2, Room 2C)
Microbial Control – Business Meeting
(Village Tower West, 15th fl., Rom. 15B)
Nematodes Division – Workshop
(Village West, Building 2, Rooms 2A/2B)

TUESDAY, 6:30 – 7:30 am

SIP 5K Run/Walk

Assemble on North Point Lane in front of the Spanos Athletic Training Facility.

TUESDAY, 8:00 – 10:00 am

Theater

VIRUS AND DBI CROSS-DIVISIONAL SYMPOSIUM

Honoring Just Vlak

Organizers and Moderators: Martin Erlandson, Kelly Bateman

Introduction - Just Vlak - impact and international collaboration on invertebrate virus research **8:00** **•65•**

Martin Erlandson

DNA Viruses of Aquatic Invertebrates **8:10** **•66•**

Kelly Bateman*, Grant Stentiford*

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The first invertebrate virus discovered from the marine environment was described infecting a crab. Since this discovery there have been numerous virus infections described infecting a range of aquatic invertebrates. Perhaps the most high profile of these is the White Spot Syndrome Virus (WSSV), which despite almost two decades since its discovery, is still considered the most significant known pathogen impacting the sustainability and growth of the global penaeid shrimp farming industry (with annual losses exceeding US $1bn). The virus was originally discovered in 1991 in *Penaeus japonicus* in China and Taipei, spreading rapidly throughout Asia and then the Americas during the mid- to late- 1990s. Due to the rapid spread of the virus and the isolation and identification by numerous laboratories the virus has been referred to by a variety of different names, until it was later agreed that these infections were caused by the same agent and the name White Spot Syndrome Virus (WSSV) was adopted. WSSV was classified at the laboratories in Wageningen University, full genome was determined and data showed that this large virus was unlike any other known viruses. A new virus family and genus were created, with WSSV virus as the sole member of the family *Nimaviridae*. Numerous publications have been written describing the protein structure, infection mechanisms, susceptible hosts and genome composition of this virus. In addition, recent work conducted on wild populations of shore crabs have identified a virus which is similar but not identical to WSSV. Whilst several crab viruses have previously tentatively been classified within the *Nimaviridae* family based upon structural characteristics, these classifications were later removed due to lack of genomic information. The discovery and description (pathology, morphology, genome) of a novel virus infecting crabs and belonging to the *Nimaviridae* not only allows for expansion of this single-taxon family but further, shines a light on the potential origin of WSSV in wild animal reservoirs.

Baculovirus Budded Virions: Entry mechanisms and progress toward understanding virus-cell interactions during entry **8:30** **•67•**

Gary W. Blissard*[1], Jeffrey Hodgson[1], Ya Guo[1,2], Zhaofei Li[2], Nicolas Buchon[3]*

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In typical baculovirus infections of lepidopteran larvae, infection of the midgut by virions of the Occlusion Derived Virus (ODV) phenotype leads to the production of progeny Budded Virions (BV). BV bud from basal membranes of the polarized midgut epithelial cells and subsequently infect tracheolar cells, hemocytes, and many other cell types. BV entry into the host cell is mediated primarily by a single major envelope glycoprotein that facilitates binding to the (unknown) host cell receptor, a process that appears to stimulate endocytosis. The endosome containing the BV is transported within the cell and progressively acidified, which triggers a conformational change in the major envelope protein and activates its membrane fusion activity. The attachment-and-fusion protein found on baculovirus BV is named F in some baculoviruses, and GP64 in other baculoviruses. F and GP64 proteins represent two distinct lineages of envelope fusion proteins, each likely acquired by baculoviruses, from either the host genome or from another virus. How BV binding initiates endocytosis and how endosomes are trafficked within the cell during entry are largely unknown. New approaches and advances in our understanding how baculovirus BV envelope proteins mediate attachment and fusion, and how BV navigate entry utilizing cellular pathways, will be reviewed.

Occlusion derived virus the baculovirus virion that is specialized for infection of insect midguts: Identifying proteins essential for oral infectivity **8:45** **•68•**

David A. Theilmann*[1], Martin A. Erlandson[2], Leslie G. Willis[1], Ajay Maghodia[1], Monique van Oers[3]*

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Baculoviruses are unique in that two virion phenotypes are produced during infection, budded virus (BV) and occlusion derived virus (ODV). In vivo, BV are initially produced in the host midgut cells and cause systemic spread to all susceptible tissues. ODV in contrast are highly specialized for oral infectivity and binding and infection of the host midgut cells. ODV can contain one or many nucleocapsids per virion envelope. The virion envelope is derived from the inner nuclear membrane and becomes associated with the ODV nucleocapsids in the nucleus. The ODV envelope contains a large subset of proteins not found in BV, many of which appear to be required for binding and attachment to midgut cells. These proteins have become known as *per os* infectivity factors (PIFs). All PIF proteins have common properties which include, being a baculovirus core gene, associated with the ODV envelope and deletion results in the virus being unable to orally infect a host. In addition, deletion of a *pif* gene does not impact virion replication, BV production or BV infectivity. To date 10 PIFs have been identified PIF0 through PIF9. Extensive work on the individual PIFs has identified specific functionality but they all appear to form a complex in the ODV envelope that is essential for oral infectivity. The core of the complex, PIF1-4, appears to serve as a scaffold to which the other PIFs associate. It is known that the PIFs are required for recognizing and binding to a midgut receptor which is required for membrane fusion. In contrast, PIF proteins do not appear to have any role in cell culture infectivity highlighting the highly specialized role of these proteins. Of significant interest is the discovery of PIF homolgues in many large DNA insect viruses, including the *Nudiviridae* and *Polydnaviridae*, suggesting that their mechanism of action involves a pathway that is conserved across a broad range of evolutionarily distant host insects.

Recent advances in the biotechnological applications of baculoviruses: vaccines and gene therapy **9:00** **•69•**

Linda A. King

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It has been more than 30 years since baculovirus expression vectors were first introduced. They now take their place as one of the most commonly used gene expression systems, in both insect and mammalian cells, across the world. In recent years, scientists working both in academia and in industry have taken the system forward far beyond the simple expression of proteins, to produce components for vaccines and therapies, for cancer as an example. Whilst today the number of vaccines on the market are small in number, many more are in various stages of R&D and clinical trials both for human and veterinary markets. There is also a growing body of research aimed at using BacMam vectors for gene therapy, for example, in the field of transplantation. BacMam vectors are being evaluated to deliver genes to alleviate the cause of ischaemic reperfusion injury that is the major cause of transplant failure. The baculovirus expression system is also being used to produce human viral vectors for gene therapy, such as adeno-associated virus. These developments and advances rely on basic science to understand

the replication and biology of baculoviruses, such as that undertaken over many years in Wageningen University under the leadership of the scientist that we are here to honor, Just Vlak.

Basic research supporting the development of baculoviruses as biopesticides **9:20** **•70•**

Zhihong Hu

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The first publication of using baculovirus for pest control was published in 1925. In 1930-1940s, baculovirus played an important role on control European spruce sawfly in North America. These events promoted the early studies of biology and pathology of baculoviruses, as well as safety considerations and the potential of using baculoviruses as pest control agents. The first viral pesticide ElcarTm was registered in USA in 1975, followed by worldwide interest. Successful examples include using Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) on soybean in Brazil, Helicoverpa armigra NPV on cotton in China, Cydia pomonella granulovirus (CpGV) on apple in Europe and North America, *etc*. In 1970s-1980s, the development of recombinant DNA technology helped to identify best strains for pest control. Later on, functional analyses discovered baculoviral genes involved in the insecticidal properties, such as genes encode for ecdysteroid UDP-glucosyltransferase (EGT), polyhedron envelope protein, chitinase, cathepsin, enhancin, ODV-E66, and per os infectivity factors. Despite the widespread interest, the use of baculoviruses for pest control has been limited. This is largely due to the shortcomings of baculoviral pesticides, namely narrow host range, slow speed of kill, sensitive to UV lights and high does needed for control high instar larvae. During late 1980s to middle 1990s, baculovirues were engineered to enhance their properties as pesticides. These include expressing insect hormones or enzymes, deletion of EGT gene, and expression of insect specific toxins. Many of such modifications successfully reduced the LT50 of baculoviruses, however, due to the consequences on the loss of yields and the strict regulation on GMO, recombinant baculoviral pesticides remained at the level of laboratory research or field trials. Recently, the studies of resistance of codling moth to CpGV pesticide revealed the complexity of virus-host interaction during application of baculoviral pesticide. Forty years have been passed since the first registration of a baculoviral pesticide, yet the key factors determine the insecticidal properties still need to be identified. In future, the combination of molecular virology, entomology and ecology will disclose the mechanisms of virus infection and insect behavior. The results of such basic research will promote development for better viral pesticides.

dsDNA Viruses of Invertebrates - Taxonomy and Phylogeny **9:40** **•71•**

Johannes A. Jehle

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Since the very first discovery of viruses infective for invertebrates, numerous groups of DNA viruses have been discovered. Some of these viruses appear to be highly diverse but to share also specific genetic, morphological and pathological characteristics, such as circular double-stranded DNA genomes, a set of common gene othologs, rod shaped nucleocapsids, viral replication in the cell nucleus, and nuclear hypertrophy. Taxonomy and classification of these viruses changed during recent decades of research, depending on classification criteria and methodology used. With the development of molecular techniques, concepts of molecular evolution and mathematical methods to deduce phylogenies, and firmly expedited by the Vlak group in Wageningen, it became obvious that a supergroup of supergroup of nuclear arthropod-specific large DNA viruses, comprising baculoviruses, nudiviruses, hytrosaviruses and nimaviruses most likely shared common ancestor.

The Forum

Bacteria Division #2

Moderator: Marianne Carey, Shuyuan Guo

Increasing virulence and producing resistance-busting mutants of *Bacillus thuringiensis* using experimental evolution **8:00** **•72•**

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Insects, and other organisms, can evolve resistance to pathogens under strong selection pressure. In turn, co-evolutionary theory predicts that pathogens should be able to adapt to host resistance and acquire 'resistance busting' mutations that enable them to successfully infect hosts once again. We designed an efficient regime to impose artificial selection for the increased virulence of *Bacillus thuringiensis kurstaki* against a Cry1Ac resistant population of diamondback moth, *Plutella xylostella* (with ≈ 10,000 fold resistance). Selection regimes were based on previous work describing the cooperative nature of virulence in *B. thuringiensis.* Population structure during selection, and bacterial mutation rate, had a strong impact on how bacteria responded to selection. Selection regimes produced mutants that had increased virulence by up to 300-fold; mutants with single order of magnitude increases in virulence were common. Preliminary sequencing results suggest that the best performing mutants have not evolved Cry toxins with new sequences. However, this type of selection regime may be valuable as a means of genetic mining for novel virulence factors or increasing the potency of microbial products.

Resistance to dual-toxin Bt cotton in the pink bollworm, *Pectinophora gossypiella* **8:15** **•73•**

Jeffrey A. Fabrick[1]*, Lolita G. Mathew*[1]*, Jeyakumar Ponnuraj*[2]*, Xianchun Li*[3]*, Yves Carrière*[3]*, Bruce E. Tabashnik*[3]

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Proteins from the bacterium *Bacillus thuringiensis* (Bt) are produced by transgenic crops targeting several major insect pests. Bt crops can suppress insect pest populations, reduce reliance on conventional insecticides, and increase yields and farmers' profits. Unfortunately, such benefits are being reduced by the rapid evolution of pest resistance to Bt toxins. In the U.S., Bt cotton has been a key tool for managing the pink bollworm and this pest has remained susceptible to both single-toxin (Cry1Ac) or dual-toxin (Cry1Ac + Cry2Ab) cotton. In India, the pink bollworm has evolved practical resistance to both single and dual-toxin Bt cotton. Here we report progress in characterizing the molecular genetics and biochemical mechanisms of pink bollworm resistance to Cry1Ac and Cry2Ab from both India and the U.S.

Functional evaluation by gene editing of a *Bacillus thuringiensis* Cry1Ac toxin receptor in *Helicoverpa zea* **8:30** **•74•**

Omaththage P. Perera[1]*, Nathan S. Little*[1]*, Calvin A. Pierce*[1]*, Heba Abdelgaffar*[2]*, Lin Niu*[2]*, Randall G. Luttrell*[1]*, Juan Luis Jurat-Fuentes*[2]

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Naturally occurring mutations in *Bacillus thuringiensis* (Bt) toxin receptors in insects are known to confer tolerance to Bt toxins. ATP Binding cassette transporter C2 (ABCC2) is a multi-domain Bt toxin receptor that confers tolerance to the Cry1Ac Bt toxin. We report the use of gene editing to truncate ABCC2 in the cotton bollworm (*Helicoverpa zea*) and test its receptor functionality. Effects on tolerance to Cry1Ac, brush border vesicle membrane binding with Cry1Ac, and fitness costs of mutations were studied using bollworm lines with edited mutations in different domains of ABCC2.

Cry11B enhances efficacy significantly to *Aedes aegypti* when added to a *Bacillus thuringiensis* strain producing the Cyt1A-BinA chimera **8:45** **•75•**

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Two naturally occurring species of bacilli, *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Lycinibacillus sphaericus* (Ls), are used worldwide for controlling larvae of nuisance and vector mosquitoes. The insecticidal activity of Bti is due to synergistic interactions among its four major proteins (Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa), while the activity of Ls is due to a binary toxin (Bin) consisting of a toxin domain, BinA, and a midgut receptor-binding domain, BinB. Although used commercially for almost three decades, reports of mosquito resistance to Bti have been rare. However, levels of resistance greater than 10,000-fold to Bin have been reported where Ls has been used intensively for mosquito control. Cyt1Aa is a lipophilic protein, and in previous studies we showed it delays the evolution of resistance to the Cry proteins of Bti, and can overcome high levels of resistance Bin. In a previous study, we fused Cyt1Aa to BinA, using the lipophile as a broad-spectrum binding domain and showed that the Cyt1Aa-BinA chimera was remarkably toxic to five major vector species of mosquitoes, *Anopheles gambiae*, *An. stephensi*, *An. quadrimaculatus*, Bin-sensitive and Bin-resistant strains of *Culex quinquifasciatus*, and *Aedes aegypti*, the latter not normally sensitive to Ls. However, toxicity against *Aedes aegypti* was not as high as against other mosquito species. Here we show that introducing another highly mosquitocidal protein, Cry11B from *B. thuringiensis* subsp. *jegathesan*, enhances the chimera's toxicity against *Ae. aegypti* significantly.

Interaction of *Bacillus thuringiensis* toxins and abamectin in Cry1Ac-resistant *Heliothis virescens* **9:00** **•76•**

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A previous study reported that a strain of *Helicoverpa armigera* with a mutation in the ABCC2 gene conferring resistance to the Cry1Ac toxin of *Bacillus thuringiensis* (Bt) was nine-fold less tolerant to abamectin than a control Bt-susceptible strain (Y. Xiao et al. (2016) PLoS Pathogens 12(2):e1005450). This suggests that abamectin might be useful in combatting Bt resistance when mutations in this ABC transporter are involved. To investigate the generality of this phenomenon, we examined the interaction in the YEE strain of *Heliothis virescens* which has the same type of Bt resistance. When expressed in Sf9 cells, the ABCC2 protein from *H. virescens* transported abamectin in inside-out membrane vesicles, confirming the previous results with *H. armigera*. The two components of abamectin were differentially transported. Bioassays utilizing abamectin incorporated into artificial diet revealed less than a four-fold susceptibility difference between YEE and the JEN2 susceptible strain. These bioassays were complicated by the unique mode of action of orally-administered abamectin. Independent of the concentration, larvae would eat just enough diet until they were paralyzed. Mandibles and body musculature stopped moving; however the heart continued beating for several days. Functionally speaking, the relevant parameter is the median effective concentration for paralysis, not the median lethal concentration. There was no significant difference in tolerance of the YEE and JEN2 strains to topical application of abamectin. This suggests that the utility of abamectin as an strategy to selectively kill Bt-resistant insects in the field will depend on how much mortality, or paralysis, occurs by the oral versus the cuticular route.

Modeling peritrophic matrix defense against pathogens infecting *per os* in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) **STU** **9:15** **•77•**

Emre Caner[1]*, Umut Toprak*[1]*, Serife Bayram*[1]*, Dwayne Hegedus*[2]*, Doug Baldwin*[2]*, Cathy Coutu*[2]*, Martin Erlandson*[2]*, David Heckel*[3]

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Many insects possess a porous structure lining the midgut called the peritrophic matrix (PM), which is composed of chitin and proteins. One essential role of PM is its capacity as a barrier against pathogens infecting *per os* such as *Bacillus thuringiensis*. It is critical to detect PM-associated defense proteins for understanding midgut defense and developing more effective *B. thuringiensis* formulations. Colorado potato beetle (CPB) is a major pest of potato and has developed resistance against the endotoxin of several *B. thuringiensis* strains. In this study, midgut specific-cDNA libraries were generated from 4th instar larvae and adults of CPB, and PM defense proteins were identified by liquid chromatography-tandem mass spectrometry and functional annotation. The PMs were found to possess pattern recognition proteins such as thaumatin, peptidoglycan recognition protein, C- type lectin, and beta-galactosidase, beta-mannosidase and REPAT proteins. Transcriptional levels of the corresponding genes were predicted using reads per kilobase per million mapped reads method (RPKM). Presence of defense proteins in the PM and their expression patterns suggest that PM may serve as a biochemical barrier in the resistance mechanisms developed against *B. thuringiensis* toxins. Finally, a model describing the role of beetle PM in defense is presented.

A chitin binding protein involved in adhesion of *Bacillus thuringiensis* to the insect peritrophic matrix **9:30** **•78•**

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As an insect pathogen, an important step of infection for *Bacillus thuringiensis* (Bt) is adhering to the host. The peritrophic matrix (PM) is a natural protective barrier of insect gut, mainly composed of chintin and mucus protein. Most Lepidopteran larval midguts are alkaline. Bt can produce several non-insecticidal extracellular macromolecules such as proteases, chitin binding proteins (CBP), lipases, etc., to facilitate the infection process. Bt HD73 strain can produce two kinds of chitin binding proteins encoded by *cbp3152* and *cbp3189*. A RNA-Seq approach showed that transcription of *cbp3189* is induced under alkaline condition. *The Cbp3189* gene was cloned and the protein expressed in *E.coli* BL21 strain. CBP3189 was purified by chitin affinity chromatography, confirming its chitin binding ability. No chitinase activity was detected from this protein, but it could bind to the isolated cell wall of the HD73 strain and the PM of *Ostrinia furnacalis* (Guenée) and *Galleria mellonella*. *Gfp* translational fusion showed the subcellular location of CBP-3189 protein is on both bacterial surface and the spore. A transcriptional fusion with *lacZ* confirmed that the promoter of *cbp3189* is induced by alkaline pH. Based on the above studies, it is speculated that the expression of CBP-3189 on the bacterial surface might trough the N-terminal chitin-binding domain stick to the PM and help Bt to be maintained in contact with the midgut cells in a susceptible insect. Interestingly, in *Galleria mellonella*, which is not susceptible to the Bt HD73 Cry toxin, the *cbp3189* deletion mutant persisted better in the gut than the wild type which might be explained by a decrease in PM binding and thereby a lower expulsion rate with the feces.

The impact of plant pyramids and planting mosaic on the evolution of resistance to Bt toxins **9:45** **•79•**

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Bt crops have been planted by farmers since 1996. Control failures due to the evolution of insect resistance have been reported in several field populations. Preventing or delaying the evolution of resistance to Bt is critical for the sustainable utilization of this state-of-the-art technology. Although Bt crop pyramids have the advantage for resistance management, deployment of different single-gene plants will be a vital in the small-hold farmers in China, especially it is usually along with the difficulty of using structured refuges. We tested how applications of Bt crop pyramids (selection with bi-mixed toxins, Cry1Ab+Cry1F, Cry1Ab+Cry1Ie) and planting mosaic (mixed selection with alternating between Cry1Ab and Cry1F and/or Cry1Ie) affected the evolution of resistance in Ostrinia furnacalis using multi-generation selection experiments. The LC50 value of each toxin estimated from a 7-day bioassay were deployed as selection pressure. There were significant differences in the rate of evolution of resistance among selection regimes. Selection with bi-mixed toxins resulted in 10-fold and 50-fold of resistance to Cry1Ab toxin after 9- and 12-generation of selection with Cry1Ab+Cry1F toxins and Cry1Ab+Cry1Ie toxins, respectively. In contrast, mixed selection with alternating between Cry1Ab and Cry1F and/or Cry1Ie resulted in 47-fold and 83-fold resistance to Cry1Ab toxin. These data indicated that resistance evolved more quickly with treatments of mixed selection with alternating between two toxins than that selection with bi-toxins. The results help to understand better the impact of plant pyramids and planting mosaic on the evolution of insect resistance to Bt crops.

Impact of parasitization of *Macrocentrus cingulum* and Cry1Ac protein on cellular immunity and humoral immunity within hemolymph of susceptible and resistant Asian corn borer **STU** **10:00** **•80•**

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The potential effects of insect-resistant, genetically engineered crops producing Cry proteins derived from *Bacillus thuringiensis* (Bt) on nontarget organisms, especially on predators and parasitoids that suppress pest populations, must be evaluated before such crops are commercially planted. The most important pest of maize in China and Southeast Asia is the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae). An insect-resistant GE maize line transformed with the *cry1Ac* gene provides good resistance against stem borers was independently developed by China Agricultural University and has entered the pilot production stage. The current research concerns the potential effects of this GE maize line on *Macrocentrus cingulum* (Hymenoptera: Braconidae), a dominant and abundant parasitoid that can suppress corn borers in maize. We studied the impact of *M. cingulum* and Cry1Ac protein on cellular immunity and humoral immunity within hemolymph of susceptible and resistant ACB larvae. According to the results, the hemocytes in hemolymph of susceptible and resistant larvae exhibited a series of pathological changes after 2 days treated by *M. cingulum* and Cry1Ac protein. Total number of hemocytes changed variously when susceptible larvae were fed on Cry1Ac before or after parasitism. Hemocytes of susceptible larvae feeding on CrylAc had significantly declined cysts capacity to Sephadex A-25 microsphere an apoptosis rate was obviously higher than other treatments. Parasitism of *M. cingulum* inhibited the PPO activity and melanism reaction in Hemocytes of both susceptible and resistant larvae, however, lysozyme activity increased after parasitism. The differences in these immune responses may be another intrinsic mechanism for the decline of host quality.

Roosevelt Room

FUNGI DIVISION SYMPOSIUM

Insect defense against fungal pathogens: mechanisms, variations and efficacy

Organizer/Moderator: Louela A. Castrillo

Defining new cuticle barrier steps in the infection process of insect pathogenic fungi **8:00** **•81•**

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Successful mycosis and the process of penetration into and out of the host insect have long been recognized to involve more than just the activities of cuticle-degrading enzymes. Recent evidence suggests that ingress and egress are two distinct steps in the infection process, requiring different genetic factors. The cuticle waxy-layer and its lipid constituents can act as growth substrates that affect conidial virulence and lipid mobilization. For example, *Beauveria bassiana* conidia grown in the presence of oleic acid displayed a ~4-5 fold decrease in mean lethal dose (LD_{50}) as compared to standard media alone. Manipulation of the ability of entomopathogenic fungi to adapt and/or detoxify host cuticular secretions has also been shown to result in an arms race between *Beauveria bassiana* and tenebrionid secretions. In this instance, cuticular quinones were detoxified by a fungal benzoquinone reductase, although the beetle, *Tribolium castaneum* secretes high enough levels of cuticular quinones to inhibit fungal growth for most wild type strains of entomopathogenic fungi. Fungal perilipin and caleosin, proteins involved in lipid assimilation, storage, and turnover, are involved in appressorial turgor pressure and hence cuticle penetration. Caleosin activity is also involved in spore dispersal, and intriguingly, melanization and/or production of secondary metabolites, including oosporein, on the insect cadaver. The production of oosporein has been shown to be a late-stage, post host death event, indicating that the fungus continues to respond to the insect even after death of the host. In this case, oosporein appears to act as an antimicrobial compound on the dead host cuticle in order to minimize growth of competing microbes. These advances have led to an expansion of our model of the pathogenic process employed by entomopathogenic fungi during infection of insect hosts.

Molecular and genomic approaches to microsporidian disease in the European honey bee, *Apis mellifera* **8:30** **•82•**

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The microsporidia *Nosema* (a type of spore forming fungus) has been implicated in the steep global population decline of honey bees that are critical pollinators of our food crops and plants. There are two species of *Nosema* that have been found to infect honey bees, *Nosema apis* and *N. ceranae*. *N. ceranae* was the more common infection of two species. Using genomic, comparative genomics and transcriptomic approaches, we studied the genome-wide genetic differences between two *Nosema* species and the dynamics of host-parasite interactions at the molecular level. The comparative genomic analysis led to the identification of genes that are unique characteristics of the individual *Nosema* species. Transcriptional profiling of host responses to *N. ceranae* infection resulted in the identification of genes and pathways implicated in host-Nosema interactions. Further, we explored the potential of RNAi as a therapy for controlling Nosema disease in honey bees. Specifically, we employed an RNAi strategy to reduce the expression of a honey bee gene, *naked cuticle* (*nkd*) which is a negative regulator of host immune function. Our studies found that *nkd* mRNA levels in adult bees were upregulated by *N. ceranae* infection (and thus the parasite may use this mechanism to suppress host immune function), and ingestion of dsRNA specific to *nkd* efficiently silenced its expression. Furthermore, we found that RNAi-mediated knockdown of *nkd* transcripts in *Nosema*-infected bees

resulted in upregulation of expression of several immune genes encoding candidate effectors such *Abaecin*, *Apidaecin*, and *Defensin-1* for the immune-related Toll pathway, reduction of *Nosema* spore loads, and extension of honey bee lifespan. The results of our studies clearly indicate that silencing the host *nkd* gene can activate honey bee immune responses, suppress the reproduction of *N. ceranae* and improve the overall health of honey bees. Our study represents a novel therapeutic for honey bee Nosema disease treatment and will have positive implications for honey bee disease management practices.

Immune priming in *Tenebrio molitor* against *Metarhizium anisopliae* **9:00** **•83•**

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Immune priming in invertebrates is their ability to mount a more vigorous immune response to a second encounter with a specific pathogen or parasite (a similar process as immune memory in vertebrates). Although at the beginning of its proposal, immune priming was not well accepted by immunologist, now there is evidence of its occurrence in different invertebrate groups such as ctenophores, porifers, nematodes, crustaceans and insects, being this later group the most studied. Now there is evidence that the immune priming protection can be species/strain-specific and can persists for a lifetime or can pass from parents to offspring. Recent research has been focused on its molecular and physiological mechanisms. However, two topics have been less studied in the literature of immune priming: the role of pathogens and its evolutionary costs. Hence, by mainly using *Tenebrio molitor* against the fungus *Metarhizium anisopliae* (MA10) as a model system, I will show that the immune primed group compared to the control group, survived for a long time period, and had more haemocytes but less ARN methylated, so I will discuss the potential link between immune response and RNA methylation. In addition, catalase seems to be implicated in host resistance because challenged insects with a more virulent derivative transformant of this fungus that overexpressed catalase (CAT) impaired immune priming. However, administering a ROS-promoter agent (paraquat) restored the protection in terms of survival of *T. molitor.* I propose that oxidative stress plays a key role in immune priming and discuss the possibility that parasites such as fungi may induce antioxidant production to overcome immune priming defenses. Finally, I will show that although immune priming provides protection to tenebrio larvae, at the adult stage the immune primed group compared to the control group were less preferred to mate, and this may reveal a trade-off between immune priming and reproduction. In general, I will use this host-parasite model system to discuss the potential outcomes and limitations of immune priming.

Honey bee behavioral defences against fungi **9:30** **•84•**

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The nests of densely populated social insect colonies provide a favorable habitat for a wide range of parasites and pathogens that have evolved to overwhelm or suppress their hosts' immune defenses. In turn, insect societies have evolved remarkable abilities to counter these challenges via dynamic defense mechanisms at both the individual level (individual immunity; *e.g.*, cell phagocytosis, immune gene up-regulation) and the colony level (social immunity). Social immunity describes colony level anti-parasitic and anti-pathogenic protection characterized by collective defensive behaviors (*e.g.* hygienic behavior, grooming), spatial organization, and regulation of contact among nest-mates. One of these behavioral defenses in honey bees is the collection and deposition of antimicrobial plant resins in the nest as a form of cement, called propolis. When honey bee colonies nest in tree cavities, they construct a contiguous propolis layer within the rough inner walls surrounding the nest area called a propolis envelope. Previous work has shown that the presence of a propolis envelope within the nest provides benefits to adult bees' constitutive baseline immune gene expression. Additionally, recent research has shown that colonies experimentally provided with a propolis envelope have significantly lower levels of a fungal pathogen, *Ascosphaera apis*, as compared to colonies without the propolis envelope. The presence of a propolis envelope on the inner walls of the nest acts as an antimicrobial layer that enshrouds the colony and benefits individual and social immune defenses of honeybees. The individual and social immunity benefits derived from propolis stem from the diverse composition of resins, each with their own complex mixtures of antimicrobial compounds. This talk will overview the range of honeybee behavioral defenses, but focus on the behavioral defense of resin collection to protect themselves against pathogenic fungi. Investigating the general and specific benefits of resin for promoting honey bees' natural defenses to pathogenic microorganisms may lead to novel and sustainable ways to improve colony health and mitigate honey bee colony loss due to disease

Marshall Room

Microbial Control Division #1

Moderators: Mary Barbercheck, Michael Brownbridge

Using NGS methods to analyze the molecular composition of CpGV isolates and making predictions for field performance **8:00** **•85•**

Johannes A. Jehle, *Gianpiero Gueli Alletti, Annette J. Sauer, Birgit Weihrauch, Eva Fritsch, Karin Undorf-Spahn, Jörg Wennmann*

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Cydia pomonella granulovirus (CpGV) is a highly important tool for an efficient control of codling moth (Cydia pomonella, L., CM) in organic and integrated pome fruit production. In recent years more than 40 orchards with CM populations resistant to CpGV products have been identified in Europe. By genetic analyses and resistance testing we identified three types of resistance (type I-III) which differ strongly in their susceptibility to different CpGV isolates of different genome groups A-E and their inheritance pattern. We used mapping of single nucleotide polymorphisms (SNPs) and next generation sequencing (NGS) of different CpGV isolates to correlate their molecular composition and virulence. Our identification method is based on 651 SNP positions either specific for one genome group, or specific for a combination of at least two genome groups. The vast majority (534 positions or 82%) were specific for a single genome group. The SNP distribution was used to quantify the composition of CpGV isolates containing viruses form different genome groups. These results allow predictions for field efficacy and recommendations for the design of resistance management strategies.

New Chinese isolates of Cydia pomonella granulovirus (CpGV) provide novel genetic diversity in the arms-race of resistance of codling moth **STU** **8:15** **•86•**

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Application of Cydia pomonella granulovirus (CpGV) products is an efficient and safe way to control codling moth (CM) in pome fruit orchard. CM field populations, however, have evolved strategies to defend against CpGV infection, and more than 40 resistant CM populations classified into two types of resistance, sex-linked (type I) and autosomal inheritance (type Ⅱ), have been identified in Europe, again causing serious economic threat to apple and pear growers. Previous research has elucidated that absence of 24 nucleotides in *pe38* is the key factor involved breaking type I resistance by certain CpGV isolates. Seven new CpGV isolates were collected in different regions in China. Their virulence was determined in three different susceptible and resistant CM populations with a discriminating concentration of 5.8×10^4 OBs ml^{-1}. Some of these isolates were breaking both type I and type II resistance. All seven isolates were Illumina sequenced and assembled using Geneious R9. Phylogenetic analyses of these isolates using the whole

genome sequences revealed considerable genetic diversity. It was observed that the isolates CpGV-ZY, -ALE, -KS1, CpGV-KS2 were grouped into CpGV genome group A containing 24 bp insertion in *pe38*. CpGV-JQ, -ZY2 and -WW were grouped into resistance-breaking genome type E without 24 bp insertion. CpGV-WW, however, could not kill CM with type I resistance efficiently as it would have been expected from the currently existing resistance models. These new CpGV isolates significantly extend the known genetic diversity of CpGV and provide new tools to analyze resistance-breaking mechanisms as well as for field control of CM.

Promoting microbial control as an important part of integrated pest management strategies in California **STU 8:30 •87•**

Sumanth S.R. Dara[1]*, Suchitra S. Dara*[1]*, Surendra K. Dara*[2]

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Conventional pest management typically relies on chemical pesticides although entomopathogenic fungi-based formulations are available against a variety of arthropod pests. Multiple studies were conducted to promote the use of entomopathogenic fungi (EPF) in strawberry and vegetable production for both pest management and other purposes. A laboratory study that evaluated reduced rates of chemical pesticides along with *Beauveria bassiana* against the western tarnished plant bug, *Lygus hesperus* led to multiple strawberry field studies where combining or rotating EPF with chemical and other control strategies was an effective integrated pest management strategy. Another study demonstrated the compatibility of *B. bassiana* with several fungicides that are commonly used for disease control in strawberry. A third study showed that *B. bassiana*, *Isaria fumosorosea*, and *Metarhizium brunneum* have a positive impact on the growth and health of potted cabbage plants growing under water stress. These studies addressed some of the concerns of growers and demonstrated the efficacy of EPF, their compatibility with fungicides, and alternative uses in agriculture.

Sweetpotato weevil microbial control **8:45 •88•**

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AgBiome is identifying and developing a microbial solution that can be utilized to control sweetpotato weevils (*Cylas* spp.) in Sub-Saharan Africa. Sweetpotatoes are one of the most important agricultural crops worldwide and in Sub-Saharan Africa, are a key dietary staple that provide crucial nutrients for health. The sweetpotato weevil is the most serious insect pest of sweetpotatoes, reducing yields by 60-100% if untreated. Furthermore, they are difficult to control since all but the adult stage of the life cycle occurs within the tuberous roots. In the United States, control methods target adult weevils, employing frequent applications of chemical insecticides. However, in Sub-Saharan Africa the primary growers of sweetpotatoes are smallholder farmers who use little to no inputs, instead relying on cultural practices such as hand-weeding and hilling. Microbial control is an attractive solution, generally affording low or no exposure risk and potentially offering season-long control through inoculation of host plants. AgBiome is utilizing an innovative microbial capture and screening program which includes isolating, fully sequencing and testing bacterial isolates from the USA and Uganda for broad-spectrum coleopteran activity by screening against larval Colorado potato beetles and Western corn rootworms. Microbes with confirmed activity will then be tested against the US sweetpotato weevil, *C. formicarius* and later against the African species, *C. brunneus* and *C. puncticollis*. Effective control options that can easily be implemented by African sweetpotato farmers are desperately needed and an efficacious microbial control agent has potential to play a key role in protecting sweetpotato crops from the sweetpotato weevil and other important coleopteran pests.

DuPont Crop Biologicals: Harnessing beneficial microbial communities **9:00 •89•**

Denny J. Bruck[1]***,*** *Richard Broglie*[1]*, Matt Ashby*[2]

DuPont Pioneer, Trait Discovery and Optimization, [1]Johnston, IA and [2]Hayward, CA

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Technological advances have enabled a deeper understanding of the complex interactions between crops, their microbial communities and the environment. The ability to deliver microorganisms or consortia of microorganisms as seed-applied products that optimize the root ecosystem systems creates new opportunities and challenges for enhancing crop productivity and sustainability in large acre field crops. One of the initial insect pest targets for the DuPont Crop Biologicals platform is the western corn rootworm, *Diabrotica virgifera virgifera*. Entomopathogenic fungal strains have been isolated, screened and advanced to the field. When applied to seed, these fungi significantly reduce root damage by *D. virgifera virgifera* and provide additive root protection to transgenic traits and chemical seed treatments.

Helping growers to get the best out of biopesticides: The UK AMBER project **9:15 •90•**

Dave Chandler[1]*, Jude Bennison*[2]*, Clare Butler Ellis*[3]*, Roma Gwynn*[4]*, Gill Prince*[1]*, Rob Jacobson*[5]*, Mark Ramsden*[2]*, Erika Wedgwood*[2]

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Globally, the commercial biopesticides industry is growing rapidly, with an increasing number of biopesticide products reaching the marketplace. In order for the biopesticides industry to make this growth sustainable, it is essential that farmers and growers understand how to use biopesticides in the most effective ways. In surveys we have conducted in the UK, we found that growers perceive biopesticides to be unreliable and difficult to use, and this is acting as a block to uptake. AMBER (Application and Management of Biopesticides for Efficacy and Reliability) is a 5 year project instigated and funded by UK growers of protected edible and ornamental crops designed to address this issue. The aim of AMBER is to have UK growers adopting new practices that have been demonstrated to improve the performance of individual biopesticide products within commercial integrated pest and disease management programmes. The project started in 2016 where we observed growers using selected microbial biopesticides on 5 different crop types as part of commercial crop production. This highlighted several opportunities for improving biopesticide performance, in particular by making changes to application practices based on better understanding of the optimum conditions required for good biopesticide performance. This included factors such as water volume, quantity of product, as well as improving awareness of the fundamentals of good application practice (choice of nozzles, tank cleaning etc.). Growers found some of the technical guidance provided by biopesticide companies confusing and difficult to understand. Our work also identified the need for simple models of the effect of biopesticides on pest growth, and better knowledge of effective doses and persistence of activity, to enable growers / agronomists to fine tune the timing and frequency of applications.

Seeing through the fog: Can we use LVM sprayers to improve application efficiency and performance of biopesticides? **9:30 •91•**

Michael Brownbridge, *Bernhardt Steinwender, Taro Saito*

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Low volume and ultra low volume spraying (also known as fogging) is considered a more efficient method for pesticide application in greenhouses compared to classic hydraulic or mechanical techniques. Automated systems require less applicator time to treat a large area while concurrently minimizing risks of worker exposure. In addition, manufacturers of low volume equipment claim that the sprays cover plant surfaces more evenly,

reach areas that are usually missed (especially the underside of leaves), and reduce waste and potential contami-nation by avoiding run off. As living organisms, microbials (e.g., bacteria and fungi) are more sensitive to abiotic factors than chemicals. High temperature and pressure can negatively affect these microbes, yet these factors are integral to the transformation of microbial suspensions into spray droplets ranging from ~10-70 µm in diameter, depending on the spray system used. The utility of two low volume mist (LVM) sprayers for application of two fungal bioinsecticides Met52 EC (*Metarhizium brunneum*) and BotaniGard WP (*Beauveria bassiana*), and the biofungicide, Cease® (*Bacillus subtilis*), was assessed. Successful insect or disease control requires that sufficient viable conidia or spores reach the target pest, either at the time of application or via pick-up from the leaf surface. The degree to which the fogging process (cold and thermal) affects the viability of microbial biopesticides therefore has significant implications for efficacy. Today growers can choose from a range of machines for the application of microbial products. Results will show how different LVM sprayers affect the viability of conidia or spores contained in microbial biopesticides, and the importance of selecting the right equipment for their application.

Development of CO_2-releasing formulations for the control of soil-borne insect pests **9:45** **•92•**

Anant V. Patel[1], *Pascal Humbert*[1], *Stefan Vidal*[2], *Michael Przyklenk*[3], *Elisa Beitzen-Heineke*[3], *Wilhelm Beitzen-Heineke*[3]

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Since effective synthetic chemicals used by conventional farmers in the past to control various soil-borne insect pests of worldwide relevance, e.g. wireworms or the western corn rootworm, have been phased out or are no longer marketed, the control options targeting these pests are strongly limited. Therefore, alternative control strategies based on biological control agents are urgently needed. Root-feeding herbivores generally use CO_2 gradients in soil to locate their host plants. Given the property of baker's yeast (*Saccharomyces cerevisiae*) to convert sugars into CO_2, it finds application in biological pest control, especially in the form of so-called Attract-and-Kill co-formulations. However, one major obstacle is to extend the CO_2 release over several weeks to obtain a long-lasting protection. *S. cerevisiae* metabolizes only mono- or disaccharides which cannot be retained by means being suitable for the encapsulation of biological control agents. A high molecular weight carbon source such as starch is retained by the polymer matrix, but *S. cerevisiae* is lacking the enzymatic equipment for degradation. To circumvent this problem, one promising approach is the co-encapsulation of an amylolytic enzyme (Humbert, 2017). Amyloglucosidase was co-immobilized with high encapsulation efficiency of 86.5±16%. The CO_2 release was shown to be dependent on the concentration of the co-encapsulated enzyme with a CO_2-production of 0.35 mL g^{-1} h^{-1} at 18 °C for a moderate concentration of 0.1 AGU/g. Besides the enzyme concentration, further factors influencing the CO_2 release from beads were revealed, e.g., incubation temperature, starch concentration, pH or bead diameter. In order to clarify the biochemical reactions taking place within the beads, scanning electron microscopy (SEM) and MALDI-ToF mass spectrometry imaging (MSI) were conducted. SEM micrographs proved the degradation of corn starch within the beads and MSI showed that *S. cerevisiae* cells are able to take up nearly all of the released glucose within the beads. The innovative CO_2-releasing beads will pave the way towards novel attract-and-kill strategies in pest control. *REF: Humbert, P., Vemmer, M., Giampà, M., Bednarz, H., Niehaus, K., & Patel, A. V. (2017). Co-encapsulation of amyloglucosidase with starch and Saccharomyces cerevisiae as basis for a long-lasting CO_2 release. World Journal of Microbiology and Biotechnology, 33(4), 71.*

10:00–10:30 am Coffee Break

TUESDAY, 10:30 am – 12:30 pm

Theater

Virus Division #2

Moderator: Peter J. Krell, Lorena Passarelli

Selected promoter expression analysis of Anticarsia gemmatalis multiple nucleopolyhedrovirus during infection of permissive, semipermissive and nonpermissive cell lines **10:30** **•93•**

Fabricio da Silva Morgado[1], *Daniel Mendes Pereira Ardisson-Araújo*[2], ***Bergmann Morais Ribeiro***[1]

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The activation of baculovirus gene promoter during infection of insect cells follows a transcriptionally controlled sequence of gene expression. Depending on the cell type, this sequence of promoter activation may be disrupted by antiviral cellular defense mechanisms and interfere with viral progeny formation. In this work, the activity of different promoters (*ie1*, *gp64*, *lef-1*, *vp39*, *p6.9* and *polh*) of the Anticarsia gemmatalis multiple nucleopolyhedrovirus isolate 2D (AgMNPV) was assessed during infection of permissive, semipermissive and nonpermissive cell lines in real-time using the firefly luciferase reporter gene. We have characterized in rich detail the AgMNPV promoters in permissive cell lines and revealed differential profiles of expression in cells with limited permissivity that correlate well with limitations in viral DNA replication. Semipermissive and nonpermissive cell lines presented delays and restrictions in late and very late promoter expression. Cells undergoing apoptosis did not inhibit late gene expression; however, viral progeny formation was severely affected.

Functional analysis of the N terminus of Autographa californica multiplenucleopolyhedrovirus DNA polymerase **10:45** **•94•**

Guoqing Chen[1], *Yang Fang*[1], *Wu Lijuan*[1], *Peter J. Krell*[2], ***Guozhong Feng***[1]

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Autographa californica multiple nucleopolyhedrovirus (AcMNPV) DNA polymerase (DNApol) plays an important role in viral DNA replication, the N terminus (residues 1 to 186) is present in all baculovirus DNApols. However, its functional role has not yet been characterized. Here we report a functional analysis of the N terminus of AcMNPV DNApol. We truncated the N-terminus of DNApol to varying lengths and reinserted them into a null-*dnapol* AcMNPV bacmid with a green fluorescent protein (GFP) reporter. Although these truncation repair mutants rescued viral DNA replication and infectious virus production, the level of viral DNA replication and production of virus and occlusion bodies were compromised. Further bioinformatic analysis showed that the first 64 amino acids at the N termini contain a conserved α(-helix)-β(-sheet)-β-β secondary structure motif and four other conserved sequence motifs are located in further downstream between aa 67 to 186. Multiple alanine point substitutions in the αβββ secondary structural region or the other four sequence motifs in AcMNPV DNApol impaired viral DNA replication and infectious virus yield and occlusion body production. Taken together, our result demonstrated that N terminus of baculovirus DNApol is important in efficient viral DNA replication and production of both infectious virus and occlusion bodies.

Mutagenesis of the carboxyl region of AcMNPV *lef-2* and its consequences for protein function STU 11:00 •95•

***Carina Bannach**, Clare Allen, Linda A. King, Robert D. Possee*

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The *late expression factor 2 (lef-2)* is an early viral gene involved in AcMNPV DNA synthesis and was originally identified by a transient *in vitro* replication assay. However, later work suggested that LEF-2 was additionally required for viral very late gene expression. Using BrdU mutagenesis, a virus designated very late deficiency 1 (vld1) was isolated, which was unable to express viral *p10* and *polh*. The original mapping and sequencing of vld1 revealed a mutation at AcMNPV genome position 3620 within *lef-2* that resulted in an amino acid change from aspartic acid to asparagine (LEF-2^{D178N}). This was thought to account for the altered phenotype of VLD1 comprising reduced budded virus production and the lack of very late gene expression. Recent resequencing of *lef-2* within VLD1 identified a second mutation at nucleotide position 3548 that resulted in an alanine to threonine change (LEF-2^{A154T}). When we attempted to construct a recombinant AcMNPV with this mutation to examine its function it was impossible to isolate an infectious virus. The alanine^{154} is conserved in most baculovirus LEF-2's so may have a critical role in its function. However, the same mutation within vld1 was permissive for virus replication. This result suggests that a combination of *lef-2* mutations (LEF-$2^{A154T; D178N}$) in VLD1 is acceptable for protein function but that LEF-2^{A154T} is not. Both of these mutations are located in the carboxyl region of LEF-2. We also examined the role of highly conserved cysteine residues in this portion of the protein and found variable requirements for their function in virus replication. Insect cells infected with these virus mutants showed different reductions in intracellular viral DNA levels as examined by qPCR, budded virus production and viral very late gene expression compared to wild type infected cells. The data indicated that different conserved amino acids at the LEF-2 C-terminus play a crucial role during the AcMNPV replication cycle.

A nucleocapsid assembly-essential *cis*-acting element in the Autographa californica nucleopolyhedrovirus *ac83* gene 11:15 •96•

***Zhihong Huang**, Mengjia Pan, Silei Zhu, Hao Zhang, Wenbi Wu, Meijin Yuan, Kai Yang*

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The mechanism of baculovirus nucleocapsid assembly remains unclear. Previous studies have shown that deletion of the *ac83* gene of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) blocks viral nucleocapsid assembly. Interestingly, the *ac83*-encoded protein Ac83 was not detected as a component of the nucleocapsid in some studies, implying a particular role for *ac83* in nucleocapsid assembly that may be independent of its protein product. To examine this possibility, Ac83 synthesis was disrupted by insertion of a chloramphenicol resistance gene into its coding sequence (with only nt 361 to 464 deleted) or by deleting its promoter and translation start codon. Both mutants produced progeny viruses normally, indicating that the Ac83 protein is not essential for nucleocapsid assembly. Subsequently, complementa-tion assays showed that the production of progeny viruses required the presence of *ac83* in the AcMNPV genome instead of its presence in *trans*. Therefore, we reasoned that *ac83* is involved in nucleocapsid assembly via an internal *cis*-acting element, which was named the nucleocapsid assembly-essential element (NAE). The NAE was identified only in alphabaculoviruses and have a conserved positional relationship with another essential *cis*-acting element (the conserved non-protein-coding element) which was recently identified. The identification of the NAE may help to connect the data of viral *cis*-acting elements and related proteins in the baculovirus nucleocapsid assembly, which is important for elucidating DNA-protein interaction events during this process.

The nucleocapsid assembly-essential element of Autographa californica multiple nucleopolyhedrovirus is located in nt 1651 to 1850 of *ac83* STU 11:30 •97•

***Silei Zhu**, Zhihong Huang, Wenbi Wu, Meijin Yuan, Kai Yang*

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In the recent study, we found that the presence of ac83 gene in the Autographa californica multiple nucleopolyhedrovirus (AcMNPV) genome, rather than the synthesis of Ac83 protein, is essential for progeny virion production. A cis-acting element, the nucleocapsid assembly-essential element (NAE), was further identified in the ac83 coding sequence. Our previous study also demonstrated that viral replication was completely blocked by deletion of nt 1651 to 2011 of ac83, while repair of nt 1351 to 1800 of ac83 could partially rescue viral viability, indicating that the NAE is located in this region. In the present study, a series of ac83-truncated viruses were generated, which had truncated fragments of nt 1351 to 2011 of ac83. It turned out that the viability of these ac83-truncated viruses was essentially unaffected. Therefore, we reasoned that the NAE is approximately 200 bp in length, located in the overlapping region (nt 1651-1850 of ac83) of these truncated fragments. Sequence analyses revealed that the NAE is conserved among alphabaculo-viruses and characterized by multiple AT-rich regions. Additionally, a conserved motif (ATATACTAC or similar sequences) was observed in both the NAE and CNE, another cis-acting element. Whether this motif is related to their functions remains to be studied. Electrophoretic mobility shift assays (EMSA) detected protein(s) that bound to the NAE in infected cells rather than uninfected cells. The results of EMSA corroborated that the NAE is a cis-acting element and indicated the NAE interacts with some viral protein(s) or virus-induced cellular protein(s).

A global proteomic analysis of the substrates of a baculovirus sulfhydryl oxidase 11:45 •98•

*Samantha A. Warnecke, Kathlyn L. Gomendoza, Elizabeth M. Martino, **A. Lorena Passarelli***

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Virions have a structural blueprint and an enclosed architecture required to protect the viral genome and transfer infection between cells. Changes in capsid or capsid-associated protein stability and/or conformation occur at specific stages during virus multiplication. Disulfide bonds within virion proteins can provide structural determinants required for virus replication. Baculoviruses are one of only a few types of viruses that encode a conserved sulfhydryl oxidase gene. This viral enzyme is able to oxidize protein thiol groups to disulfide bonds. The formation of disulfide bonds usually occurs in the endoplasmic reticulum, a site with an oxidizing milieu, as proteins are trafficked to the cell membrane. In contrast, oxidation reactions are not favored in the nucleus or cytoplasm of cells, where the environment is reducing. Autographa californica multiple nucleopolyhedrovirus (AcMNPV) encodes a functional sulfhydryl oxidase, Ac92. Deletion of *ac92* affects both virion types that are produced during virus replication, resulting in non-infectious budded virus and singly, rather than multiply, enveloped occlusion-derived virions. Using an in vitro assay, we found proteins in the budded virus of AcMNPV that contain disulfide bonds. To identify the virion proteins containing disulfide bonds, we utilized an unbiased proteomic assay in which proteins containing thiol-reactive groups were alkylated and differentially separated using two-dimensional gel electrophoresis. Mass spectrometry was subsequently used to identify modified proteins. Continuing these studies will provide insight into the mechanisms of virus assembly and virion stability in the budded and occlusion-derived virions.

Autographa californica multiple nucleopolyhedrovirus *ac51* gene encodes a nucleocapsid protein and is required for efficient egress of nucleocapsids to form budded virions **12:00** **•99•**

Jianxiang Qiu, Zhimin Tang, Yi Cai, Wenbi Wu, Meijin Yuan, ***Kai Yang***

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Alphabaculoviruses are lepidopteran-specific nucleopolyhedroviruses and the anterograde transport of their nucleocapsids is rarely known. In the present study, an alphabaculovirus unique gene, Autographa californica multiple nucleopoly-hedrovirus (AcMNPV) orf51 (*ac51*), was identified to be involved in anterograde transport of AcMNPV nucleocapsids. Our study indicated that *ac51* is regulated by both early and late promoters and maintains its transcripts from the early to the late stage of infection. *ac51* product (Ac51) was detectable from 12 hours post-infection (h p.i.) to 72 h p.i. Ac51 was distributed predominantly in the nuclei of infected cells and also locates surrounding the nuclear membrane in the cytoplasm. Moreover, Ac51 is a nucleocapsid protein of budded virions (BVs). Upon *ac51* deletion, infectious BV production by 96 h p.t. was reduced by approximately 1,000-fold compared with that of wild-type AcMNPV. Neither viral DNA synthesis nor viral gene expression were affected. Transmission electron microscopy showed that virogenic stroma formation, distinguishable nucleocapsid assembly and the formation of occlusion-derived virions and occlusion bodies were not impaired by *ac51* deletion. However, in vAc51KO-transfected cells, the number of nucleocapsids in the nucleus was comparable with that of wild-type AcMNPV, while BV production in the supernatants in a virus life cycle was dramatically decreased, indicating a role for *ac51* in the egress of nucleocapsids to form BVs. Taken together, our results support the conclusion that *ac51*, encoding a nucleocapsid proteins, is not involved in the nucleocapsid assembly, but plays an important role in the egress of nucleocapsids to form BVs.

Deciphering the genetic factor for morphology of granulovirus occlusion body **STU** **12:15** **•100•**

***Keiko Tsuruta*[1]*, Jörg T. Wennmann*[2]*, Maki N. Inoue*[1]*, Yasuhisa Kunimi*[1]*, Johannes A. Jehle*[2]*, Madoka Nakai*[1]

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In 1999, we found a granulovirus (GV) that produced an unusual morphology of occlusion bodies (OBs) in *Adoxophyes* sp. (Lepidoptera: Tortricidae) larva in a tea field in Miyazaki Prefecture, Japan. This isolate is a morphological mutant of Adoxophyes orana granulovirus, AdorGV (AdorGV-M) with 99.7% nucleotide sequence identity the English isolate of AdorGV (AdorGV-E). Whereas AdorGV-E OBs have a typical ovo-cylindrical shape with a size of 0.3 x 0.5 µm, and each OB contains one virion, AdorGV-M has cuboidal-shaped OBs with a diameter of 0.8-2.2 µm. In this study, we investigated the potential genetic factor responsible for difference of the OB morphology between the two isolates. Based on sequence comparison between AdorGV-E and AdorGV-M, five candidate genes were initially identified. Among them, *pep-p10* was considered to be the most likely target. Because studying gene functions of AdorGV is hampered by the lack of susceptible cell lines and bacmid technology, we used Cydia pomonella granulovirus (CpGV) and Cp14R cell line as model to generate *pep-p10* recombinants. A *pep-p10* knockout mutant (bacCpGV$^{hsp\text{-}GFP}$Δpep-p10) was constructed from CpGV bacmid (bacCpGV$^{hsp\text{-}GFP}$) and used for cell transfection. The results revealed that both bacCpGV$^{hsp\text{-}GFP}$ and the knockout mutant bacCpGV$^{hsp\text{-}GFP}$Δpep-p10 produced viable budded viruses (BV). The infected Cp14R cells were observed under transmission electron microscopy (TEM), and the results showed that both bacCpGV$^{hsp\text{-}GFP}$ and bacCpGV$^{hsp\text{-}GFP}$Δpep-p10 produced also OBs, however with completely different morphology. BacCpGV$^{hsp\text{-}GFP}$ produced typical normal-sized, ovo-cylindrical OBs, whereas bacCpGV$^{hsp\text{-}GFP}$Δpep-p10 produced OBs with a cuboidal shape and a diameter of 0.6-1.9 µm. We propose that deletion of *pep-p10* resulted in the generation of cuboidal OBs and that this gene is most likely involved in the OB morphology of GVs.

The Forum

NEMATODES DIVISION SYMPOSIUM

Nematode Omics

Organizer and Moderator: Patricia Stock

Looking for nematode biology in tera-bases of sequence data **10:30** **•101•**

***Makedonka Mitreva*[1,2]

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There has been constant increase in the number of nematode genomes published (and in progress) which is a trend that correlates to ever-improving molecular techniques for obtaining biological material, performing DNA extraction and library construction, and predominantly to ever-evolving sequencing chemistry and instrumentation, as well as to decreased sequencing costs. These nematode genomes provide an invaluable resource to enable development of postgenomic tools to investigate the biology of parasitic nematode diseases and accelerate discovery for the development of anthelminthics, vaccines and diagnostics. However, identifying good candidate targets in high throughput data is challenging and requires an integrated multi-omics approach (including nematode genomics, transcriptomics, functional genomics, proteomics, interactomics etc), and innovative bioinformatic analysis. Here I present our progress on knowledge based drug target identification, prioritization and experimental validation exploring metabolic chokepoints as targets. We first reconstruct metabolic pathways for 56 nematode species, using ~700 unique enzymes encoded by their genomes. Comparative genomics identified phylogenetically conserved and restricted pathways that underline differences in life-cycle and mode of parasitism. A total of 185 enzymes are pan-phylum conserved, and 88 of them are conserved chokepoints. The prioritization of the chokepoints for experimental validation included level of conservation in nematodes and host, orthology in drug target databases, RNAi phenotype, expression profile across tissue and developmental stages, function in multiple pathways of interest, gene copy number, etc. The chokepoints were linked to inhibitors in drugbanks and subsequently, the predictions are being validated in multiple nematode species. Preliminary work on a broadly conserved chokepoint enzyme CPT (carnitine palmitoyl transferase) resulted in compiling of a small library of 22 CPT inhibitors that we screened against five parasitic nematode species spanning the phylum Nematoda. A worm motility assay identified 9 hits, including 4 with potential for broad applicability across clades since the are effective against gastrointestinal and filarial species. Parasite-specific sequence variations are now explored to increase selectivity to the parasites over the host ortholog. The prioritized drug targets and drug-like compounds have potential to expedite the discovery of new anthelmintic drugs with broad-spectrum efficacy.

Lateral gene transfer from bacteria to nematodes … and beyond! **11:00** **•102•**

Julie C. Dunning Hotopp

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Lateral gene transfer (LGT) is the transfer of DNA between divergent organisms. In 2007, we demonstrated that bacterial DNA from *Wolbachia* endosymbionts is found in the genomes of numerous arthropods and filarial nematodes. Overall, LGT is increasingly described as occurring from bacteria to animals, but particularly *Wolbachia* and its arthropod and nematode hosts. At least 10.6% of the *Wolbachia* genome has been transferred to its *Brugia malayi* nematode host yielding >0.5 Mbp of *Wolbachia* sequence in the nematode's 89 Mbp genome. Most LGTs are gene fragments or otherwise degenerate sequences with respect to protein coding potential, and many

are present multiple times in the *B. malayi* genome. A handful of LGTs contain full length *Wolbachia* genes with the potential to encode full-length functional proteins. At least four LGTs have evidence of life stage-specific regulation of transcription at levels like other nematode transcripts, raising the possibility that they might be functional. In *Drosophila ananassae*, a remarkable 2% of the nuclear genome is of bacterial origin, including 20% of the known material on a *Drosophila* autosome. In combination, these results suggest that extensive amounts of bacterial DNA may reside in invertebrate nuclear genomes. Based on these findings, we hypothesized that there could even be integration of bacterial DNA in human somatic cells. Using publicly available sequence data from the human genome project, the 1000 Genomes Project, and The Cancer Genome Atlas (TCGA), we have presented evidence that bacterial DNA integrates into the human somatic genome through an RNA intermediate and that such integrations are detected more frequently in (a) tumors than normal samples, (b) RNA than DNA samples, and (c) the mitochondrial genome than the nuclear genome. As such, LGTs on invertebrate genomes may serve as a model for understanding insertional mutagenesis of somatic vertebrate genomes, including the human genome.

Transcriptional evidence of symbiont-modulated metabolism in the entomopathogenic nematode *Steinernema carpocapsae* (Nematoda: Steinernematidae) **11:30** **•103•**

***BF Peterson*[1], *JG McMullen*[2], *MN Yousefelahi*[3], *SP Stock*[1,4]**

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Steinernema carpocapsae nematodes, and their bacterial *symbionts*, *Xenorhabdus nematophila,* are lethal insect pathogens. Outside the insect host, symbiont-colonized infective juveniles (IJs) live in the harsh soil environment for extended periods of time without feeding. Therefore, nutrients accumulated before leaving the insect cadaver are the only food source available to IJs and their bacterial symbionts. We hypothesized that *X. nematophila* colonization causes a reallocation of these nutrients and impacts their utilization in IJs. To address this, we examined *S. carpocapsae* IJs from three rearing conditions: 1) *in insecta* with *X. nematophila*, 2) *in vitro* on lawns of *X. nematophila*, and 3) *in vitro* aposymbiotic on rich medium. Resulting IJs were subjected to standard RNAseq and differential gene expression analysis protocols. Our findings emphasize the influence that *X. nematophila* has on the IJ metabolism; genes related to carbohydrate, lipid, and protein metabolism, were differentially regulated with symbiont colonization. We also measured total lipid, protein, and glycogen content across rearing conditions and assayed catabolism of potentially important disaccharides, like maltose. These results support our hypothesis that *X. nematophila* influences *S. carpocapsae* IJ metabolism, with the unanticipated finding that carbohydrate utilization may be a key factor for IJ longevity and symbiont provisioning.

The mechanisms of developmental plasticity: from switch genes and epigenetics to the interplay of organisms and their environment **12:00** **•104•**

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Developmental plasticity is increasingly recognized as primary mechanism for the emergence of novelty. However, molecular mechanisms underlying this phenomenon remain elusive. The nematode *Pristionchus pacificus* exhibits developmental plasticity for its mouth-form and feeding strategies. Individuals develop one of two alternative mouth-forms, a predatory eurystomatous (Eu, wide-mouthed) or a bacteriovorous stenostomatous (St, narrow-mouthed) form. Using genetic screens, we have identified developmental switch genes that regulated plasticity. *eud-1* mutants are all-St, whereas mutants in the downstream nuclear-hormone-receptor *nhr-40* are all-Eu. More recent work indicates that *eud-1* expression is under epigenetic control involving the histone acetyltransferase *lsy-12*, which acts through an antisense RNA at the *eud-1* locus itself and up-regulates *eud-1* expression. Here, we present our most recent molecular findings on i) the molecular mechanisms underlying plasticity, ii) the interaction of this regulatory network with the environment and iii) first insight into the involvement of small RNAs and Argonaute proteins.

Roosevelt Room

Fungi Division #2

Moderators: Nemat Keyhani, Drauzio E.N. Rangel

Light during mycelial growth produce conidia of *Metarhizium robertsii* with increased stress tolerance, germination speed, and virulence **10:30** **•105•**

Ariel de Souza Oliveira, ***Drauzio Eduardo Naretto Rangel***

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Light conditions during fungal growth are known to cause several physiological adaptations in conidia. In this study, conidia of the entomopathogenic fungi *Metarhizium robertsii* (ARSEF 2575) were produced on: 1) potato dextrose agar (PDA) medium in the dark; 2) PDA medium under white light (4.98 W m^{-2}); 3) PDA medium under blue light (4.8 W m^{-2}); 4) PDA medium under green light (2.2 W m-2); 5) PDA medium under red light (2.8 W m^{-2}); and 6) minimum medium (MM = Czapek medium without sucrose) or MM supplemented with 3% lactose (MML) in the dark. The conidial production for each treatment as well as the speed of conidial germination, stress tolerance, and virulence to the insect *Tenebrio mollitor* (Colleoptera: Tenebrionidae) were studied. For stress tolerance, the conidial suspensions of each treatment were exposed from 0 (control) and 100 to 210 minutes (with increments of 10 minutes) to an irradiance of 0.60 W/m^2. After irradiation, the plates were incubated for 48 hours in the dark at 26 °C. For the osmotic stress, the conidial suspensions of each treatment were dropped on PDA medium supplemented with KCl concentrations of 0 (control) 1.0, 1.3, 1.4, 1.5, 1.9, and 2.1 M, and the germination was counted after 24 h. Conidia produced on MM in the dark were the most tolerant to both UV radiation and osmotic stress. Conidia produced under blue light were the second most tolerant to UV radiation, followed by the UV tolerances of conidia produced under visible light. Conidia produced under green or red lights were the least UV tolerant and less tolerant than conidia produced in the dark. For KCl tolerance, conidia produced under visible or blue light were the second most tolerant, followed by conidia produced under green light. Again, conidia produced under red light were the least tolerant to KCl. The fungus grown under blue light produced more conidia than the fungus grown in the dark. The conidial production of the fungus grown under white or red light was similar to the conidial production in the dark. The MML afforded the least conidial production. Conidia produced on MML or on PDA medium under white or blue light germinated faster than conidia produced on PDA medium in the dark or under red light. Conidia produced on MML or PDA medium under white light were also more virulent than conidia produced on any other treatment. In conclusion, the white and blue light treatments during mycelial growth produced conidia with increased stress tolerance, faster germination speed, and were more virulent to the insect, with similar result as conidia produced on MM or MML. Support: FAPEG, CNPq, FAPESP.

Over-expression of genes may reveal increased tolerance against UV-B radiation in *Metarhizium* spp. conidia produced on culture medium supplemented with riboflavin (vitamin B2) **STU** **10:45** **•106•**

***Ronaldo A. Pereira-Junior*[1,2*], *Carla Huarte-Bonnet*[1,2], *Flávia R. S. Paixão*[1,2], *Christian Luz*[1], *Drauzio E. N. Rangel*[1], *Donald W. Roberts*[3], *Nicolás Pedrini*[2], *Éverton K. K. Fernandes*[1]**

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The success of entomopathogenic fungi is limited by their vulnerability to abiotic stresses, including ultraviolet radiation (UV) in sunlight, viz. UV-A and UV-B, which can significantly reduce the viability and delay germination of conidia. In an effort to protect conidia from solar irradiation, we evaluated the effect of adding riboflavin to culture medium for conidial production on the tolerance of these conidia to UV-B radiation, as well as the expression of photolyase (*Phr*), laccase (*Lcc*), and polyketide synthase (*Pks*) genes in *Metarhizium acridum* (ARSEF 324) and *M. robertsii* (ARSEF 2575). Conidia of both isolates were produced on potato dextrose medium without riboflavin (PDA) or PDA supplemented with riboflavin (PDA + Rb) at different concentrations and subsequently suspended in Tween 80® or riboflavin solution. Conidia were also produced on PDA medium supplemented with yeast extract (PDAY), which contains complex B vitamins. The conidia were exposed to UV-B (3.9, 5.46 or 6.24 $kJ.m^{-2}$), visible light (16 Klux) for 2 hours, or to UV-B followed by visible light exposure. Relative UV-B tolerance was determined by comparing percent germination of conidia on the various media with and without UV exposure. Conidia from both *Metarhizium* isolates produced on PDA + Rb, regardless its concentration, or PDAY were considerably more tolerant to UV-B than conidia produced on PDA medium without supplement. The conidial tolerance of *Metarhizium* isolates to UV-B did not change when conidia were suspended in riboflavin solution, suggesting that these fungi need to produce and accumulate metabolites inside the conidia to confer photoprotection. The expression of *MaLcc3* and *MaPks2* for *M. acridum*, as well as *MrPhr2*, *MrLcc1*, *MrLcc2,* and *MrLcc3* for *M. robertsii* was higher when the isolates were cultivated on PDA + Rb and exposed to UV-B followed by exposure to visible light, or exposed to only visible light, than conidia produced on PDA medium alone. The addition of riboflavin to substrates used for mass production of *Metarhizium* spp. and consequently enhancing the tolerance of conidia against UV-B radiation may be a viable approach to improving the effectiveness of these fungi in biological control programs.

RNAi-mediated knock down of insect immune-related genes in *Tenebrio molitor* increases entomopathogenic fungal virulence in *Beauveria bassiana* JEF-007 STU 11:00 •107•

***Mi Rong Lee**, Yi-Ting Yang, Se Jin Lee, Sihyeon Kim, Jong Cheol Kim, Jae Su Kim**

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Toll signaling pathway is responsible for defense against Gram-positive bacteria and fungi. Gram-negative binding protein 3 (GNBP3) 1, 3-β-D-glucan pattern recognition proteins (GRP) has strong relationship for fungal cell wall component (β-1, 3-glucan), which can move prophenoloxidase (proPO) cascade and cause Toll signaling pathway. Myeloid differentiation factor 88 (MyD88) is essential adaptor protein used by signal transduction pathways of the Toll-like receptor (TLR). In this study, we monitored the response of TmGNBP3 and TmMyD88 genes of the insect immune system against Beauveria bassiana JEF-007 in Tenebrio molitor using RT-PCR. TmGNBP3 was up-regulated after fungal infection. To better understand the roles of Toll signaling pathway in T. molitor immune system, TmGNBP3 and TmMyD88 were knocked down by RNAi. The results of RNAi showed that target gene expressions were decreased at 2 days post-dsRNA injection, and dramatically reduced at 6 days post-dsRNA injection. Therefore, T. molitor was compromised by B. bassiana JEF-007 at 6 days post-dsRNA injection. Silencing of the TmMyD88 and TmGNBP3 resulted in reducing the resistance of the host to fungal infection. These results indicate that not only TmGNBP3 but also TmMyD88 is required in T. molitor for survival against B. bassiana JEF-007, and the knock down of these genes possibly contributes to the enhanced fungal insecticidal activity.

***Drosophila* usage of chemical cues in removing fungus *Beauveria bassiana* from the body surface** 11:15 •109•

***Aya Yanagawa**[1], Marie-Ange Chabaud[2], Tomoya Imai[1], Toshimitsu Hata[1], Tsuyoshi Yoshimura[1], Frederic Marion-Poll[3,4]*

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Grooming behavior serves to remove harmful microbes from an insect's body surface. Although this role has not been reported clearly in solitary insects yet, it is well known that solitary insects like *Drosophila melanogaster* dissipate the attached foreign organisms by self-grooming behavior. In this study, we examined whether grooming serves to protect flies by reducing the risk of fungal infection in *D. melanogaster.* First, we confirmed that fungal conidia were removed by grooming. Fruit flies were treated with harmful entomopathogenic fungus, *Beauveria bassiana.* Then the removal of fungal conidia from the insect's body surface through grooming was quantified through microscopic observation. Second, the role of insect perception in fungus removal was examined to learn how flies find those micro-sized organisms attached on their surface. Since size of conidia does not allow flies to find them visually, we studied a role of chemical cues using the wildtype fly Canton-S, the taste deficiency mutant *poxn 70,* and the olfactory deficiency mutant *orco1*. The results showed that flies remove fungal conidia from their body surfaces via grooming behavior, and indicated that it is olfactory cues rather than gustatory cues, which have a significant role in fungal removal in *D. melanogaster*.

The *Drosophila* model system for analyzing natural variation in resistance to *Metarhizium* spp. 11:30 •110•

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We have used broad and narrow host range *Metarhizium* spp, generalist and narrow host plant specialist *Drosophila* spp, 188 *Drosophila melanogaster* Genetic Reference Panel (DGRP) lines, and a panel of mutant *D. melanogaster* to explore the genetic basis of natural variation in *Metarhizium* host specialization and insect disease resistance. Features of interest include: 1) substantial individual variation in disease resistance between flies collected in the same habitat, with implications for the evolution of disease resistance; 2) strong genetic control over microenvironmental plasticity, particularly in fly species that have a wide environmental range; 3) males are typically more resistant than females; 4) little difference between sporulation capacity on resistant lines and susceptible lines, so there is no penalty for the fungus to kill quickly, and 5) variation between DGRP lines in resistance to *M. anisopliae* was correlated with resistance to *Pseudomonas aeruginosa*, oxidative stress sensitivity, sleep duration and number of nightly 'naps'. We identified a host of candidate genes associated with variation in disease resistance, many of which are known to interact physically and/or genetically which enabled us to place them in a biologically informative genetic network. Overall, our results suggest that fly's differ in their ability to control and/or tolerate replicating fungi during infection, which is achieved mostly through the coordinated interplay of morphological and physiological restraints, and phagocytic effectors that function in subtly different ways in different lines. However, the majority of polymorphisms with major effects on disease resistance were rare, suggesting a general cost to defense involving trade-offs.

Host responses to entomopathogenic fungi 11:45 •111•

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Insects employ a variety of defensive systems to deter and/or repond to potential microbial pathogens. Entompathogenic fungi have evolved measures to overcome host defenses, resulting in the selection of host countermeasures, ultimately leading to an arms race between the host and the pathogen. Litle, however, is known about specific host tissue responses to fungal infection. Transcriptomic analyses were performed to investigate the global gene expression responses of hemocytes, the fat body, and the central nervous system of the socially flexible locust, *Locusta migratoria*, during infection by the locust-specific fungal pathogen, *Metarhizium acridum*.

Temporal patterns of gene expression were examined throughtout a time course of infection that included (a) spore attachment and consolidation (4 h), (b) germination on the insect cuticle surface (12 h), (c) appressorium, cuticle penetration and initial ingress into the hemocoel (24h/36h) and (d) mycosis (48h/72 h). Specific tissues were dissected during this time interval and RNA_Seq analyses performed. These data revealed that the fat body responds to fungal infection mainly through activation of genes involved in innate immune, energy metabolism and development. Hemocyte gene expression responses mainly affected genes involved in membrane regulation, activation of cellular immune responses, and release of humoral immune factors. Surprisingly, significant changes in central nervous system (CNS) tissues were seen throught the infection time course including at the earliest time point (4 h). Both neurological pathways and a significant number of immune related deifferentially expressed genes (DEGs) were identified in the CNS global gene expression response to fungal infection. The transcriptomics data were verified via qRT_PCR quantification of select genes. A number of host genes are currently being examined via RNA interference knockdown in order to identify potential targets for increasing the efficacy of entomopathogenic fungi to target insect hosts.

Endophytic association of *Metarhizium robertsii* under varying C and N soil conditions and plant immune responses **12:00** **•112•**

[1]*Larissa Barelli,* [1]*Sha Sha Hu,* [2]*David Liscombe,* [1]***Michael J Bidochka***

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Many vascular plants are able to form close symbiotic associations with endophytic fungi. *Metarhizium* is a common soil fungus that is both a plant endophyte and an insect pathogen. Previously we have shown that the endophytic capability and insect pathogenicity of *Metarhizium* are coupled to provide an active method of nitrogen transfer to a host plant via fungal mycelia. Here we investigated nitrogen transfer from insects infected by *Metarhizium robertsii* under soil conditions with excess carbon (1% glucose) or nitrogen (1% ammonium nitrate) to host plants (haricot beans). Highest amounts of insect nitrogen were transferred to plants without soil treatment without C or N at 14 days in a soil microcosm containing *M. robertsii* and insects (*Galleria mellonella*). Interestingly highest fungal association with host plants was found in soil with excess carbon at 21 days. Also highest fungal amounts were in soil amended with excess carbon at 21 days. Other time points (0, 7, 14 and 28) were not statistically different with respect to fungal colonization. We are also interested in the question as to why plants allow beneficial fungi such as *Metarhizium* to colonize roots while pathogens elicit a strong immune response. We used the OPLS-DA method to tease out which compounds were driving the differences between plants colonized by *Metarhizium* as opposed to plants without *Metarhizium.* A Principal Component Analysis plot was used to illustrate global differences between samples. Overall, thousands of mass spectral features (compounds) were detected but out of all of these, *Metarhizium* seems to enhance precursor (4-coumarate and tartaric acid) pools an accumulation of trans-coumarate, after day 7. We also observed jasmonate action- a hydroxyjasmonate (12-hydroxy or 11-hydroxy) accumulated at higher levels in *Metarhizium* colonized plants. No salicylic acid or derivatives were detected in these samples.The results help to understand better the endophytic interaction of *Metarhizium* in agroecosystems and plant immune responses to fungal endophytes.

Marshall Room

Diseases of Beneficial Invertebrates Division #2

Moderators: David Bass, Colleen Burge

Health monitoring and disease management of endangered white abalone *(Haliotis sorenseni*) in a captive breeding program **10:30** **•113•**

Blythe C. Marshman [1]*, Malina M. Loeher* [1,2]*, Kristin M. Aquilino* [2]*, Jim D. Moore* [1,2,3]

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White abalone (*Haliotis sorenseni),* a marine snail species native to waters off Southern California, significantly declined in the 1970s as a result of overfishing. In 2001, they were the first marine invertebrate to be federally listed as an endangered species and, since their listing, the population has continued to decline in the wild. The White Abalone Recovery Program, a NOAA-sponsored mission led by the Bodega Marine Laboratory (BML), is dedicated to restore the species throughout its native range through outplanting of captively reared progeny of wild-collected parents. One of the biggest challenges faced by this project is the susceptibility of the species to the rickettsiales-like prokaryote *Candidatus* Xenohaliotis californiensis (Xc), an intracellular bacterium that can cause a chronic degenerative disease called Withering Syndrome (WS). The WS disease, which can affect both wild and captive abalone, is exacerbated by elevated seawater temperatures and limited food sources. To help combat WS within captive breeding populations involved in the program, the CDFW Shellfish Health Lab performs routine testing on fecal samples using qPCR to detect emerging infections within populations at BML and partner facilities. To treat the disease, infected abalone undergo an oxytetracycline (OTC) antibiotic bath treatment that has been shown to not only eliminate the Xc infection, but also protect treated abalone from subsequent reinfection with Xc for at least four months post-treatment. The 21-day treatment, consisting of eight baths, has the advantage of not only administering a uniform dose to each abalone, but also allows for whole captive populations to be treated simultaneously. Shell-boring organisms, including bivalves, sponges, and polychaetes, exert additional stress on their abalone hosts and may facilitate opportunistic microbes that cause shell lesions. A shell waxing protocol was developed to rid the affected abalone of these pests and it has been effective in reducing the deleterious health impacts, including mortality, caused by lesions. Due to their efficacy while presenting minimal risk, we believe that both the OTC bath and shell waxing treatments can be used on captive abalone populations to aid in restoration efforts.

Candidatus Xenohaliotis californiensis* viability and infectious dose in *Haliotis rufescens **10:45** **•114•**

Nina S. Lottsfeldt*, Mariah E. Weavil-Abueg, Lisa M. Crosson, Carolyn S. Friedman*

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Withering syndrome (WS) is a fatal disease that affects a number of NE Pacific abalone species (*Haliotis spp.).* The etiological agent is the *Rickettsiales*-like bacterium (RLO) *Candidatus Xenohaliotis californiensis*, an obligate intracellular parasite. Our study aimed to: (1) determine how long the WS-RLO remains viable in seawater under two temperature conditions, 14°C and 18°C, and (2) create a standardized protocol for exposing abalone in the laboratory with known concentrations of WS-RLO using infectious dose 50 (ID50) methodology. To determine WS-RLO viability, duplicate 500mL samples of effluent seawater from a California abalone farm were filtered daily for a total of 8 days. DNA and RNA were extracted from all filters and quantified via qPCR as a proxy for viability. To determine WS-RLO ID50, triplicate tanks of 20 uninfected red abalone were exposed for 3 hours to 1L of WS-RLO homogenate (infected abalone post-esophageal tissue and feces

suspended in sterile seawater) at four concentrations: 0, 10^3, 10^4, and 10^5 WS-RLO DNA copies/mL. Abalone fecal samples were collected bi-weekly from each tank to monitor WS-RLO DNA loads and abalone post-esophageal tissues were sampled at day 61 post-exposure. Results from our viability experiment indicated WS-RLO DNA loads in seawater were highest at day 2 at both 14°C and 18°C then rapidly declined below the qPCR assay limit of detection by days 5 and 4, respectively. WS-RLO cDNA loads were 30-fold lower than WS-RLO DNA loads indicating that of the total WS-RLO DNA present, only a small proportion was potentially viable. Results from our ID50 experiment concluded that only 2.2-3.2 x 10^3 WS-RLO DNA copies/mL are required to generate a 50% infection prevalence. These findings provide critical information to enable a better understanding of WS-RLO transmission dynamics including concerns over WS spillover and spillback between wild and farmed abalone populations, and can aid management decisions for the successful protection and restoration of threatened and endangered abalone species.

Complementary methods for the detection and characterisation of novel haplosporidian parasites of the edible mussel, *Mytilus edulis* **STU 11:00 •115•**

***Georgia M. Ward*[1,2], Stephen W. Feist[1] , Ander Urrutia[1], Stuart Ross[1], Matthew Green[1], David Bass[1,2]**

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Haplosporida (Rhizaria, Cercozoa) is an order of protistan parasites of aquatic invertebrates, including molluscs, crustaceans and annelid worms. A number of haplosporidian species are known to be responsible for significant mortalities in commercially exploited oysters, including *Haplosporidium nelsoni* and *H. costale* (*Crassostrea* spp.) and *Bonamia ostrea* (*Ostrea* spp.). While these pathogens are well characterized within the host, parasite life cycle, modes of transmission and geographic distribution are much less clear. Similarly environmental DNA (eDNA) studies have hinted at significant uncharacterised genetic diversity within the order, however the relationships between these novel haplosporidian lineages and their hosts remains largely unexplored. This study uses the example of *Minchinia* sp., a novel parasite of the edible mussel *Mytilus edulis*, to demonstrate the efficacy of combining traditional histopathology with haplosporidian-targeted PCR screening, *in situ* hybridisation (ISH) and eDNA approaches to explore the prevalence, phylogenetics and life-cycle of haplosporidian parasites.

Development of biomarkers for Ostreid herpesvirus 1 resistance in Pacific oysters **11:15 •116•**

***Natalie D. Rivlin*[1], Colleen A. Burge[1], Collin J. Closek[2], Carolyn S. Friedman[3]**

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The Ostreid herpesvirus 1 (OsHV-1) has emerged as an infectious disease agent associated with mass mortalities of larval and seed Pacific oysters, *Crassostrea gigas*. Although OsHV-1 is a global pathogen of bivalve molluscs, it has been observed in only 2 adjacent bays in the US: Tomales Bay (TB) and Drakes Estero, California. In TB, OsHV-1 has caused severe (50-60%) losses of Pacific oyster seed nearly annually since the early 1990's, with mortality events recurring during the summer months. Seasonal elevated water temperatures are consistently associated with oyster mortalities in TB, and may trigger viral replication and/or transmission of OsHV-1 to naïve juvenile oysters. The present research investigates host susceptibility of Pacific oysters exposed to OsHV-1 and aims to develop biomarkers for OsHV-1 resistance. Individuals from two US west coast Pacific oyster families (hybrid pair-mated families) were exposed to OsHV-1 in TB over the course of a summer, and sampled periodically throughout mortality events. The two families exhibited differential mortality. Viral replication of OsHV-1 was quantified from whole-body tissue samples using qPCR, which revealed differential copy number between the two families. Samples are currently being assessed using genetic and transcriptomic methods to screen for OsHV-1 resistant genes. Characterizing resistance traits will help to provide tools and solutions to the US and global oyster industries and will be informative to Pacific oyster breeding programs.

Hytrosavirus genetic diversity and eco-regional spread in *Glossina* species **STU 11:30 •117•**

***Meki, I.K.*[1, 2], Kariithi, H.M.[1, 3], Vlak, J.M.[2], van Oers, M.M.[2, *], Parker, A. G.[1], Vreysen M.J.B.[1], Abd-Alla A.M.M.[1,*]**

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Tsetse control via the sterile insect technique requires tsetse mass production, which can be hindered by infections with *Glossina pallidipes* Salivary gland hypertrophy virus (GpSGHV; *Hytrosaviridae*) that can cause collapse. Although GpSGHV asymptomatically infects many *Glossina* species, the rare SGH symptoms are preferentially triggered in *G. pallidipes*. Hypothetically, *G. pallidipes* significantly drives GpSGHV evolution that can lead to emergence of more virulent virus variants transmissible to multiple *Glossina* species. Here, we report GpSGHV diversity in seven tsetse species derived from 20 African geographical locations. Species identities were verified using optimized PCR-based technologies including the use of nuclear internal transcribed spacer sequence, microsatellite markers, and diagnosis of *Wolbachia* infection. Based on differences between GpSGHV-Uganda and GpSGHV-Ethiopia genomes, three genes and two variable number tandem repeat regions were PCR-amplified and sequenced to identify GpSGHV haplotypes and their phylogenetic relatedness. Of the identified 14 GpSGHV haplotypes, 13 were present in *G. pallidipes* from 13 of the 20 sampled locations. GpSGHV diversity was highest within the *G. pallidipes* populations; the reference haplotype was the most widely distributed (in 8 out 20 locations). These data are valuable in establishment of new tsetse colonies, and in prediction/ prevention of SGH outbreaks in facilities producing multiple *Glossina* species.

Impact of *Glossina pallidipes* salivary gland hypertrophy virus on the performance of heterologous host, *G. f. fuscipes* **11:45 •118•**

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Tsetse flies (Diptera: Glossinidae) are the vector of African trypanosomosis, the cause of sleeping sickness in humans and nagana in animals. The lack of effective vaccine and the development of resistance by trypanosomes to the available drugs makes vector control the most effective approach for sustainable management of this disease. The sterile insect technique (SIT) is an effective method to suppress or eradicate tsetse flies in the frame of an area-wide integrated pest management programme. SIT relies on the mass production of the target insect and release of sterile males in the targeted area to compete with wild males in mating with wild females. Tsetse mass rearing is a challenge for SIT application and an understanding of the reproductive biology and associated pathogens is essential to improve mass rearing. *Glossina fuscipes fuscipes* is an important vector of sleeping sickness in central Africa. GpSGHV affects the fecundity, productivity and therefore performance and sustainability of *G. pallidipes* colonies. In this study, we evaluated the impact of GpSGHV on the performance of a *G. f. fuscipes* colony including productivity, mortality, survival, flight ability and insemination rates. The results indicate that GpSGHV infection has a significant impact on mortality, productivity, survival and mating ability but not on adult flight ability or insemination rates in *G. f. fuscipes*. These results explore the important role of GpSGHV in the sustainability of *G. f. fuscipes*

colonies and the need to implement measures to avoid virus infection to ensure the successful establishment of mass rearing of this species for SIT programmes.

Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere **12:00**

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Phylum Apicomplexa comprises a group of unicellular, often highly pathogenic, obligate parasites exploiting either one host (monoxenous) or two hosts (heteroxenous) to complete a full reproductive cycle. Life cycles of species infecting molluscs are poorly understood, although both monoxenous and heteroxenous species are known. For decades, cyclical mass mortality events have occurred in various scallop populations, some of which have been recently attributed to apicomplexan infections. An apicomplexan, causing severe myodegeneration, recently reduced the Iceland scallop population by 90%, caused mass mortalities in queen scallop in Faroese waters and is the probable aetiology behind mass mortality events of sea scallops in the eastern USA and the Gulf of Alaska. We report the first dual mollusc life cycle for an apicomplexan: a species highly pathogenic in pectinid bivalves, but apathogenic when infecting the common whelk as *Merocystis kathae*. The sympatric distribution of the common whelk and the scallop in the North Atlantic makes transmission extremely effective, occurring via the gastrointestinal tract, by scavenging and predation in whelks and unselective filter feeding in scallops. Infective sporozoites from whelks utilize host haemocytes to reach muscular tissue, where asexual reproduction occurs. Phylogenetically, this apicomplexan is robustly placed within the Aggregatidae and its inclusion in analyses considerably increases resolution between other basal apicomplexans groups (gregarines), which raises concerns over the validity of some related taxa. Scallops seem able to regulate low-level infections of *Merocystis* as they exist in normal scallop populations between epizootics but disease outbreaks occur during prolonged or high levels of exposure from localised infected whelks. Hence, a targeted removal of whelks from valuable scallop grounds would be advantageous to minimize the occurrence of *M. kathae* epidemics and prevent damaging economic losses.

TUESDAY, 12:30 – 5:00 pm
EXCURSIONS

Boxed lunches are provided for excursion participants. Buses for the San Diego Zoo and Midway excursions depart at 12:30 pm from the Price Center, and will drop off participants at the Birch Aquarium at 5:30 pm.

TUESDAY, 6:00 – 10:00 pm
BARBEQUE at Birch Aquarium

Buses will leave the Village housing area at 5:30 pm for the Birch Aquarium for those not attending an excursion. Buses will return from the Aquarium as needed between 9 and 10 pm.

WEDNESDAY, 8:00 – 10:00 am

Theater

Bacteria Division #3

Moderators: Juan Ferré, Satomi Adegawa

Functional characterization of Vip3Ab1 and Vip3Bc1 N-terminal and C-terminal domains **8:00** **•119**

Marc Zack*, Megan Sopko, Meghan Frey, Xiujuan Wang, Jennifer Arruda, Ted Letherer, Sek Yee Tan, and Ken Narva*

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Vegetative insecticidal proteins (VIPs) are soluble insecticidal proteins produced by *Bacillus cereus* and *B. thuringiensis* with demonstrated activity on lepidopteran insects. In general, it is accepted that Vip3 function by a mode of action different than that of the more-studied 3-domain crystal Bt family of insecticidal proteins. Therefore, the Vip3 family is valuable for development of insect-resistant crops such as corn and cotton. To date, 3 subsets of the VIP3 family have been identified; VIP3A, VIP3B, and VIP3C. We have recently shown that Vip3Bc1, a new member of the Vip3B family, has an insecticidal spectrum unique from that of Vip3A proteins. In addition, we have shown that Vip3Bc1, like Vip3Ab1, forms a ~340 kDa tetramer that is present before and after proteolysis. In the present work, we extend our understanding of Vip3 toxins by using chimeras of Vip3Ab1 and Vip3Bc1 to explore the relative roles of amino and carboxy terminal protein domains. We produced proteins in which we exchanged the N-terminal ~200 amino acids, named Vip3_AB and Vip3_BA, to denote the N-terminal changes. We then analyzed proteolytic stability, tetramer formation, and insecticidal activity. Vip3_AB was processed as expected by gut enzymes and formed tetramers before and after digestion. However, Vip3_BA was present primarily in monomeric form with some presence of tetramer. Monomers, but not tetramers, were rapidly proteolyzed by gut enzymes. Both chimeras lost lethal insecticidal activity. However, Vip3_BA caused significant growth inhibition of insects susceptible to Vip3Ab1 despite the majority of protein being present as a protease-sensitive monomer. Therefore, this work indicates a specific interaction between N-terminal and C-terminal domains of Vip3 proteins that is required for proteolytic stability and insecticidal activity. In addition, we have shown that the C-terminal portion of these proteins, often referred to as a core, directs specificity but is rapidly degraded and loses all activity on susceptible insects. These data provide novel insights towards Vip3 mechanism of action and insecticidal specificity that will support the development of new insecticidal traits.

Stability of the *Bacillus thuringiensis* Vip3Aa protein to protease digestion **8:15** **•120•**

Yolanda Bel, Núria Banyuls, Maissa Chakroun, Baltasar Escriche, ***Juan Ferré***

ERi de Biotecnología y Biomedicina (BIOTECMED), Departament de Genètica, Universitat de València, 46100 Burjassot, España.
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Vip3 proteins are secretable proteins from *Bacillus thuringiensis* whose mode of action follows similar steps as the Cry proteins. Once in the midgut of the insect, Vip3 proteins are activated. The activation of Vip3Aa has been shown to be necessary to form pores *in vitro*, and differences in the rate of activation have been related with differences in susceptibility amongst lepidopteran species. It had been described that the *in vitro* activation of Vip3A proteins with trypsin or insect midgut juice produces a major fragment of about 62–65 kDa, along with other fragments, mainly one of about 20 kDa that corresponds to the N-terminal region. In the present study, the activation process for the Vip3Aa16 protein has been closely examined in order to better understand the Vip3Aa protein stability and to shed light on its structure. At high concentrations of trypsin or midgut juice, if the action of proteases is not properly neutralized, the results of SDS-PAGE analysis may lead to think that the protoxin is completely processed. However, when the proteolytic reaction is efficiently stopped, the result reveals that the protoxin

is only cleaved at a primary cleavage site, regardless of the amount of trypsin used. The 66 kDa and the 19 kDa peptides generated by the proteases co-eluted after gel filtration chromatography, indicating that they remain together after cleavage. The 66 kDa fragment was found to be extremely resistant to proteases. The trypsin treatment of the protoxin in the presence of SDS revealed the presence of secondary cleavage sites at S-509, and presumably at T-466 and V-372, rendering C-terminal fragments of approximately 29, 32, and 42 kDa, respectively. The fact that the predicted secondary structure of the Vip3Aa protein shows a cluster of beta sheets in the C-terminal region of the protein might be the reason behind the higher stability to proteases compared to the rest of the protein, which is mainly composed of alpha helices.

Vip3Ca induces apoptosis in midgut epithelial cells from *Spodoptera exigua* and in cultured *Spodoptera frugiperda* (Sf21) cells 8:30 •121•

Patricia Hernández-Martínez[1], Joaquín Gomis-Cebolla[1], Juan Ferré[1], ***Baltasar Escriche[1]***

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Bacillus thuringiensis produces a wide range of insecticidal proteins which are active against a number of agriculturally pest species. Nowadays, products based on either crystalline insecticidal proteins (Cry proteins) and/or vegetative insecticidal proteins (Vip proteins) are used to control insect pests. Whereas the mode of action of Cry proteins has been extensively studied, little is known about the mechanisms of action of Vip3 proteins. In the present work, the mechanism responsible for Vip3Ca toxicity in both *Spodoptera exigua* larvae and in *Spodoptera frugiperda* cultured insect cells (Sf21) was studied. To elucidate the mechanism of cell death in the midgut epithelial cells, *S. exigua* larvae were exposed to different sublethal concentrations of Vip3Ca. Then, midguts were sectioned and stained using the DeadEndTM Fluorimentric TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) system. The results showed that in control larvae, and in the larvae exposed at the two lower concentrations used (10 and 100 ng/cm^2), no TUNEL-positive cells were observed. However, TUNEL-positive cells were observed in the gut of the larvae intoxicated at the highest concentration used (10000 ng/cm^2). These results suggest that the epithelial cell response is dose-dependent. In parallel, using the same methodology, we showed that the Vip3Ca protein can also induce apoptosis in Sf21 cells, suggesting that this *in vitro* system can be useful to further characterize the biochemical basis of the cell death.

Novel synergistic activity between *Bacillus thuringiensis* Cry9Aa and Vip3Aa toxins against *Chilo suppressalis* (Walker) STU 8:45 •122•

Zeyu Wang[1,2]*, Longfa Fang[1], Zishan Zhou[1], Sabino Pacheco[2], Mario Soberón[2], Alejandra Bravo[2], Jie Zhang[1]*

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A novel synergistic interaction among two different proteins produced by *Bacillus thuringiensis* (Bt) is described in this work. The Cry9Aa, a three-domain Cry toxin, and Vip3Aa, a vegetative insecticidal toxin, exhibit highly significant synergism against *Chilo suppressalis* with synergy factor of 35.7. These two proteins bound specifically to BBMV of *C. suppressalis*, and do not share binding sites since there is no-binding competition between them. We demonstrate that both Cry9Aa and Vip3Aa interact with each other by Ligand-blot, ELISA and Pull-down assays. This interaction showed an affinity value (Kd) of 40.1 nM. The specific interaction sites between these proteins were identified by analyzing binding of Cry9Aa to overlapping fragments of Vip3Aa and by analyzing binding of Vip3Aa to individual domains of Cry9Aa heterologous expressed in *E. coli* cells. To further narrow the binding sites of Cry9Aa and Vip3Aa we are using cellulose membrane-bound peptides. Ongoing research is focused to functional studies involving site-direct-mutagenesis followed by synergistic bioassay. The results indicate, for the first time, that the binding between Cry9Aa and Vip3Aa could be the basis of their synergism against *C. suppressalis*. Our data contribute to further understanding of synergy activity among Bt toxins and will be highly relevant to propose novel combinations of toxins to be used in the Pyramids scheme that would help to effectively control this pest and delay development of resistance.

A genomic and proteomic approach in the mining of new insecticidal proteins from *Bacillus thuringiensis* STU 9:00 •123•

Gomis-Cebolla, J[1]*, Ricietto, APS[1], Ferré, J[1].*

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The next generation sequencing (NGS) allows rapid sequencing of whole genomes with profitable cost-effective ratios and in combination with the development of a large number of freeware NGS tools can enable the rapid detection of insecticidal protein genes at the genome level. Nine samples were selected from Spanish collections of *Bacillus thuringiensis* (*Bt*) based in their *vip1* and *vip2* gene content (Hernández-Rodríguez et al.,2009 J. Appl. Microbiol. 107:219-225). As a first step, we determined which type of *vip1* and *vip2* genes contain every *Bt* isolate and we selected those *Bt* isolates with low similarity to the described *vip1* and vip2 genes (E-SE10.2 and O-V84.2). With these *Bt* isolates we performed a combined genomic and proteomic approach. These two *Bt* isolates were sequenced to determine the full gene content and then a proteomic analysis of the secreted and crystal insecticidal proteins was conducted to validate the automatic genomic annotation of Rast Server. As a result, we obtained a set of vegetative proteins, specifically two couples of Vip-like proteins (Vip2Ac-like_2/Vip4-like and Vp2Ac-like_3/Vip4Aa1-like_1). If insect bioassays prove that they hold insecticidal activity, these proteins could be considered as first representatives of new binary toxins, Vip6Aa-Vip5Aa and Vip6Ba-Vip5Ba, respectively.

Implication of the *Bacillus cereus & B. thuringiensis* siderophore Bacillibactin and its Siderophore Bonding Protein (SBP) FeuA in growth and virulence under iron restricted conditions 9:15 •124•

Laurent Consentino, Christophe Buisson, Agnès Rejasse, ***Christina Nielsen-Leroux***

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The ability of *B. cereus* (Bc*)* and B*. thuringiensis* to colonize various mammals and insects is linked to the presence of several adaptation factors, one of which is the capacity to acquire iron. Iron is an essential for several cellular functions and thus for all living organisms. Meanwhile free iron is toxic and is bound to molecules like hemoglobin, ferritin, transferrin etc. Previously, an *in vivo* screen of *B. cereus* led to the identification of a novel protein, IlsA, which is specifically expressed in the insect hemocoel and under iron restrictive conditions *in vitro*. It was further shown that IlsA is localized on the surface of *B. cereus* and affinity tests revealed that IlsA interacts with both hemoglobin and host ferritin. Furthermore, we showed that *B. cereus* siderophores (bacillibactin, encoded by the operon *entA-dhbBCF*) is particularly important for ferritin iron uptake and for full virulence in *G. mellonella* and may favor IlsA-related iron uptake (Segond *et al.* 2014). Siderophores are small molecules with strong affinity for ferric iron. To enter the bacteria the siderophore/Iron complex generally binds to a Siderophore Binding Proteins (SBP) at the surface of the cell. To get further insight into the role of the SPB FeuA of *B. cereus* in iron uptake from ferritin a Bc ATCC 14579 strain mutant ΔfeuA and a ΔfeuAΔentA double mutant along with complemented strains were constructed and analysed *in vitro* and *in vivo*. The results indicate that the ΔfeuA mutant is affected *in vitro* almost similarly to the bacillibactin entA-mutant with horse-spleen ferritin as the sole iron source. Addition of extracellular bacillibactin restored the growth kinetics of only the ΔentA mutant. Interestingly, *in vivo* infection (injection of exponentially growth bacteria into the hemocoel) of *G. mellonella* shows that the feuA mutant and particularly the double mutant ΔfeuAΔentA were as virulent as the willdtype strain. These results may indicate that *in vivo* the

double mutant could be stimulated to express other virulence /adaptation factors or may be less sensitive to insect defense mechanisms. These hypotheses are currently under investigation.

A revolution in global health: mass-producible purified natural Bt crystals as a drug product **9:30** **•125•**

Yan Hu, Ambily Abraham, Jason Noon, Zeynep Mirza, Hanchen Li, Florentina Rus, David Gazzola, Deysy Tatianan Pinto Rodriguez, Gary Ostroff, ***Raffi V. Aroian***

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Soil-transmitted helminths (hookworms, whipworms, Ascaris) are nematodes that live in the human intestine and are the most common parasites on earth. They are major contributors to childhood stunting and poverty worldwide. Low efficacy and emerging resistance to current chemical drugs mandates development of new therapeutics. We have demonstrated that spore-crystal lysates expressing the three-domain Bt crystal protein Cry5B can cure hookworm and Ascaris infections in animal models of human disease and significantly impact whipworms. This makes Bt and Cry5B potential major players on the global health stage. The primary challenge for the development of a Bt-based therapeutic is the identification of an appropriate API (Active Pharmaceutical Ingredient) containing Cry5B. Cry5B spore-crystal lysates, although mass producible, face a difficult road as an FDA approvable drug given the phylogenetic proximity of Bt to *Bacillus cereus* and the difficulty in defining drug substance specifications for a crude mixture of live spores, bacterial lysate contaminants and Cry5B as the API. In the course of defining what a suitable Bt Cry protein drug substance might look like, we ascertained that an ideal solution might be purified natural Bt crystals that lack spores and Bt contaminants. However, heretofore we knew of no such means to purify Bt crystals free of spores and contaminants by a pharmaceutically scalable, inexpensive process that retains full bio-activity. Such purified crystals could be formulated for oral delivery in room-temperature stable enteric capsules to treat STH infections. Here, we describe the discovery and development of a method to purify naturally formed protein crystals from Bt or other heterologous microbial protein production systems that contain one or more Cry proteins free from spores and other bacterial contaminants (>95% pure at the protein level). The simple process is inexpensive and compatible with industrial and pharmaceutical production methods. Tests *in vitro* and *in vivo* against parasitic nematodes with Purified Cry5B Crystals (PCC) demonstrate that PCC is highly bioactive, even more so than Cry5B spore-crystal lysates. The discovery of PCC not only opens up the path for development of Cry protein drugs, but also potentially novel and potent applications of purified Bt crystal insecticides.

Novel insecticidal proteins from plants for crop protection against major lepidopteran pests **9:45** **•126•**

Jennifer Barry[1]*, Lu Liu*[2]*, Eric Schepers*[1]*, Amy Lum*[2]*, Janet Rice*[1]*, Nasser Yalpani*[1]*, Ryan Gerber*[3]*, Nuria Jimenez*[1]*, Fikru Haile*[1]*, Xiuli Qi*[1]*, Adane Kassa*[1]*, Matt Heckert*[2]*, Weiping Xie*[2]*, Scott H. Diehn*[1]*, Virginia C. Crane*[1]*, Howard Damude*[4]*, Carol Pilcher*[1]*, Russ Booth*[1]*, Mark Nelson*[1]*, Albert L. Lu*[1]*, Timothy M. Nowatzki*[1]*, Gusui Wu*[1]

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Many lepidopteran insect species are economically important pests of crops worldwide. The loss can be in billions of US dollars annually due to reduced crop productivity and increased pest management costs. Transgenic crops developed with *Bacillus thuringiensis* (*Bt*) insecticidal protein encoding genes have been widely adopted and they offer benefits to both farmers and the environment by increasing productivity and reducing reliance on conventional pesticides. However, rapid and widespread adoption of these transgenic crops has increased the risk of evolution of pest resistance to *Bt* proteins and in certain instances it has resulted in reduced effectiveness. We report here the discovery of a new family of potent insecticidal proteins from plant species. These proteins show activity against lepidopteran insect pests including corn earworm (CEW) (*Helicoverpa zea*), fall armyworm (FAW) (*Spodoptera frugiperda*), soybean looper (SBL) (*Chrysodeixis includens*) and velvetbean caterpillar (VBC) (*Anticarsia gemmatalis*). Transgenic soybean (*Glycine max* L. Merill) and corn (*Zea mays* L.) plants expressing those proteins show protection from feeding damage by these insect pests. This family of novel proteins is useful for developing new transgenic crops for lepidopteran pest control.

The Forum

MICROSPORIDIA DIVISION SYMPOSIUM

The past and future frontiers in microsporidiology (Retrospective look at Microsporidia research from the first meeting 50 years ago)

Organizer: Yuliya (Julia) Sokolova

Moderators: Leellen Solter and Susan Bjornson

Highlights of research on microsporidia from aquatic hosts in North America **8:00** **•127•**

James J. Becnel

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The earliest record of microsporidia from North America was probably *Glugea stepani* from a fish in 1901. There were few additional reports of microsporidia until Kudo, a pioneer in microsporidian research in North America, published more than a dozen significant papers on mostly aquatic microsporidia from 1920-1925. Kudo (1924) listed 21 species of microsporidia that had been described from North America with the great majority (18) from aquatic hosts. The aquatic hosts were mostly mosquitoes and blackflies but also included parasites of mayflies and microcrustacea. Over the next 30 years progress was slow with only about 13 new species described from insects in North America with only 4 of these from aquatic hosts. During this time, Sprague, a student of Kudo, did publish a number of descriptions of microsporidia from marine and freshwater Crustacea. As part of the movement beginning in the late 1950's to investigate biological control options for pest arthropods, microbial control efforts led to many new reports of microsporidia in host from North America. For the aquatic host, the focus was mainly on mosquitoes beginning with the California Department of Public Health's Fresno laboratory. This was followed by U. S. Department of Agriculture, Agricultural Research Service Laboratories formed in Lake Charles, Louisiana and Gainesville, Florida and eventually at many University programs. The Society for Invertebrate Pathology became a focal point for researchers on microsporidia and this association was formalized with the establishment of the Microsporidia Division in 1969. Much was learned about microsporidian biology and taxonomy in aquatic hosts in North America led by researchers such as Kellen, Chapman, Anthony, Hazard, Sprague, Undeen and a number of students that have carried on the research. Highlights will be presented on the many advancements made on microsporidia found in aquatic hosts by this relatively small but dedicated group of microsporidiologists.

Microsporidia in marine invertebrates **8:20** **•128•**

Kelly Bateman*, Grant Stentiford*

European Union Reference Laboratory (EURL) for Crustacean Disease, Cefas, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK. *Corresponding author: Kelly.bateman@cefas.co.uk*

Microsporidia are a diverse parasite phylum infecting multiple host groups and causing important diseases in aquatic organisms. Almost half of the known microsporidian genera infect aquatic hosts with approximately 50 genera in arthropods and at least 21 genera found in aquatic non-arthropod invertebrates, protists and hyperparasites of aquatic hosts. To date, most of the focus has been on hosts of commercial or ecological significance (e.g., farmed or fished decapod crustaceans) meaning that Microsporidia remain vastly under-reported in our less enigmatic hosts. Nevertheless, access to materials collected from hosts from a wide range of aquatic environments

and the development of pathological and molecular diagnostics is continually expanding our understanding of the phylum and, providing exposing an ever-increasing diversity in pathogen morphology, lifecycle and parasitic strategy. Whilst the majority of taxa were erected on the basis of morphological descriptions of various life cycle stages (meronts, sporonts, sporoblasts and spores), more recent taxonomic assemblages have been based upon phylogenetic data, normally relating to sequencing of the partial SSU rRNA gene. SSU-based phylogenies appear to perform well for placement of new genera (of which there are apparently many) within the phylum. However, where species are more closely related, concatenated phylogenies, based upon multiple gene sequences is proving useful for taxonomic clarity. The increasing availability of whole genome sequences for members of the Microsporidia is assisting construction of concatenated phylogenies based upon provision of gene sequences from across the parasite genome. Overall, whilst our understanding of diversity in the aquatic microsporidians has steadily expanded with the application of molecular diagnostic tools, we predict an era of accelerated discovery based upon use of these tools on a wider range of host and environmental matrices. However, recent work combing morphological characteristics with phylogentic data has demonstrated plasticity in morphological traits making them unreliable metrics for the purpose of classification.

The (relatively) early history of microsporidian researchers in North America **8:40** **•129•**

Joseph Maddox

Illinois Natural History Survey, Prairie Research Institute, University of Illinois, Champaign, IL, USA (retired)
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An informal review of the activities of microsporidian researchers with an emphasis on events before 2000. Questions (and some answers) about the phylogeny and classification of microsporidia, the role of microsporidia as biological control agents, and the formation of the Microsporidia Division of the SIP will be discussed.

The path to registration of a microbial pesticide **9:00** **•130•**

John E. Henry

Retired,
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This narrative describes the initiation and execution of a project on biological control of grasshoppers with an intent of providing non-chemical approaches to managing the densities of grasshoppers. The project led to the selection and development of the pathogenic protozoan, *Nosema locustae*, as a tool for applied use. It presents the criteria used to select this organism and reviews the extensive testing that established that applications of the organism affected both short term and long term host population regulation. With the involvement of the US Environmental Protection Agency (EPA) the organism was registered as the active ingredient in a microbial pesticide.

History and highlights of microsporidia research in Russia **9:20** **•131•**

***Yuliya Y. Sokolova*[1,2]**

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The focused research on Microsporidia (M) in Russia counts from mid-60ies, when Irma Issi defended her PhD dissertation The microsporidiosis of Cabbage White and other insects, and its biological importance, and established a Microsporidia research group, Issi's lab, within the Laboratory for Microbiological Control, Institute for Plant Protection, St.Petersburg, Russia. This lab has been running by her for more than 50 years now, and became an incubator for young microsporidiologists: >30 PhD students from all over the former Soviet Union have passed through the lab and graduated under Prof. Issi's supervision. The research have been focused on: (1) host population control by M and possible application of M in microbiological control; (2) interactions of M with the insect host: impact on immunity and physiology; (3) host-parasite relations at the cellular and subcellular levels; (4) taxonomy of M. One of the brightest research achievements of the lab was a long-term (over 25 years) observation on the epizooty of *Nosema mesnili* in *Pieris brassicae* that demonstrated how the microspridium took the control over the host population. Peaks and declines in host and parasite densities were accompanied by alterations in the microsporidium host cycle and switches of transmission routes. Unfortunately this research was published only in Russian. Dr.Issi's monograph Microsporidia as a Phylum of Parasitic Protists (1986) focused on the taxonomy, systematics and origin of M, was translated to English by Dr.Lipa and printed in 1991 by the request of SIP Microsporidia Division. Irma's studies were enthusiastically continued by her graduates, Vladimir Voronin, Petr Kilotchitsky (both specialized in microsporidia from freshwater hosts), Tamara Pankova (M from East-Siberian host), Viatcheslav Dolgikh (gave a new breath to the lab by establishing advanced biochemical, cell and molecular biology approaches), and myself (life cycles, taxonomy and cell biology of M), to mention only a few. The next generation of Dr.Issi's prodigious grandkids, Yuriy Tokarev, Elena Nassonova and Anastasiya Simakova, have already established themselves as international scholars. I will also focus on the three major recent highlights of Russian microsporidiology: structure and functional of secretory compartment of M, biochemistry of host-parasite relations, and studies on metchnikovellids.

Microsporidiosis: Perspective from human infections **9:40** **•132•**

***Louis M. Weiss*[1,2]**

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Microsporidiosis, infection by members of the phylum Microsporidia, affects both invertebrate and vertebrate hosts. Phylogenetic studies indicate that these pathogens are related to the Fungi, most likely as a sister group with the Cryptomycota. Given the wide host range of these organisms it is not surprising that they are pathogens of humans. These organisms were initially reposted in humans in 1959 with the description of a child with encephalitis, but until the HIV epidemic human infections were only sporadically reported. Gastrointestinal infections due to *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* were associated with chronic diarrhea and wasting in patients with AIDS. The number of reported cases increased exponentially until the widespread use of combination antiretroviral therapy (cART) resulted in a decline in infection in this population. There are over 200 microsporidian genera, of which the following have been reported to cause infection in humans: *Nosema* (*N. corneum* renamed *Vittaforma corneae*; *N. algerae* reclassified as *Brachiola algerae* and then *Anncaliia algerae*), *Pleistophora*, *Encephalitozoon*, *Enterocytozoon*, *Septata* (reclassified as *Encephalitozoon*), *Trachipleistophora*, *Brachiola* (reclassified as Anncaliia), *Anncaliia*, *Tubuli-nosema*, *Endoreticulatus* and *Microsporidium*. Infection in humans has now been documented in both immune compromised patients (including those with transplantations) and immune competent patients. These infections have involved almost all organ systems causing diseases such as encephalitis, myositis, sinusitis, keratitis, and conjunctivitis. As our understanding of these ubiquitous pathogens increases our recognition of their role as human pathogens and our ability to diagnose these infections is increasing.

THIS SYMPOSIUM CONTINUES IN THIS ROOM AFTER THE COFFEE BREAK

Microsporidia associated with bumble bees in the southern Neotropical region 10:30 **•133•**

Santiago Plischuk, Marina Haramboure, ***Carlos E. Lange***

Centro de Estudios Parasitológicos y de Vectores (CCT La Plata CONICET-UNLP-CICPBA), La Plata, Argentina
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With 40 years of history in Argentina and long-term studies restricted to a few host insect orders, Microsporidiology as a whole is still a young discipline in the country. Initial research was prompted by experimental use of the grasshopper biocontrol agent *Paranosema locustae*. Other sustained efforts

studied microsporidia associated with black flies, mosquitoes, fire ants, and more recently with honey bees and bumble bees. Bumble bees (*Bombus* spp.) play a key role as highly efficient pollinators in a variety of natural and man-made ecosystems. Of the approximately 250 species known worldwide only nine native species have been reported to inhabit Argentina, Chile, and Uruguay which seem to depict a somewhat low diversity for southern South America when compared to the Palearctic and Nearctic regions. Two additional Palearctic species were introduced late in the XX century in Chile, became naturalized, and spread to Argentina across the southern Andes. Less than a decade ago knowledge of microsporidia associated with bumble bees in the southern Neotropic was nonexistent. Since then surveys started and the occurrence of at least three microsporidia was recorded. The predominantly fat tissue pathogen *Tubulinosema pampeana* was discovered in native *Bombus atratus* and *B. bellicosus* in the Pampas, the enteric *Nosema ceranae* was registered in natives *B. atratus*, *B. bellicosus*, *B. morio* and *B. brasiliensis* in the Pampas and North of the country, and systemic *Nosema bombi* in exotics *B. terrestris* and *B. ruderatus* in western Patagonia. Since *B. terrestris* is extremely ubiquitous and has already reached the northern Patagonian Atlantic coast it is likely that its range expansion will continue further possibly allowing new microsporidia-host associations of unpredictable outcomes. The presentation will review the status of both microsporidia and hosts, emphasizing the importance of immediate intensification of surveys before *B. terrestris* reaches a wider distribution.

Microsporidia infection in *C. elegans*: How an obligate intracellular parasite makes itself at home **10:50** **•134•**

Emily Troemel

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Our lab studies *Nematocida parisii*, which is a natural pathogen of the genetically tractable nematode *C. elegans*. We have found that *N. parisii* restructures host cells through causing the host intestine to become a syncytium, and by hijacking host exocytosis for spore exit. These and other strategies have enabled this obligate intracellular parasite to 'make itself at home' in the host organism.

Phylogeny of Microsporidia: impact of molecular approaches **11:10** **•135•**

Charles R. Vossbrinck[1], *Yuri Tokarev*[2]

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Prior to the use of molecular characters to study phylogeny taxa were described based on phenotypic character states. As single-celled microorganisms, defining homology between characters is difficult and it appears that the Microsporidia may be gaining and loosing character states rapidly resulting in polyphyletic taxa. In addition there is no fossil data for microsporidia to clarify any relationships. We will briefly review former classification systems for the microsporidia. We suggest developing a microsporidial classification based upon a molecular phylogeny and placing the character states on the tree in a cladistic fashion.

Roosevelt Room

Microbial Control Division #2

Moderators: Wendy Gelernter, Travis Glare

Toxin-mediated control of insect host population by the endosymbiotic bacteria *Spiroplasma poulsonii* **STU** **8:00** **•136•**

G. Garcia-Arraez*, F. Masson, J.C. Paredes, B. Lemaitre*

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Spiroplasma is a group of widespread arthropod-associated bacteria. They exhibit a wide range of interactions with their hosts, from parasitism to mutualism. All species lack cell wall components, which make them invisible to the immune system of their insect hosts. *Spiroplasma poulsonii* (*S. poulsonii*) is an endosymbiotic bacterium that infects specifically the fruit fly, *Drosophila melanogaster*. *S. poulsonii* is found extracellularly in the hemolymph but can colonize the germline during oogenesis to be vertically transmitted from mother to offspring. *S. poulsonii* can disrupt the sex ratio of its host by inducing male killing, which consists in the specific death of all male embryos. While male-killing has been reported in many species, the molecular mechanisms underlying this phenotype are still unknown. We have isolated a new *S. poulsonii* variant called blind-killer that kills only 25% of the offspring of young female flies increasing to 70% for the offspring of two-weeks old females. Interestingly, the sex ratio of the offspring is not affected by the infection, since both male and female embryos die in similar proportions. In order to gain insight into the blind-killing phenotype, we have sequenced and compared the genome and transcriptome of both male-killer and blind-killer strains. Intriguingly, a family of toxin encoding genes, called *Ribosome Inactivating Proteins* (RIPs), were found to be differently expressed between the two strains. To date, Spiroplasma RIPs have been involved in the protection exerted by this endosymbionts against entomopathogenic nematodes in *Drosophila neotestacea.* It was proposed that these RIP toxins can specifically cleave the ribosomal 28S RNA of the nematodes. We are now investigating the role RIPs in male-killing.

DNA-based genotyping for Cry1Fa resistance in field populations of *Spodoptera frugiperda* **STU** **8:15** **•137•**

Rahul Banerjee[1], *Lucas Hietala*[2], *Robert Meagher*[3], *Rod Nagoshi*[3], *James Hasler*[4], *Kenneth Narva*[4], *and Juan Luis Jurat-Fuentes*[1,2]

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Populations of the fall armyworm (*Spodoptera frugiperda*) in North and South America have developed field-evolved resistance to transgenic corn producing the Cry1Fa insecticidal protein from *Bacillus thuringiensis*. Our group identified mutations in the *SfABCC2* gene that are genetically linked with Cry1Fa resistance in Puerto Rico and Florida and developed genotyping assays to detect these alleles in fall armyworm samples. We report the use of these assays to screen for the Puerto Rico and Florida alleles in field collected samples of fall armyworm, including archived and recent collections. Our results allow the detection of resistance evolution in Puerto Rico, testing its spread to southeastern US, and the detection of additional field-evolved resistance alleles. These findings represent the first report of successful molecular monitoring for field-evolved resistance to transgenic Bt crops and facilitate our understanding of resistance dispersal and frequency in field population of *S. frugiperda*

Pathogen growth rate is constrained by host diet **STU** **8:30** **•138•**

Robert Holdbrook[1], *Catherine E. Reavey*[1], *Joanna L. Randall*[1], *Yamini Tummala*[1], *Sheena C. Cotter*[2], *Stephen J. Simpson*[3], *Kenneth Wilson*[1]

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The use of bacteria and other pathogens as biological pesticides against insect crop pests is growing due to environmental concerns over traditional chemical pesticides. However, we have only a rudimentary understanding of how factors such as host nutrition impact on the efficacy of biopesticides. The Egyptian cotton leafworm, *Spodoptera littoralis*, is a major pest throughout much of Africa. Several studies have shown that the outcome of host-pathogen interactions in this and related species varies with dietary macronutrient composition, with individuals on a higher-protein diet inhibiting disease progression for longer. Using the gram-negative entomopathogenic bacterium *Xenorhabdus nematophila*, we investigated the nutrition dynamics in the host-pathogen relationship from the perspective of the parasite. Synthetic haemolymphs (bloods) were designed to replicate the in vivo host nutritional state when fed on a range of diets varying in their macronutrient compositions. We found that the in vitro

population growth profiles of *X. nematophila* cultivated in these synthetic bloods varied in a con-sistent manner, reflecting our in vivo findings; bacterial replication rate decreased inversely with solution protein content. This implies that the insect's preference for dietary protein may not only boost immune function, but may directly inhibit pathogen proliferation, further increasing the host's chances of survival.

Poor growth of *Bacillus thuringiensis* in larval cadavers **8:45** **•139•**

Alejandra Zamudio-Ramírez, Javier Luévano-Borroel, Rosalina García-Suárez, A. Nadin Lule-Chávez, Luis A. Verduzco-Rosas, J. Francisco Castillo-Esparza, Mónica García-Montelongo, Sebastián E. González-Villarreal, Leandro G. Ordoñez-Acevedo, ***Jorge E. Ibarra****.*

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Entomopathogens may be important biotic factors in the regulation of insect population densities in nature and agro-ecosystems. They share important features such as virulence, infectivity, co-evolution with their host, ability to cause epizootics, transmissibility, and a strong relationship with their host's biology. Many show some degree of specificity to their hosts, and their horizontal transmission is based on their ability to massively grow in the host so the cadavers become into inocula to other hosts. Although the most successful entomopatho-gen used in insect control is *Bacillus thuringiensis*, it does not show some of the typical features of a natural entomopathogen. This report shows that B.t. is deficient in its ability to grow massively in its host, hindering its ability for horizontal transmission. B.t. was quantified in cadavers of *Manduca sexta* killed by this bacterium, from 0 to up to 12 days after death, and in three different settings: cadavers left on the artificial diet, cadavers removed from the artificial diet after death, and cadavers left on tomato leaves. Cadavers left on the artificial diet showed an increasing number of B.t. that stabilized at about 1 million CFU per cadaver. Cadavers removed after death from the artificial diet decreased rapidly the B.t. numbers to stabilize at about 1,400 CFU per cadaver. When cadavers were left on tomato leaves, the number of B.t. CFU decreased to about 500 CFU per cadaver. In none of these settings, B.t. overgrew saprophytic bacteria in the cadavers. In the experiment with cadavers left on tomato leaves, proportions of Bt:saprophytic bacteria were 2.4:1000 at 0 days after death (DAD); 1.4:1000 at 3 DAD, and 0.3:1000 at 6 DAD. These results clearly indicate a poor growth of B.t. in larvae killed by itself, especially when tested under near-field conditions. These results also may answer some doubts about the ability of B.t. to cause epizootics in nature.

Formulation, field evaluation and commercialization of coleoptera toxic *Bacillus thuringiensis* **9:00** **•140•**

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A formulation with high efficacy and low cost would add to the characteristic features of *B. thuringiensis*. The native isolate of *B. thuringiensis* having better Coleopteran insecticidal activity were used for the formulation and field evaluation. Primarily used the amarantus stalk and corn stalk extract as a source for mass production *Bt*. Both stalk extracts supported growth of native *Bt* strains. Corn stalk extract yielded $1.12x10^{-9}$ cells in JK Bt IIHR isolate. Whereas Amarantus stalk extract yielded $1.01X10^{-9}$ cells in AND Bt IIHR isolate and $9.58X10^{-9}$ cells/ml in MD native *Bt* isolate. In the present study, granular, talc and liquid formulations of *B. thuringiensis* were prepared. The cultures were purified by restreaking on LB agar plates several times and maintained on LB agar slants with 20% glycerol at 4ºC. Liquid formulations of native *Bt* was prepared by using five different stabilizers, *viz.*, glycerol, groundnut oil, mustard oil, mineral oil, and sunflower oil. Granular formulation was done using sodium bentonite granules. Whereas wettable powder formulation was done using talc powder. Liquid formulation bioassay were conducted against the adults of brinjal ash weevil *Myllocerus subfasciatus* and *M. discolour* (Coleoptera: Curculionidae). Granular and talc formulation bioassay were carried out against the larvae of Arecanut white root grubs (*Leucopholis lepidophora* B. & *Leucopholis burmeisteri*) and white grub (*Holotrichia serrata*) Coleoptera: Scarabaeidae. Formulations were very effective against the tested insects. Successfully conducted the field trial against Arecanut white root grubs and brinjal ash weevil. The *Bt* based formulation offer advantages over harmful chemical insecticides being target specific, biodegradable and economical.

Screening of four soil bacteria for secondary metabolites (SMs) against pulse beetle for post-harvest preservation of green gram **9:15** **•141•**

Shripad M. Upasani

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Green gram (*Vigna radiata* (L) Wilczek) is major constituent of human diet in India. Production is not meeting the demand, hence grains has to protect biologically in storage saving 25% grain being lost every year. Four bacteria namely *Bacillus farraginis* NAIMCCB-00813, *Lysinibacillus sphaericus* NAIMCC-B-00111, *Stenotrophomonas acetaminiphila* NAIMCC-B-00473 and *Paenibacillus polymyxa* NAIMCC-B-00864 were tested for their secondary metabolites having pesticidal activity. Among four bacteria *B. farraginis* and *Stenotrophomonas acetaminiphila* shown well growth and yielded comparatively high amount of secondary metabolites. Pesticidal activity of all crude secondary metabolites checked against *Callosobruchus chinensis* Linn., which is cultured on Green gram (*Vigna radiata* (L) Wilczek); in previous study at School of Life Sciences, North Maharashtra University Jalgaon, it has been found to be most susceptible pulses to the infestation of *Callosobruchus chinensis* Linn. Secondary metabolites from *Bacillus farraginis* shown 95% adult mortality at 6 mg ml^{-1} in 2 h, *Lysinibacillus sphaericus* shown 66% adult mortality at 8 mg ml^{-1} in 7 h. *Paenibacillus polymyxa* shown 53% adult mortality at 10 mg ml^{-1} in 8 h whereas *Stenotrophomonas acetaminiphila* shown 97% adult mortality at 3 mg ml^{-1} in 1.5 h. Also only 1%, 2%, 10% and 17% adults has been seen emerged in green gram treated by *B. farraginis, Stenotrophomonas acetaminiphila, Lysinibacillus sphaericus* and *Paenibacillus polymyxa* secondary metabolites respectively in ovicidal activity. As *Bacillus farraginis* and *Stenotrophomonas acetaminiphila* found to be the significant bacteria for management of *Callosobruchus chinensis* efforts has been made to identify their active ingredients by UVVIS spectroscopy, TLC, HPLC, FTIR and HRLCMS. It has been seen that SMs extracted from bacteria are volatile and their action on beetle is fumigant. Solid formulations have been shown best results in protecting grains while kept in bags of green gram in the simulated grainary conditions.

Molecular Identification, characterization and implementation of *Spodoptera litura* associated NPV for the management of major lepidopterist insect pests of major crop in Pakistan **9:30**

Jam Nazeer Ahmad*[1,2], Robert L Harrison[3,] Muhammad Jafir[1] Ishita Ahuja[4], Atle Bones[4], Samina Jam Nazeer Ahmad[1,2]*

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The microbial control of major insect pest may serve as a potential replacement of insecticides when insect pests gain resistance against synthetic pesticides. In the present study, for the first time, native Nucleopolyhydroviruses infected insects were collected from fields of one crop growing region of Pakistan and brought to laboratory for identification and laboratory trials. The experiments were conducted to investigate the effect of native Nucleopolyhedroses virus (NPV) isolated locally from field populations of army worm (*Spodoptera litura* -Lepidoptera: Noctuidae). For molecular study, DNA was isolated from NPV infected insects and used for Polymerase chain reaction by using Lef8, Lef9 and Polh primers pairs, RFLP and then sequencing. Our results indicated that our native NPV were closely related to *Spodoptera litura* associated NPV (splNPV) already identified from other countries. For laboratory trials, three dose rates of NPV (1×10^7; 1 x

10^8; and 1 x 10^9 POB mL^{-1}) were applied against 2nd and 4th instar larvae *spodoptera litura* and *Helicoverpa armigera* reared in our laboratory. The data was recorded after every 24 h for mortality, pupation, adult emergence and egg eclosion for both insects' larval instars. All the bioassays were carried out at 25±2°C, 75% r.h. and L16: D8 h photoperiod. The medium and higher dose rate of NPV for Spodoptera and higher dose rate for *Helicoverpa* population exhibited good effect against various stages of insects. Our results showed that the army worm population was highly susceptible and life stages were severely affected against identified splNPV. We also found maximum mortality of *Helicoverpa* population at high dose rate. Moreover, it may be suggested that SplNPV can be equally tested against some other lepidopterist pests. It can also be combined with other microbial mixtures and insecticides that can prove as a potential Integrated Pest Management (IPM) strategy against *S. litura* and *Helicoverpa armigera* in crop growing areas of Pakistan.

Eicosanoids act in insect/pathogen interactions: cloning and characterization of a phospholiplase A_2 from *Heliothis virescens* **9:45**

David Stanley[1], *Cynthia Goodman*[1], *Joseph A. Ringbauer, Jr*[1], *Tamra Reall*[1], *Yongguyn Kim*[2]

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Phospholipase A_2s (PLA_2s) make up a large superfamily of enzymes responsible for hydrolyzing fatty acids from the *sn*-2 position of phospholipids. They are very well appreciated in the biomedical literature and understudied in insect species. PLA_2s act in insect venoms and in a range of physiological functions, including digestion and as the first step in the biosynthesis of prostaglandins and other eicosanoids, which are key mediators of cellular and humoral insect immune reactions to pathogens and other microbes. Here, we report on cloning, expression, and characterization of a recombinant PLA_2 from *H. virescens* tissues. We also characterized PLA_2s in two insect cell lines, a *Heliothis zea* (HzAM1) and a *H. virescens* (HvAM1) line. The three PLA_2 preparations were sensitive to the usual biophysical parameters, source protein concentration, reaction time, temperature and pH. Reactions in the presence of selective secretory and intracellular PLA_2 inhibitors indicate most of the activity in the cell lines is due to cellular PLA_2s. The cloned gene encodes a 202-amino acid protein that includes a signal peptide, a calcium-binding domain, and a catalytic site. Phylogenetic analysis shows it is a new PLA_2 that clusters into the secretory PLA_2s. Because PLA_2 is the first step in eicosanoid biosynthesis, we infer that PLA_2s are crucial elements in insect/pathogen relationships.

10:00–10:30 am Coffee Break

WEDNESDAY, 10:30 am – 12:30 pm

Theater

Virus Division #3

Moderators: Katsuhiko Ito, Ikbal Agah Ince

Ascovirus P64 homologs: A novel family of large cationic proteins that condense viral genomic DNA for encapsidation **10:30** **•142•**

Dennis K. Bideshi[1, 2], *Tatsinda Spears*[3], *Heba Zaghloul*[4], *Yeping Tan*[1], *Yves Bigot*[5], *Brian A. Federici*[1,3,4]

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Most eukaryotic dsDNA viruses use small virus or host protamine-like proteins or histones to condense their genomic DNAs (gDNAs) during virogenesis. These arginine- and lysine-rich proteins are highly basic and typically less than ~20 kDa. Ascoviruses are large dsDNA (~125-186 kbp) viruses that cause a chronic and fatal disease in lepidopteran larvae. At present, little is known about the molecular basis for condensation and encapsidation of their large gDNAs. Previous proteomic and functional studies showed that Spodoptera frugiperda ascovirus (SfAV-1a) virions lack small cationic proteins, but instead contain a relatively large novel virus-coded DNA-binding protein (P64; 64 kDa, pI = 12.2) proposed to condense its gDNA during virion maturation. The N-terminal domain of P64 contains four virus-specific 2-cysteine adaptor motifs, whereas the C-terminal domain has 14 modular repeats rich in arginine and serine that potentially mediate gDNA sequestration. In this study, we used centrifugation, electrophoretic mobility shift assays and transmission electron microscopy to demonstrate P64's basic C-terminal domain condenses SfAV-1a gDNA. We further show that P64 homologs occur in other ascoviruses. Collectively, our data indicate P64 homologs are a novel family of viral gDNA condensing proteins.

Transcriptome analysis of the Spodoptera frugiperda ascovirus *in vivo* provides insights into how its apoptosis inhibitors and caspase promote increased synthesis of viral vesicles and virion progeny **STU** **10:45** **•143•**

Heba Zaghloul[1 4], *Robert Hice*[1], *Peter Arensburger*[3], *Brian A. Federici*[1 2]

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Ascoviruses are ds DNA insect viruses that primarily attack caterpillars of the family Noctuidae. Ascovirus virions are large (400 x 150 nm) and typically transmitted on the ovipositor of endoparasitic wasps during egg laying. However, the most unique characteristic of ascoviruses is their novel cytopathology, whereby after infection the nucleus lyses, after which the cell cleaves into a cluster of vesicles in which virions continue to assemble after the vesicles move from infected tissues into the hemolymph. A virus-encoded caspase induces this unique cytopathology, but other genes involved in pathogenesis and viral vesicle formation remain unknown. Suitable cell lines that reproduce typical ascovirus vesicles *in vitro* have not been developed. Thus, we used strand-specific RNA-Seq analysis to study transcription of the type species, *Spodoptera frugiperda* ascovirus-1a (SfAV-1a), *in vivo* during infection of *S. frugiperda* 3rd instars. We focused on 44 core ascovirus genes and 26 others putatively involved with viral vesicle membrane formation. Differential expression patterns revealed three temporal classes, namely, early, late and very late. Three genes that inhibit apoptosis (IAP-like) were expressed early, whereas the caspase gene was expressed very late. This correlates well with apoptotic events associated with viral vesicle formation. A Diedel-homolog gene, the only known virokine, was also highly expressed, implying this protein may help SfAV-1a evade its host's innate immune system. Interestingly, 15 bi-and poly-cistronic messages were also identified in the SfAV-1a transcriptome, indicating possible usage of non-canonical translation mechanisms by ascoviruses.

Expression profile of *nsd-2* gene encoding the putative Bombyx mori bidensovirus receptor after virus infection **11:00** **•144•**

Katsuhiko Ito[1], *Takeshi Fijii*[1], *Takeshi Yokoyama*[1],*Keiko Kadono-Okuda*[2]

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Bombyx mori bidensovirus (BmBDV) is a pathogen that replicates only in the midgut columnar cells and causes fatal disease in the silkworm. The resistance to BmBDV is determined by a single gene, *nsd-2* (resistance gene), which is charac-terized as non-susceptibility irrespective of the viral dose.

Previously we have identified *nsd-2* by positional cloning using *B. mori* genome information and found that this gene encodes a putative amino acid transporter which may work as a receptor for BmBDV. In this study, to better understand the relationship between BmBDV and the putative virus receptor, we performed an expression analysis of viral transcripts and receptor, $+^{nsd-2}$ (susceptibility gene), in the midgut after virus infection. We first fed an artificial diet smeared with a BmBDV suspension to newly ecdysed fourth instar larvae (susceptible strain for BmBDV), and investi-gated the expression pattern of the BmBDV-derived transcript in the midgut every day. The quantitative RT-PCR analysis showed the unique expression pattern; the expression of the viral transcript increased soon after viral infection and then decreased until the end of the fourth instar, and then the viral transcript increased again after becoming the fifth instar. Next, we compared the expression pattern of $+^{nsd-2}$ between the midgut of the virus-infected and uninfected silkworm. The qRT-PCR analysis revealed that the expression levels of $+^{nsd-2}$ drastically decreased from the fifth instar in the midgut of the virus-infected larvae. On the other hand, there was no change of the expression levels of $+^{nsd-2}$ in the midgut of the virus-uninfected larvae. Additionally, we investigated the expression patterns of other genes whose expression in the midgut have been already reported, but similar differences were not detected. These results suggested that BmBDV might specifically regulate the expression level of the virus receptor, $+^{nsd-2}$, during infection. We believe that a study of the relationship between virus and receptor will contribute to elucidation of the BmBDV infection mechanism.

The transcription strategy of Bombyx mori Bidensovirus and a characterization of the viral structural proteins **11:15** **•145•**

***Qian Yu**, Peng Lü, Yali Xing, Qin Yao*

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Bombyx mori bidensiovirus (BmBDV) is a species of Bidensovirus that has been was placed into a new genus within the new family *Bidnaviridae* by the International Committee on Taxonomy of Viruses. BmBDV causes fatal flacherie disease in silkworms, which causes large losses to the sericulture industry. BmBDV contains two sets of complementary linear single-stranded DNAs of approximately 6.5 kb (viral DNA 1, VD1) and 6.0 kb (viral DNA 2, VD2). VD1 and VD2 are encapsidated in separate icosahedral non-enveloped capsids, which are similar in size and shape. However, the complete mRNA procession profile remains unclear. In this work, we investigated the expression strategies of BmBDV non-structural proteins and structural proteins. Total RNA was obtained from the midgut of infected silk worm at 5^{th} instar. RACE methods were used to identify the 5′ and 3′ transcription ends. Meanwhile, a total of six structural proteins were separated by two-dimensional electrophoresis and shown to be encoded by the BmBDV VP gene via mass spectrometry. The transmission electron microscopy results showed that co-expression of the BmBDV VP and SP structural proteins in *Spodoptera frugiperda* sf9 cells resulted in the formation of 22–24 nm virus-like particles. Furthermore, a mutation of the major structural protein-encoding VP gene, in which the second in-frame ATG codon was mutated to GCG, abrogated the production of several structural proteins, indicating that this strategy of expressing BmBDV VP is dependent on a leaky scanning translation mechanism.

Cloning and rescue of the genome of Bombyx mori bidensovirus and characterization of a recombinant virus **STU** **11:30** **•146•**

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Bombyx mori bidensovirus (BmBDV) is the first segmented ssDNA virus identified in insect, which was classified into the *Bidnaviridae* family by International Committee on Taxonomy of Viruses (ICTV) in 2012. In this study, we constructed a full-length genomic clone of BmBDV which contains the complete terminal structure. A recombinant BmBDV that expressed the green fluorescence protein (GFP) gene was constructed. Then, the insect cell xx was transfected with the linearized genome using continuous culture and expanded cell culture methods. Western blotting analysis detected the expression of the major structural protein, VP, and green fluorescence was observed in infected cells. Furthermore, re-infected silkworm larvae showed typical densonucleosis symptoms, and more importantly, the direct visualization of green fluorescence allowed us to detect recombinant plasmids that were expressed in the silkworm midgut. These results suggest that infectious virus particles have been rescued from the infected BmN cells after co-transfection of the linear genome. We also found that BmBDV cannot transform mammalian cells, which means that BmBDV is safe to mammals. Overall, this study showed BmBDV has the potential to be used as biological pesticides, and also provided a platform for researching viral replication mechanisms, viral gene functions, and viral infection mechanisms.

Study the interaction between Bombyx mori bidensovirus structural protein and the host protein $+^{nsd-2}$ **STU** **11:45** **•147•**

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Bombyx mori bidensovirus is major pathogen that causes chronic densonucleosis disease of silk worm. In 2012, the International Committee on the Taxonomy of Viruses (ICTV) established a new family, *Bidnaviridae*, and designated BmBDV as the type species in the new genus *Bidensovirus.* VP as the major structural protein of BmBDV could decide the host specificity by structural protein interaction with the host receptor. The minor capsid protein P133 closely related to host recognition and the virus into the host. The lacking of the $+^{nsd-2}$ gene encoded trans-membrane protein could make the silkworm resistance from BmBDV infection, showing the $+^{nsd-2}$ gene may contain the recognition site of BmBDV. However, the mechanisms of the interaction between BmBDV and membrane protein are still unclear. In this study, a BmN cell strain stably expressing the host protein $+^{nsd-2}$ has been successfully established. The expression plasmids pIB-HA-VP and pIB-FLAG-P133 were then transfected into the BmN($+^{nsd-2}$) cell strain, respectively. The interaction between BmBDV structural protein and the host protein $+^{nsd-2}$ was analyzed by Co-IP and pull-down technics. Co-IP results revealed that structural protein VP of BmBDV could interact with the host $+^{nsd-2}$ protein. Morevoer, these results reveal a proof of concept for the infecting mechanism of the host cell by BmBDV at the molecular level.

Heliothis zea Nudivirus-1 miRNAs promote latent infection via epigenetic regulation **STU** **12:00** **•148•**

***Pei-Chi Wu**[1], Yueh-Lung Wu[1]*

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Heliothis zea nudivirus-1 (*Hz*NV-1) is a7n unique insect virus that can induce both lytic and latent infections in many insect cell lines. During latent infection, the persistency-associated gene 1 (*pag*1) expresses several microRNAs (miRNAs) and is the only detectable *Hz*NV-1 gene. Two of the miRNAs derived from *pag1* have been found to suppress and silence *Hind*III-I 1 (*hhi1*), an early gene of *Hz*NV-1. *Hhi1* induces strong apoptosis and increases the lytic to latent ratio. *Pag1* reduces the apoptosis caused by *hhi1* and further promotes the establishment of latent infections. Previous reports have shown that persistent infection of herpes simplex virus (HSV) is highly associated with epigenetic regulation of the chromatin structure and is regulated by latent-specific miRNAs. Since *pag1* is a non-coding transcript, it could regulate host chromatin structures through miRNAs to further establish latent infections. In this study, we found that histone modification associated enzymes changed upon *pag1* transfection. We then tested the histone acetylation/methylation protein expression levels through Chromatin Immunoprecipitation (ChIP) assay to investigate the histone modifications of insect hosts. This study not only provides a better understanding of the *Hz*NV-1 infection mechanism, but also the relationship between viral miRNAs and their hosts.

Functional analysis of non-polyadenylated invertebrate iridovirus mRNAs: The role of a CAUUA-containing hairpin **12:15** **•149•**

Ikbal Agah Ince[1]*, Zeynep Kanlıdere*[1]*, Ayca Zeynep İlter Akülke*[1]*, Arzu Özgen*[1,2]

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It is observed that the transcripts of *Invertebrate iridescent virus 6* (IIV6) are not polyadenylated, in line with the absence of canonical polyA motifs (AATAAA) downstream of the open reading frames (ORFs) in the genome. Previously, we used ligation-based amplification of cDNA ends (LACE) to determine the 3' ends of IIV6 the transcripts of the virion protein genes from infected Drosophila Schneider 2 (S2) cells. Many of the transcripts ended directly after a CAUUA motif (CATTA in cDNA). *In silico* analysis showed that the complementary motifs CATTA and TAATG were strongly enriched in the 100 base pair regions downstream of the IIV6 ORFs in comparison to their presence in the whole viral genome. When we have investigated the hairpin formation at 3'end of individual ORFs *in silico* among the related species suggests that a 3' hairpin sequence, often with a CAUUA motif, is a conserved feature in invertebrate-infecting iridoviruses (IIV3, IIV 9 and IIV31). The CAUUA motif as part of the stem-loop structure is likely to determine the pre-mRNA cleavage, or alternatively the direct termination point. We hypothesized that this motif may play a role in transcript 3'end formation. For the functional analysis of the hairpin structure, we selected two CATTA containing virion protein genes, 274L and 261R, to unravel the role of the conserved CAUUA motif in 3' hairpin structure formation using site directed mutagenesis. Each 3' UTR region is sub-cloned into pIZ vector into the downstream region of firefly luciferase gene as a phusion driven by Opie2 promotor. Single or double mutations introduced to CATTA motif on the 3' UTRs. We have examined whether these mutations on the CATTA motif affects viral 3' end formation or not.

Roosevelt Room

Microbial Control Division #3

Moderator: Trevor Jackson, Stefan Jaronski

Global patterns of field-evolved resistance to Bt crops:Successes and failures **10:30** **•150•**

Bruce E. Tabashnik [1]*, Jeffrey A. Fabrick*[2]*, Yves Carrière* [1]

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From 1996 to 2016, farmers worldwide planted a cumulative total of >830 million hectares of genetically engineered corn, cotton, and soybean that produce insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt). These Bt crops kill some key insect pests, yet are not toxic to most other organisms including people. Bt crops can suppress pests, reduce insecticide sprays, and increase farmer profits. However, their benefits are diminished or lost when pests evolve resistance. Here we review data monitoring resistance to nine Bt proteins in 15 major pest species targeted by Bt crops on six continents. We report a surge since 2005 in cases of resistance with practical consequences for pest management in the field. This surge in practical resistance is associated with cross-resistance, increased cumulative exposure to Bt crops, and increased monitoring. In addition, practical resistance to Bt crops is associated with non-recessive inheritance of resistance and a scarcity of refuges of host plants that do not produce Bt proteins. To maximize the benefits of Bt crops, we encourage implementation of large refuges of non-Bt host plants together with alternative control tactics, particularly when inheritance of resistance is not recessive and alleles conferring resistance are not rare.

Discovery of new insecticidal traits **10:45** **•151•**

Rebekah Kelly*, Vadim Beilinson, Mary Kroner and AgBiome team*

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Insect pests are a serious threat to agricultural production, and insecticidal proteins are among the most promising tools for limiting the damage they cause. Since the commercialization of biotech crops in 1994, however, only a small number of insecticidal proteins have made it to market. Moreover, emerging pest resistance to the current products points to a pressing need for new and unique modes of action. AgBiome is working to fill that need by discovering and screening plant-associated microbes to provide new solutions for insects, nematodes, and disease. We have built a large and diverse proprietary strain collection of over 40,000 fully sequenced microbial isolates. These microbial isolates are the foundation of our Biological and Trait discovery programs. Using our innovative microbial-capture and gene-identification technology, we have discovered more than 3,000 potential insecticidal genes to test in our high-throughput Lepidopteran, Coleopteran, and Hemipteran assays. Currently, we have identified over 20 active genes that are being evaluated for new modes of action and efficacy in plants.

Exploiting mosquito biology with transgenic *Metarhizium pingshaense* **STU** **11:00** **•152•**

Brian Lovett[1]*, Etienne Bilgo*[2]*, Abdoulaye Diabate*[2]*, Raymond St. Leger*[1]

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We compared arthropod genes encoding insect-specific sodium, potassium and calcium channel blockers for their ability to improve the efficacy of *Metarhizium* against insecticide-resistant anophelines. Some of our most potent strains, engineered to express multiple toxins, are able to kill mosquitoes with a single spore. A strain expressing the EPA approved Hybrid (Ca++/K+ channel blocker), was studied for efficacy in a MosquitoSphere in a malaria endemic region of Burkina Faso. Compared to the wild-type fungus, Met-Hybrid killed anopheline mosquitoes in half the time and at much lower spore doses in semi-field conditions, which increased the percent of lethally infected mosquitoes and the effective persistence of the pathogen. In the MosquitoSphere, we can reliably use males to deliver a fatal dose of transgenic fungus to female mosquitoes during mating: revealing an avenue of application that would easily integrate with established vector control techniques. Importantly, Met-Hybrid had an outsized reduction on blood feeding in infected mosquitoes compared to wild-type fungus. These lethal and pre-lethal effects of Met-Hybrid prevent >90% of disease transmission in just 5 days, surpassing the WHO threshold for successful vector control agents. This NIH funded, international effort represents an important step in the progression of transgenic mosquito control technologies into field application.

Functional response of the predator *Chrysoperla carnea* (Stephens) in an endophytic entomopathogenic fungus aphid management system **STU** **11:15** **•153•**

Natalia González-Mas, *María Cuenca-Medina, Enrique Quesada-Moraga*

Departamento de Ciencias y Recursos Agricolas y Forestales, Universidad de Córdoba

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The recently discover endophytic behavior of entomopathogenic fungi (EF) have led to complementary uses in horticulture complementary to the well-developed spray applications of commercially available mycoinsecticides. Fungal suspension spraying, seed dressing and even soil drenching with EF conidia may lead to endophytic colonization, whereas the possible effect of such endophytic colonization targeting the pest on the trophic cascades remains unknown. The trophic cascade effects of endophytic colonization of melon leaves targeting *Aphis gossypii* on attraction, settling and predatory capacity of the generalist predator *Chrysoperla carnea* have been investigated by offering *Beauveria bassiana* challenged aphids, either by direct spray with a fungal suspension or by feeding on endophytically colonized melon plants. Results shown that the proportion of *Chrysoperla carnea* larvae that prefer fungal colonized plants (70%) was higher when

compared with control (30%) three hours after the preys were offered (χ^2=0.025). However no differences were observed in the average number of herbivorous prey eaten by the predators (P<0.05).Our results demonstrate that fungal colonization has an impact on natural enemy's behavior attracting them to entomopathogenic fungal colonized plants which could improve their efficacy when these two techniques of biological control are applied simultaneously. Aphid control strategies based on the use of endophytically colonized plants are apparently safe for predators, whereas further research is needed to evaluate the overall success of IPM strategies based on the simultaneous use of these biocontrol agents.

Potential of entomopathogenic nematodes Rhabditida: Steinernematidae, *Steinernema carpocapsae* and Heterorhabditidae, *Heterorhabditis bacteriophora* for control of *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) in Georgia **11:30** **•154•**

Nona Mikaia

Tbilisi, Georgia; Sokhumi State University Department of Natural Sciences and Health Care

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Planococcus citri, the citrus mealybug, is the most important species of mealybug known to infest citrus in West Georgia. Various laboratory bioassays were conducted to determine the potential of entomopathogenic nematodes to control *P. citri.* Adult female *P. citri* were screened for susceptibility to two introduced from Germany nematode species. *Planococcus citri* was found to be most susceptible to *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, causing 92% and 71% mortality, respectively. The development of nematodes after infecting adult female *P. citri* showed both *H. bacteriophora* and *S. carpocapsae* were able to complete their life cycles inside the host. Further bioassays illustrated a linear relationship between mealybug mortality and the concentration of nematodes applied, with the highest level of control using a concentration of 150 infective juveniles (IJs)/insect. As nematodes would be used as an above-ground application to control *P. citri* in citrus orchards, available water is a major limiting factor. Insecticidal activity proved to be dependent on the available surface moisture after nematode application. The water activity (aw) bioassay indicated that *S. carpocapsae* to be two times more tolerant to lower levels of free water, compared to *H. bacteriophora.* After application, nematodes have a limited time frame in which to locate and infect hosts, as the level of available free water gradually decreases, as trees dry out. *Steinernema carpocapsae* proved able to locate and infect *P. citri* quicker than *H. bacteriophora.* Nematode activity was not significantly affected when exposed to 20°C and 25°C. IJs were able to infect *P. citri* at an exposure time as short as half an hour. Results also showed that the first 2 to 3 h application is the most decisive time for establishing successful infection of mealybugs by potential use of nematodes for the control of *P. citri.*

A physiological time model to predict the virulence of entomopathogenic fungi under conditions of fluctuating temperature **STU** **11:45** **•155•**

Declan Perry*, Dave Chandler*

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Temperature determines the speed of kill of entomopathogenic fungi (EPF) used as biopesticides. Unfortunately, little research has been done to understand and predict how biopesticide efficacy is affected by the varying temperature conditions that are typical of many field crop environments, and this is preventing us making best use of fungal biopesticides in IPM. Moreover, because candidate EPF strains are often selected in screens done at only one temperature, we may also be failing to identify 'winning' strains that are best adapted to variable field conditions. As part of a wider project to investigate EPF for the control of the diamondback moth (*Plutella xylostella* L.) (DBM), we have investigated thermal- and physiological-time models to understand and predict EPF virulence under varying temperatures. A number of non-linear statistical models used to describe biological processes in ectotherms were fitted to data on the effect of temperature on: (i) DBM larval development rate; (ii) growth and germination of different EPF strains pathogenic to DBM larvae; and (iii) fungal virulence to DBM larvae (measured as speed of kill and lethal dose). Of these, we found that a particular model described by Briere (1999) gave the best fit overall and allowed the cardinal temperatures to be identified with greater precision than before now. A plot of the relationship between the optimum temperature for virulence vs. the optimum for germination was not significantly different from zero (linear regression, P = 0.13). Fungal isolates differed in their thermal profiles for growth, germination and virulence, and we were able to use the model to select EPF strains whose thermal virulence 'profile' closely matched that for DBM development. Next, a day-degree model was developed, using mortality data at fixed temperatures, to predict DBM mortality at various variable temperature regimes. A simple DD model based on a sigmoidal equation was accurate in predicting mortality during a variable temperature regime over seven days. These results help to understand and address the sources of variability when taking EPF from the lab to field and should be a valuable tool for biopesticide development.

Plant cell-wall degrading enzymes improve endophytic establishment of *Metarhizium brunneum* in potato plants **STU** **12:00** **•156•**

Vivien Krell[1]*, Desireé Jakobs-Schoenwandt*[1]*, Stefan Vidal*[2]*, Anant V. Patel*[1]

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Biocontrol with endophytic entomopathogenic fungi is a promising option to protect plants systemically from insect herbivores. Yet, current applications are limited especially by low plant colonization. Inspired by penetration mechanisms of plant pathogenic fungi, we aimed at enhancing potato plant penetration and colonization by the endophytic entomopathogenic *Metarhizium brunneum* strain Cb15 through supplementation of plant cell-wall degrading enzymes pectinase and cellulase or corresponding substrates into beads containing mycelial biomass. We found that after bead application to potato tubers and incubation at 18-23 °C with a LD cycle of 16:8 for 21 days, *M. brunneum* was re-isolated from surface-sterilized roots, tubers, and shoots in 75.0 ± 9.4 %, 33.3 ± 9.8 %, and 29.2 ± 15.1 % of samples, respectively. Additional verification of re-isolated *M. brunneum* was conducted with qPCR. Pectinolytic and cellulolytic enzymes were successfully induced by addition of corresponding substrates, but activity levels were low and no correlation between enzymatic activity and fungal penetration was found. However, incorporation of cellulase into beads led to a substantial increase in plant penetration by 25.0 % in roots, 54.2 % in tubers, and 16.6 % in shoots. This was accompanied by a 3.0-fold enhanced spore formation on the surface of beads to 1.91 x 10^8 ± 0.26 x 10^8 per bead. Finally, a stronger root development of treated plants was observed indicating a fertilizing effect mediated by the formulation. This study will pave the way to understanding and eventually use of enzymes as penetration aid for endophytes.

Biological control of ambrosia beetles that vector laurel wilt fungus in avocado using entomopathogenic fungi **12:15** **•157•**

Pasco B. Avery[1]*, Daniel Carrillo*[2]*, Rita H. Duncan*[1]*, Alison Lukowsky*[2]*, Verónica B. Fuentes*[1, 3]*, Cecilia Gámez*[1, 3]*, Ronald D. Cave*[1]

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Laurel wilt (LW) is a disease threatening the avocado industry in Florida. The causative agent of the disease is a fungus vectored by ambrosia beetles that bore into the trees. Until recently, management strategies for the vectors of the laurel wilt fungus relied solely on chemical control and sanitation practices. Beneficial entomopathogenic fungi (EPF) are the most common and prevalent natural enemies of pathogen vectors. Laboratory experiments demonstrated that commercial strains of EPF can increase mortality of the

primary vector of the pathogen, *Xyleborus glabratus*, and alternative vectors, *Xylosandrus crassiusculus, Xyleborus volvulus, and X. bispinatus* (Coleoptera: Curculionidae: Scolytinae). Field experiments revealed that EPF do not prevent beetles from boring into the trees. However, EPF-infected beetles die inside the trees without reproducing, providing control similar to that by chemical insecticides but persisting longer on the bark of avocado trees. To improve efficacy, EPF must be integrated into the management strategy for LW and other avocado pests, which includes the use of several fungicides, insecticides, and adjuvants. We determined antagonistic and synergistic relationships among EPF and agrochemicals used by avocado growers. Several producers have incorporated this biocontrol strategy to manage ambrosia beetles and other pests of avocado. The main limitation of EPF is they do not control ambrosia beetles already breeding inside trees. The search for improved delivery systems that could increase efficacy of EPF will be discussed.

WEDNESDAY, 12:30 – 1:30 pm
LUNCH BREAK

Roosevelt Room

Nematode Division – Business Meeting

The Forum

Student Workshop on Science Communication

The road to more effective science communication **12:30** **•158•**

Bruce Lieberman

[1] Bruce Lieberman, Science Writer & Editor

Presenter: bruce.lieberman@yahoo.com; www.blieberman.com

In this workshop, Bruce Lieberman will review how scientists can better communicate the significance, the details, and the impact of their work to a lay audience. He'll share examples of clear and concise science writing, by scientists and journalists alike, and offer tips on how researchers can make their work more understandable and compelling to anyone. Bruce will also discuss how scientists can effectively interact with media professionals, whether reaching out to news organizations through their institution's public affairs office or calling a reporter or editor directly. He'll also cover some of the changes underway in the media business, how these changes impact a scientist's ability to share their work with the public, and how a scientist can reach out directly to people through social media. As part of the workshop, Bruce will conduct a short exercise during which a few volunteers will be asked to describe what they're working on now; the group will then review these brief presentations. Finally, all attendees will leave the workshop with handouts on what's been covered, including practical tips they can take with them, written examples of great science writing, and web links to other valuable resources on science communication.

Bruce Lieberman is a freelance science writer and editor with more than 25 years of experience in the news business. He worked as a reporter at daily newspapers for many years before becoming an independent writer and editor in 2010. Bruce has written about astronomy, climate change, and biology for national publications and online. He has also worked on science writing projects for universities, biomedical labs, and foundations. As an editor, Bruce has worked with state and regional government agencies throughout California, and he has consulted with them to improve written communications. Bruce earned his Master's Degree at Columbia University's Graduate School of Journalism in 1998, and his Bachelor's Degree in Rhetoric from the University of California at Berkeley in 1988.

WEDNESDAY, 1:30 – 3:30 pm

Theater

Virus Division #4

Moderators: Monique van Oers, Johannes Jehle

SIP EARLY CAREER AWARD WINNER

Insane in the brain: how baculoviruses manipulate brain function and behavior in caterpillars **1:30** **•159•**

*Yue Han[1], Hanneke Suijkerbuijk[1,2], Hans M. Smid[2], Monique M. van Oers[1], **Vera I.D. Ros**[1]*

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Modification of host behaviour is a widely adopted strategy of viruses to enhance their own transmission. The examples of behavioural manipulation are rapidly accumulating, covering a broad spectrum of viruses and hosts. Nevertheless, surprisingly little is known on the underlying causative physiological, neuronal, hormonal or molecular mechanisms. A typical case of behavioural manipulation is found in insects infected with baculoviruses. Infected caterpillars show enhanced mobility and climb to the top of plants or the forest canopy prior to death, a phenomenon known as 'tree-top disease'. As a consequence, the virus is spread over a larger area, thereby increasing the chance to infect a new caterpillar. The baculovirus-insect system provides an excellent platform to study viral manipulation of insect host behaviour. It allows the comparative analysis between wildtype viruses and single gene knock-out mutants. I will review the current knowledge on the mechanism of baculovirus-induced behavioural manipulation, including an overview of viral genes found to modify host behaviour and how these may affect host pathways**.** I will show data on virus spread in the larval brain and discuss how the virus might manipulate host behaviour by affecting the host central nervous system.

MAURO MARTIGNONI AWARD WINNER

Timely trigger of zombie behaviour: the importance of light exposure timing in baculovirus-induced tree-top disease **STU** **2:00** **•160•**

***Yue Han**[1], Stineke van Houte[1,2], Monique M. van Oers[1], Vera I.D. Ros[1]*

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Host behavioural manipulation is a common strategy used by parasites to enhance their survival and/or transmission. Baculoviruses induce hyper-activity and tree-top disease (pre-death climbing behaviour) in their caterpillar hosts. However, little is known about the underlying mechanisms of this behavioural manipulation. Previously, we showed that the baculo-virus *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) induced tree-top disease at 3 days post infection in third instar *S. exigua* larvae and that light from above plays a key role in triggering this behaviour. Here we further investigated the temporal requirements for the presence of light to trigger this behaviour and found that light from above was needed between 43-50 hours post infection to induce tree-top disease. Infected larvae that were not exposed to light from above in this period finally died at low positions. Exposure of larvae to light prior to this period did not affect the final positions where they died. We hypothesize that virus achieve this via invading host central nervous system. Currently, we are comparing transcriptomes from larval heads between manipulated and non-manipu-lated groups to identify host/viral molecules that potentially are involved in this process. Overall we conclude that light in a particular time frame is needed to trigger SeMNPV-induced tree-top disease in *S. exigua* larvae.

The baculovirus *per os* infectivity factors form a complex to resist proteolytic degradation by larva-derived alkaline proteases **STU 2:15 •161•**

Bob Boogaard[1], *Jan W.M. van Lent*[1], *David A. Theilmann*[2], *Martin A. Erlandson*[3], *Monique M. van Oers*[1]

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Baculoviruses orally infect caterpillars in the form of occlusion-derived viruses (ODVs). The ODV envelope contains a number of proteins essential for oral infectivity, called *per os* infectivity factors (PIFs). PIF1, PIF2, PIF3 and PIF4 form a stable complex. The P74 protein (PIF0) associates loosely with this complex, but is not needed for its formation in contrast to the other constituents. We show here that PIF-proteins are prone to proteolytic degradation when they are not part of an intact complex. When wildtype ODVs released from larva-derived occlusion bodies (OBs) were analysed, both the complex and PIF monomers were found. However, in ODVs from a *p74*-deletion mutant, only the complex was found and no PIF monomers. But when these ODVs were released from OBs isolated from cultured insect cells or heat-treated larva-derived OBs, the PIF monomers were found again, suggesting that larva-derived alkaline proteases degraded the monomers. Disappearance of PIF monomers was also observed in larva-derived *pif2*-, *pif3*-, *pif4*- and *pif6*-deletion mutants. The PIF monomers were found again in these mutants when the OBs were heat-treated prior to ODV release, just as previously observed. So baculoviruses might protect their PIFs against insect-derived alkaline proteases by complex formation, to safeguard oral infectivity.

Silencing chitin deacetylase, insect intestinal mucin and response to pathogen genes in *Spodoptera littoralis* (Lepidoptera:Noctuidae): Increases and decreases in the biological activity of baculoviruses **2:30 •162•**

Umut Toprak[1,2], *Serife Bayram*[1], *Dwayne Hegedus*[2]

[1]Ankara University, Molecular Entomology Lab. Faculty of Agriculture, Dept. of Plant Protection, Ankara, Turkey; [2]Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK Canada
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Baculoviruses are invertebrate-specific viruses with great potential for use as a biopesticide. Upon ingestion of the occlusion bodies by an insect, they dissolve and virions are released in the midgut. In order of virions to initiate an infection in midgut epithelial cells, they must first pass the peritrophic matrix (PM), an inner porous tube serving as a barrier against pathogen invasion within the midgut. PM is composed of chitin microfibrils and multiple proteins. Previous studies showed that several proteins such as chitin deacetylases (CDAs), insect intestinal mucins (IIMs) and response to pathogen (REPAT) interfered with the baculovirus infections. We recently showed that various CDAs, IIMs and REPATs are tightly associated with the PM in *Spodoptera littoralis* (Lepidoptera: Noctuidae), a destructive pest of vegetables and field crops in Mediterranean Region Countries. In order to understand the potential role of these proteins in the PM, we attempted to silence genes encoding a CDA (SpliCDA823), an IIM (SpliIIM3594) and a REPAT (SpliREPAT872) and then checked the status of the baculovirus infections. Silencing of SpliIIM3594 induced the mortality, while silencing of SpliCDA823 reduced the mortality. No effect on the baculoviral activity was detected upon silencing the SpliREPAT872; however, this silencing was transient. This study showed that baculovirus infections interfere with insect mucins and chitin deacetylases by possibly disruption of the PM. *This study was supported by The Scientific and Technological Research Council of Turkey (Project #: 112O305).*

Dissecting the host cell endocytic trafficking pathway of baculovirus by single-virus tracking and live-cell imaging **STU 2:45 •163•**

Fujun Qin[1,2], *Congrui Xu*[1,2], *Shili Yang*[1,2], *Gaobo Zhang*[1,2], *Chengfeng Lei*[1], *Jia Hu*[1], *Hanzhong Wang*[1], *Xiulian Sun*[1]

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The Baculoviruses are enveloped DNA viruses that have been widely applied for insect control, eukaryotic expression, and gene therapy. Based on the use of limited inhibitors of endocytosis, previous study showed that the budded viruses (BVs) form of *Autographa californica multicapsid nucleopoly-hedrovirus* (AcMNPV) entered into insect cells mainly through clathrin-mediated uptake. However, the mechanism and the dynamics related to BV particles entering and trafficking through the endocytic network in the host cells remain unclear. In this study, in order to investigate the cell entry pathway, several Sf9 cell lines expressing distinct endocytic markers and recombinant baculoviruses with labeling tags were constructed. Single-particle virus tracking revealed that most of BVs (82%) randomly diffused on cell surface before being delivered to a preformed clathrin coated pit (CCP). After internalization into cytoplasm, BVs were primarily transported to Rab5-positive early endosomes, in which the nucleocapsids of BV particles (78%) were released into cytoplasm. Subsequently, a minority of viruses (22%) progressed to mature into intermediate endosomes and late endosomes, in which membrane fusion was also detected. Moreover, actin facilitated the processes of scission of CCP from the plasma membrane, and propelled clathrin coated vesicular (CCV) away from the membrane, whereas microtubules are required for the transport of virus-bearing endocytic carrier vesicle (ECV) to the perinuclear region. In summary, this is the first study that investigates the host cell entry process of baculovirus BVs at the single particle level in live cells, and describes the mechanistic and kinetic insights of the infection of BV. These findings will contribute to a better understanding of the infection pathway of baculoviruses.

Comparative study of fast-killing versus slow-killing SfAV isolates **STU 3:00 •164•**

Hiroki Ishii[1], *Eiko Arai*[1], *Ikbal Agah Ince*[2], *Xiao-Wen Cheng*[3], *Dennis Bideshi*[4,5], *Maki N. Inoue*[1], *Brian Federici*[4], *Madoka Nakai*[1]

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Ascoviruses (AVs) are double-stranded DNA viruses with circular genomes. They primarily attack lepidopteran larvae. AVs are transmitted by female parasitic wasps via oviposition and cause a chronic disease that terminates mortality. It is likely that a prolonged infectious period results in a higher rate of transmission by parasitoids that vector these viruses. However, we found that an atypical fast-killing AV (Spodoptera frugiperda ascovirus-X: SfAV-X) kills host rapidly after infection. The 50% survival time (ST_{50}) of SfAV-X infected 4th instar *Spodoptera litura* larvae was 2.6 dpi, while ST_{50} of the slow-killing AV (SfAV-F), which is an isolate closely related to SfAV-X, was 22.0 dpi. Our poster at SIP2016 showed that the genome replication speed of SfAV-X was significantly faster than that of SfAV-F. However, it showed SfAV-X infected larvae died as soon as genome replication plateaued (the interval was 0.9 dpi), while SfAV-F infected larvae died long-after SfAV-F genome replication plateaued (interval was 18.1 dpi). So the fast-killing phenotype results from genome replication speed and other factors. In this study, to identify other factors that cause the fast-killing phenotype, we compared tissue tropism and AV proteins between SfAV-X and SfAV-F. Firstly, we observed virions in tissues by Transmission Electron Microscopy (TEM). Because it is known that the tissue tropism of SfAV-1a, the slow-killing AV, is limited primarily to the fat body, we hypothesized that the fast-killing AV could infect other tissues resulting in a fast-killing infection. However, our observations showed SfAV-X only infected the fat body, so broader tissue tropism was not the cause of fast-killing. Secondly, we compared AV

hemolymph proteins of SfAV-X and SfAV-F after viral genome replication plateaued. This experiment identified 51 AV proteins in hemolymph of infected larvae, 7 of which were synthesized at a significantly higher rate in SfAV-X than in SfAV-F. Thus, our current hypothesis is that one or more of these 7 proteins are responsible for the faster killing speed of SfAV-X.

Microplitis bicoloratus bracovirus regulates NF-κB signaling pathway **3:15** **•165•**

Dong-Shuai Yu[1], Ya-Bin Chen[1]*, Ming Li[1]*, Ming-Jun Yang[2], Yang Yang, Jian-Sheng Hu,* ***Kai-Jun Luo***

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Polydnaviruses (PDVs) play a critical role in altering host gene expression to induce immunosuppression. However, PDV gene(s) to affect host genes remain largely unclear. Here, the whole-genome sequencing of Microplitis bicoloratus bracovirus (MbBV), which is known as an apoptosis inducer, was performed. The MbBV genome showed 17 putative double stranded DNA circles and 179 fragments with a total size of 336,336 bp encoding 116 open reading frames (ORFs). Based on conserved domains, 9 gene families were identified, in which IκB-like viral ankyrin (vank) family included 28 members and was one of the largest family. Among 116 ORFs, 13 MbBV genes were expressed in the MbBV-induced apoptosis hemocytes and further analyzed. Three vank genes (vank86, vank92, vank101) were expressed in hemocytes collected from Spodoptera litura larvae parasitized by M. bicoloratus, in which host NF-κB/IκBs including relish, dorsal, and cactus, were also persistently expressed. When Spli221 cells were infected with MbBV viral particles, mRNA levels of host and viral NF-κB/IκB genes were persistent and also varied in the virus-induced pre-apoptosis cell Spli221 from 1 to 5 hours post infection; then both presented a time-dependent expression manner in virus-induced apoptotic cells. Upon these analyses, we found that viral IκB-like transcription could not inhibit host NF-κB/IκB expression, suggesting that they might be able to regulate the different transcriptional mechanisms.

The Forum

Bacteria Division #4

Moderators: Ming Sun, Marianne Carey

Two mosquitocidal *Clostridium bifermentans* strains contain a Cry toxin and a *Clostridium* Neurotoxin-like loci **1:30** **•166•**

Estefania Contreras*, Swati Chawla, Nadia Qureshi, Jianwu Chen, Sarjeet Gill*

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Mosquito-borne diseases have been significantly impacted human civilization despite of centuries of intensive control effort. Diseases such as Dengue, Yellow fever, Chikungunya or Zika which are transmitted most commonly by an infected *Aedes* female mosquito, Filariasis and West Nile fever by *Culex*, and Malaria by *Anopheles* mosquito species, still remain serious public health problems with constant threats of re-emergence. *Bacillus thuringiensis israelensis* and *Lysinibacillus sphaericus* formulations have become the predominant non-chemical means for *Culex* and *Aedes* mosquito control. However, new biological insecticides with different mode of actions are needed in order to broaden susceptibility range and avoid mosquito resistance. In our work, we compared the toxicities of two *Clostridium bifermentans* mosquitocidal strains and *Bacillus thuringiensis israelensis* against *Aedes aegypti*, *Anopheles gambiae* and *Anopheles stephensi*. *Clostridium bifermentans malaysia* was the most toxic strain to *Anopheles gambiae*. In order to find its toxic components, we sequenced the genome of the two *C. bifermentans* mosquitocidal strains, a non mosquitocidal *C. bifermentans* wild type strain and a non toxic *C. bifermentans malaysia* mutant that we developed using gamma irradiation. Our sequencing data showed that *C. bifermentans* mosquitocidal strains contain a plasmid containing two toxin loci. The first one is composed by Cry16A, Cry17A and two hemolysins which are toxic as a complex to *Aedes aegypti*, but not to *Anopheles* mosquitoes. Interestingly, the second toxin locus has similarity to Clostridium Neurotoxin loci, which we renamed Ctox locus.

***Brevibacillus laterosporus* insecticidal toxin genes and their expression during pathogenesis** **1:45** **•167•**

Luca Ruiu[1]

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The endospore-former *Brevibacillus laterosporus*, morphologically featured by a typical canoe-shaped parasporal body, is an insect pathogen acting by ingestion through a toxin-mediated mechanism involving specific interactions with the host midgut epithelium. Genome sequencing of some *B. laterosporus* strains showing insecticidal activity against different targets, led to the identification of several putative toxins and virulence factors, including mosquitocidal toxins, vegetative insecticidal proteins, chitinases, and several polyketides and nonribosomal peptides. In addition, proteins of the spore coat canoe-shaped parasporal body complex (SC-CSPB) were shown to be involved in pathogenesis, thus representing additional virulence factors. Our recent genome sequencing and annotation of strain UNISS 18, a well documented pathogen of mosquitoes and flies, highlighted a variety of putative insecticidal toxin genes. Following a comparison with other *B. laterosporus* genomes, we conducted *in vitro* and *in vivo* gene expression and proteomic analyses that revealed which proteins may have a leading role in the pathogenic process. *This study was financially supported by Italian Ministry of Education, University and Research (MIUR) (PRIN project 2015 BIOPIC, 2015BABFCF).*

Identification of a genetic locus encoding a *Serratia entomophila* Penicillin-Binding Protein region associated to toxicity towards insect larvae **2:00** **•168•**

M. Eugenia Nuñez-Valdez[1], *Victor Cruz-López[1] Mauricio Díaz-Sánchez[1], Zitlhally Rodríguez-Segura[1], Jeanwu Chen[2], Sarjeet Gill[2]*

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The bacterium *S. entomophila* strain Mor4.1 (SeMor4.1; Enterobacteriaceae) is a entomopathogenic bacteria isolated in Mexico from larvae of the soil dwelling pest *Phyllophaga* spp (Coleoptera: Scarabaeidae). Insecticidal activity against larvae of *Phyllophaga* spp, *Anomala* sp and larvae of the lepidopteran *Manduca sexta* has been observed by injecting either, the bacteria or the cell free culture broths, suggesting that Mor4.1 secretes toxin-like factors with a wide spectrum of action at the level of the hemocoel. In order to identify SeMor4.1 toxic factors a SeMor4.1 genome library was prepared in *Escherichia coli*. Five insecticidal clones were selected by injection bio-assays. Further subcloning of the C8 clone render the clone E9 encoding an amino terminal region of a putative Penicillin-Binding Protein 1b (PBP) inserted in the pBluescriptSK+. Cell-free culture broth from E9 was evaluated (50µg protein/larva) by injection bio-assays in larvae of the Coleoptera *Phyllophaga blanchardi* and the Lepidoptera *Spodoptera frugiperda* and *Galleria mellonella*. Results showed that E9 caused 70%, 90% and 65% mortality after three days from the onset of bioassay in *P. blanchardi*, *S. frugiperda* and *G. melonella* larvae, respectively. No significant mortality was observed for the control larvae. No toxic activity was observed by oral inoculation. The characterization of SeMor4.1 toxins is important to determine their potential for the control of insect pests. *Acknowledgments: We thank CONACYT (CB-2014-243027) for supporting this work.*

A novel type six secretion system in the insect-pathogenic bacterium *Xenorhabdus bovienii* **2:15** **•169•**

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Xenorhabdus bovienii (XB) has a fascinating dual lifecycle- as a beneficial symbiont of nematodes and a potent pathogen of soil-dwelling insect larvae. *Steinernema* nematodes harbor XB in a specialized intestinal receptacle and vector XB into insects, releasing the bacteria into the insect body cavity, where they produce a variety of insecticidal toxins and antimicrobials. We recently identified a novel type six secretion system (T6SS) in the genome of XB. Because T6SSs allow bacteria to directly manipulate eukaryotic host cells or other bacteria, we speculate that the novel T6SS contributes to XB's interactions with its invertebrate partners or the normal flora of host insects. Using qRT-PCR, we found that the T6SS is highly expressed in XB in a medium mimicking insect hemolymph. To identify potential T6-secreted proteins, we generated a mutant XB strain lacking Hcp, a key structural component of the T6SS, and compared its secretion profile to that of wild type XB. Using 1D gel electrophoresis coupled with mass spectrometry, we identified several proteins in the wild type supernatant that are absent in the Δ*hcp* supernatant, including one with domains characteristic of T6-secreted effectors. We are currently exploring the functions of these proteins and the contribution of the novel T6SS to the complex XB lifecycle.

Influence of exogenous pathogens on the expression of *BmToll9-2* gene in larval *Bombyx mori* **2:30** **•170•**

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Aim: Toll-like receptors are important members in Toll signaling pathway in insects and play an important role in activating the innate immune response to invading pathogenic microorganisms. This study aims to explore the influence of exogenous pathogens on the expression of the Toll-like receptor gene *BmToll9*-2 in larvae of the silkworm, *Bombyx mori*. Methods: Exogenous pathogens lipopolysaccharide (LPS) and *Escherichia coli* were respectively injected into the body cavity of the 5th instar larvae to induce their immune response, and the expression levels of *BmToll9*-2 in the midgut, fat body and epidermis at different time after injection were detected by real-time quantitative PCR. Results: The real-time quantitative PCR results showed that injection of LPS could induce the expression of *BmToll9*-2 in the midgut of the 5th instar larvae, and the best induction effect was at 6 h after injection. Injection of *E. coli* could also induce the expression of *BmToll9*-2 in the midgut of the 5th instar larvae, and the best induction effect was at 3 and 6 h after injection. Injection of LPS and *E.coli* could also induce the expression of *BmToll9*-2 in the fat body and epidermis, and the best induction effect was at 24 h after injection. Conclusion: Expression of *BmToll9*-2 in *B. mori* larvae is up-regulated after injection of LPS and *E. coli*, suggesting that *BmToll9*-2 receptor may be involved in the recognition process of Toll-like receptors to exogenous pathogens such as LPS and *E. coli* in insects.

Symbionts in waiting: the dynamics of incipient endosymbiont complementation and replacement in minimal bacterial communities of psyllids **2:45** **•172•**

Jennifer L. Morrow, Aidan A.G. Hall, ***Markus Riegler***

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Obligate bacterial primary (P-) endosymbionts that are maternally inherited and codiverge with hosts are widespread across insect lineages with nutritionally restricted diets. Secondary (S-) endosymbionts are mostly facultative but in some hosts they complement P-endosymbiont function and therefore become obligate. Phylogenetic evidence exists for host switching and replacement of S-endosymbionts. The community dynamics that precede endosymbiont replacement and complementation have been little studied across host species, yet they are fundamental to the evolution of endosymbiosis. We performed bacterial 16S rRNA gene amplicon sequencing of 25 psyllid species (Hemiptera, Psylloidea) across different developmental stages and ecological niches by focusing on the characterisation of the bacteria other than the universally present P-endosymbiont *Carsonella* (Gammaproteobacteria). Most species harboured only one dominant representative of diverse gammaproteo-bacterial S-endosymbionts that was consistently detected across all host individuals and populations (*Arsenophonus* in eight species, *Sodalis* or *Sodalis*-like bacteria in four species, unclassified Enterobacteriaceae in eight species). The identity of this dominant obligate S-endosymbiont varied across closely related host species. Unexpectedly, five psyllid species had two or three co-occurring endosymbiont species other than *Carsonella* within all host individuals, including a *Rickettsiella*-like bacterium (Gammaproteobacteria) in one psyllid species. Based on standard and quantitative PCR, all psyllids carried *Carsonella*, at higher titres than their dominant S-endosymbionts. Some psyllids also had Alphaproteobacteria (*Lariskella*, *Rickettsia*, *Wolbachia*) at varying prevalence. Incidence of other bacteria, including known plant pathogens and potential entomopathogens was low. Ecological niche of gall-forming, lerp-forming and free-living psyllid species did not impact endosymbiont communities. Two flush-feeding psyllid species had population-specific differences, and this was attributable to the higher endosymbiont diversity in native ranges and the absence of some endosymbionts in invasive ranges. Our data support the hypothesis of strict vertical transmission of minimal core communities of bacteria in psyllids. We also found evidence for S-endosymbiont replacement across closely related psyllid species. Multiple dominant S-endosymbionts present in some host species, including at low titre, constitute potential examples of incipient endosymbiont complementation or replacement. Our multiple comparisons of deep-sequenced minimal insect bacterial communities exposed the dynamics involved in shaping insect endosymbiosis.

Invertebrate pathology in the absence of infection: The role of gut bacteria in mosquito development **3:00** **•173•**

Michael R. Strand*, Kerri L. Coon, Luca Valzania, David A. McKinney, Kevin J. Vogel, Mark R. Strand*

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Mosquitoes host communities of microbes in their digestive tract that consist primarily of bacteria. Several mosquito species including *Aedes aegypti* do not develop beyond the first instar in the absence of a gut microbiota. In contrast, several species of bacteria, including *Escherichia coli*, rescue development of axenic larvae into adults. The molecular mechanisms underlying bacteria-dependent growth in contrast are unknown. Here we designed a genetic screen around *E. coli* that identified high affinity cytochrome *bd* oxidase as an essential bacterial gene product for mosquito development. Bioassays showed that bacteria in non-sterile larvae and gnotobiotic larvae inoculated with wild-type *E. coli* reduced midgut oxygen levels below 5%, whereas larvae inoculated with *E. coli* mutants defective for cytochrome *bd* oxidase did not. Experiments further supported that hypoxia stimulates growth and ecdysone-induced molting. Altogether, our results identify aerobic respiration by bacteria as a previously unknown but essential process for mosquito development.

Roosevelt Room

Fungi Division #3

Moderators: Jae Su Kim, Richard Humber

Developing a strain improvement system for the entomopathogenic fungus *Beauveria bassiana:* a way to get better biocontrol agents? STU 1:30 •174•

***Laura Reyes**, Dave Chandler*

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Biocontrol agents (BCAs) based on entomopathogenic fungi (EPF) are playing an increasing in Integrated Pest Management programmes. At present, the commercially available EPF all consist of wild type strains isolated from nature, however, there is potential to breed more effective strains by recombining wild types with complementary characteristics. The aim of this research is to develop a system to improve strains of the entomopathogenic fungus Beauveria bassiana through genetic recombination. A group of 50 *Beauveria* strains were genotyped using multi-locus sequencing and mating gene analysis, and then phenotyped with respect to their virulence, thermal biology, tolerance of UV light, and spore production. Five strains were selected with complementary phenotypes and potentially compatible mating types for parasexual recombination studies. Spontaneously generated nitrate non-utilizing (nit) mutants have been produced from these strains using a potassium chlorate-amended selective medium and their vegetative compatibility is currently being investigated prior to parasexual recombination studies. Eight fungal strains have also been selected to investigate the potential for inducing sexual recombination by pairing complementary mating types on different media (oatmeal agar, malt extract agar, and Czapek Dox agar +/- biotin) known to induce ascospore formation in other anamorphic ascomycetes. Work is currently underway to develop parasexual and sexual recombination systems for selected strains with complementary phenotypes. The long-term aim is to produce recombinant strains that are more efficient as biocontrol agents.

Regulation of gene expression in *Beauveria bassiana* strain infecting *Riptortus pedestris*, bean bug STU 1:45

***Se Jin Lee**, Sihyeon Kim, Mi Rong Lee, Jae Su Kim**

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Beauveria bassiana (*Bb*), a major species of entomopathogenic fungi, has been known as highly virulent against a variety of insect pests. So far, many studies have focused on its insecticidal activity and virulence, but little consideration was given to the molecular mechanisms to figure out how it works in pathogenesis. Herein, to understand the transcriptional interaction between *Bb* and bean bug (*Riptortus pedestris*), the genomic DNA isolated from a *Bb* strain JEF-007 was subjected to whole genome sequencing using Pacbio sequencing, and transcriptomes of the infecting fungal strain and fungi-infected bean bug were analyzed. The genomic DNA of *Bb* JEF-007 was 36.5 Mb long and predicted to have 39 scaffolds and 10,857 genes. A total contigs of non-infected/infected bean bug and non-infecting/infecting fungal strain were 141,722 and 37,503, respectively. Differential expressed gene (DEG) analysis showed that 2,302 and 2,381 genes were up- and down-regulated respectively in the infecting *Bb* JEF-007 and virulence-related genes such as halogenase, cytoxic lectin, bacterial-like toxin and proteins related to cell wall formation were validated to be highly up-regulated. DEG analysis of the infected bean bugs showed that 3,588 and 3,296 genes were up- and down-regulated, respectively, and cuticle protein, cysteine-rich secreted protein and pigment-dispersing factor precursor were highly up-regulated possibly to survive against the fungal infection. This work can provide a deep insight on molecular mechanisms of infecting *Bb* and how the insect responds to the fungal infection in transcriptional level.

Alkane-grown *Beauveria bassiana*: Mycelial pellets formation, oxidative stress induction and cell surface alterations STU 2:00 •176•

***Carla Huarte-Bonnet**, Juan C. Ponce, Marianela Santana, Eduardo Prieto, Nicolás Pedrini*

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Entomopathogenic fungi attack insect hosts via attachment to the epicuticle, usually composed by aliphatic hydrocarbons. *B. bassiana* is able to grow on insect-like alkanes improving its virulence. In this study we described some physiological and molecular processes involved in fungal growth, nutritional stress response and cell surface alterations found in alkane-grown conidia. Fungi were observed by transmission electron microscopy (TEM) and atomic force microscopy (AFM), and their hydrophobicity was measured on the cell surface. Additionally, the expression pattern of several genes associated with oxidative stress, hydrophobicity and peroxisomal biogenesis was analyzed by qPCR. We found a novel type of growth in alkane-cultured *B. bassiana*, similar to mycelial pellets described in other filamentous fungi grown in alkane-free media. Pellets were able to germinate in media without a carbon source and produce viable conidia. TEM showed that pellets were formed by hyphae cumulates with an apparent surface thickening. Cell surface appeared to be more hydrophobic and exhibited different surface topographies as was observed by AFM. We also found a significant induction in several genes encoding for catalases, superoxide dismutases, hydrophobins and peroxins. Additional studies are being conducted to better understand the relationship between alkane growth adaptation and fungal cell changes, in order to improve the efficacy of fungal penetration through the cuticle and thus enhance virulence against insect pests.

Entomopathogenic fungal granules for biological control of *Protaetia brevitarsis seulensis* larvae STU 2:15 •177•

***Sihyeon Kim**, Se Jin Lee, Jong Cheol Kim, Mi Rong Lee, Jae Su Kim*

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Many turfgrass pests, such as *Ectinohoplia rufipes*, *Exomala orientalis* and *Popillia quadriguttata*, cause serious damage to grass fields in Korea. Given the residual side-effects of synthetic pesticides in turfgrass soil, more safe control agents, for example entomopathogenic fungi, need to be developed. In this study, *Protaetia brevitarsis seulensis* as an alternative insect was used to screen out highly virulent entomopathogenic fungi. In a virulence assay where the insect larvae were exposed to the fungal mass of nine genus of entomopathogenic fungi on cultured dishes, *Beauveria, Metacordyceps* and *Metarhizium* isolates showed high virulence against *P. brevitarsis seulensis*. One *Metathizium* isolate showed ca. 60% virulence 7 days after the exposure. Compared to the contact-exposure, the spray treatment of *Metathizium* isolate showed relatively lower virulence against the larvae. In addition, the selected isolate showed insect stage-dependent virulence in laboratory conditions; high virulence against eggs and 1st larvae but low virulence against 2nd and 3rd larvae. A semi-filed assay was conducted to investigate the potential of soil application of the selected isolate. These results provide a basic information for the control of *P. brevitarsis seulensis* using entomopathogenic fungi.

***Metarhizium brunneum* (Ascomycota: Hypocreales) treatments are safe for the generalist predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)** 2:30 •179•

***Inmaculada Garrido-Jurado**[1], Álex Ríos-Moreno[1], Enrique Quesada-Moraga[1]*

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Metarhizium sp. has shown great success in the control of insect pests as a biocontrol alternative to hazardous chemical pesticides. This genus can secrete secondary metabolites such as destruxin A, which information on their fate in the food chain and their risk to human and animal health is

scarce. In the present work, predator-prey bioassays were performed to evaluate the behavior and survival of *Chrysoperla carnea* (Stephens) larvae when feeding on larvae of the polyphagous pest *Spodoptera littoralis* challenged by *M. brunneum* BIPESCO5 and EAMa 01/58-Su strains. In addition ecotoxicological studies based on HPLC-MS were performed to monitor the fate of destruxin A in the prey-predator system. The maximum concentration of destruxin A produced by BIPESCO5 and EAMa 01/58-Su strains in infected *S. littoralis* larvae was approximately 0.014 and 0.031 µg/g, respectively, whereas the metabolite was not detected in *C. carnea* larvae consuming *M. brunneum*-challenged *S. littoralis* larvae. Furthermore, *C. carnea* larvae preferred consuming healthy prey versus *M. brunneum*-challenged prey as revealed by both the higher predator ratio feeding on *S. littoralis* control larvae and the higher *per capita* number of prey control larvae consumed by the predator compared to the *M. brunneum*-challenged larvae. Hence, the *M. brunneum* treatments against *S. littoralis* larvae were safe for *C. carnea* due to both the lack of fungus-related mortality in the predator and the lack of movement of destruxin A from the prey to the predator.

Ecological biocontrol as a strategy to develop fungal biopesticides for successful pest management (e-Biopesticide) **2:45** **•180•**

***Jae Su Kim*[1]**, *Se Jin Lee*[1], *Jong Cheol Kim*[1], *Sihyeon Kim*[1], *Mi Rong Lee*[1], *So Eun Park*[1], *Seok Ju Lee*[1], *Taek Su Shin*[2], *Tae Hoon Kim*[2], *Pan Jung Ha*[2], *Tae Hyun Park*[2]

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Now we are trying to deal with problems in pest management, such as ecological toxicity of synthetic pesticides, increasing pest resistance and stronger pesticide regulation in many countries. As one of the solutions, many global companies have been merging and acquiring some biopesticide companies, Pasteuria, Agraquest, Prohphya, Itaforte, and Becker Underwood. Some collaborative works have been producing biopesticides with enhanced quality. In the R&D of microbial insecticides, bacterial and viral insecticides received many interests and outstanding commercial products have been developed. However little consideration has been given to the fungal insecticides, although this group has much higher potential in controlling hemipteran and thysanopteran insects by the hyphal penetration through the integument. High production cost of fungal insecticides might be a technical barrier. Application of the fungal insecticides to unfavorable conditions might result in lower performance in pest management. Herein this work, I suggest ecological biocontrol considering long-term colonization in nature rather than quick pest control. Additionally other important technical aspects need to be strongly considered, such as economic downstream process, effective control and environmentally safe, so finally proposing 4e-biopesticide. In terms of ecological biocontrol, entomopathogenic fungal pesticides can be applied to the soil and water in agricultural fields, water in urban cities and hydric areas in forest, where the use of synthetic pesticides is not easily accepted and getting disapproved before long. We have some achievements in the management of thrips and other pests. With this field-oriented works, our group is also working on fungal molecular biology to elucidate the mode of actions and interaction between insects and entomopathogenic fungi. This ecological biocontrol can be a strong background for successful development of fungal biopesticides.

Isolation, morphological and molecular characterization of entomopathogenic fungi as a potential control strategy against *Lobesia botrana* in Argentinean vine growing areas **STU** **3:00**

***Rodrigo J. López Plantey*[1,3,4]**, *Daciana Papura*[2], *Andrés E. Riquelme*[3], *Antonella Balloni*[3], *Pablo H. Pizzuolo*[1,4], *Joana J. Boiteux*[1,4], *Denis Thierry*[2], *Gabriela S. Lucero*[1,4]

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The appearance of new pests in a culture requires actions to prevent establish-ment, spreading internal or across international borders. The European grapevine moth *Lobesia botrana* was recently introduced in Argentina in 2010, when it was declared as quarantine pest. The current methods for controlling grape moth's populations include conventional chemical ovicides or larvicides, but also alter-native methods as mating disruption and Bt toxin. The use of entomopathogenic fungi as a control strategy of Lepidoptera is known from ancient times. Argen-tinean wine production is mainly concentrated in the western area of the country, next to the Cordillera de los Andes with a high specificity in the local environ-mental conditions. The aim of present work was to isolate and characterize natives entomopathogenic fungi from Argentinean grapevine environment, which could be propose as biological control agents against *L. botrana.* Soils from vineyards over 15 years old were sampled across 1200km on the Argentinean provinces of Mendoza, San Juan, Catamarca, La Rioja, Salta, Río Negro and Neuquén. A total of 45 samples were prepared and conserved at laboratory. Petri dishes with prepared soils samples were used according to the insect bait method (3 repetition per sample), where 15 larvae L4/L5 of *L. botrana* were placed for 7 days at controlled temperature (25°C), photoperiod (16:8) and humidity (>60%). Larvae with fungus symptoms were separated and cultivated until fungus isolation and purification. A total of 32 strains of entomopathogenic fungi were isolated which were characterized by dichotomous key and molecular techniques using a partial sequence of nuclear protein-encoding gene, translation elongation factor 1-alpha. Three genders were identified by morphological characterization and all the strains were placed by BLAST® in one of these three species: *Beauveria bassiana, Metarhizium robertsii* and *Paecilomyces lilacinus*. The results help to start the physiological and pathogenic studies of these strains against *L. botrana* and their use as biological control.

3:30–4:00 pm Refreshments Break

WEDNESDAY, 4:00 – 6:00 pm POSTERS

West Ballroom

All presenting poster authors should be present at or near their posters.

All posters must be removed by 6:30 pm on Wednesday

VIRUS DIVISION POSTERS

Genomics of alphabaculovirus isolates infecting *Malacosoma disstria* in North America **Vir01** **•181•**

***Martin A. Erlandson*[1]**, *Doug Baldwin*[1], *Andrew Keddie*[2], *George Rohrmann*[3], *David A. Theilmann*[4]

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The forest tent caterpillar (FTC), *Malacosoma disstria*, is a major defoliator of deciduous trees in Canada and the USA and is characterized by cyclic population outbreaks. The alphabaculovirus species infecting FTC, *Malacosoma disstria nucleopolyhedrovirus* (MadiNPV), is one of the major natural regulators of FTC population cycles. We have sequenced the

complete genomes of two strains, pp3A and pp11A, from the MadiNPV-A92 isolate. The MadiNPV-pp3A and -11A genomes are closely related with an average predicted ORF identity of 99% and have 41% G+C content, and are 135,623 and 136,414 bp, respectively, and contain 135 and 139 ORFs, respectively. Phylogenetic analysis of polyhedrin, lef-8 and lef-9 sequences, generated using degenerative primers, of NPVs isolated from FTC, as well as sequences from the NCBI database of MaamNPV (*M. americanum* - eastern tent caterpillar, North America), MacaNPV (*M. californicum* - western tent caterpillar, North America), and ManeNPV (*M. neustria* - lackey moth, Eurasia) indicated that the MadiNPV isolates constitute a distinct alphabaculovirus species that is most closely related to ManeNPV. Our previous studies indicated that budded virus of MadiNPV pp3A and pp11A isolates had differential replication dynamics in three cell lines from FTC. However, the only major differences in the gene content of the MadiNPV isolates was the presence of 3 additional ORFs of unknown function in pp11A and a large 910 aa ORF in pp3A of unknown function that is represented by 2 truncated ORFs in pp11A. Whether these ORFs play a role in the differences in virus replication dynamics noted in the various host cell lines will require further study.

Oddities in the genome of the alphabaculovirus infecting *Malacosoma californicum pluviale* **Vir02** **•182•**

***Elisabeth A. Herniou*[1], *Julien Thézé*[1,2], *Aurélien Chateigner*[1], *Shannon Escasa*[3], *Martin Erlandson*[4], *David Thielmann*[5], *Jennifer S. Cory*[3,6]

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The Western tent caterpillar *Malacosoma californicum pluviale* is well known for its cyclic population dynamics associated with its specific alphabaculovirus McplNPV. Over the years considerable effort has been put into determining the complete genome of McplNPV, but so far assembly has remained unsatisfactory. Here, based on combined 454 and Illumina data, we were able to assemble a circular consensus sequence of 135,827 bp and comprising 144 ORFs. One of the striking features of this genome is the disruption of the cluster of 4 genes that is conserved in all other baculovirus genomes completely sequenced to date. A second characteristic of McplNPV concerns its homologous repeat regions. The genome of McplNPV contains 12 HRs formed of large palindromic repeats and ranging in size from 233 to 928 bp. Whole genome phylogenetic analyses showed McplNPV belong to the group II of the genus Alphabaculovirus but is distant from other baculovirus genomes.

Genomic comparisons among Phthorimeae operculella granuloviruses isolated from different hosts in field **Vir03** **•183•**

***Gloria P. Barrera*[1], *Juliana Gómez*[1], *Mariano N. Belaich*[2], *Pablo D. Ghiringhelli*[2], *Carlos Espinel*[1], *Laura Villamizar*[3]

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Some baculoviruses infect different hosts as a mechanism to increase their genetic diversity and to improve their fitness and maintenance in nature. However, most species from Betabaculovirus genus are characterized by showing a reduced host range. In the case of Phthorimaeae operculella granulovirus (PhopGV), previous works showed that infects different Gelechiidae´s insects including Tuta absoluta (Meyrick) and *Tecia solanivora* (Povolny) with different biological activity. With the goal to contribute in the understanding of genetic causes associated with the infection of PhopGV in different hosts, two field isolates recovered from *T. absoluta* (TuabGV) and *T. solanivora* (TesoGV) were studied. To this, both genomes were completely sequenced and compared between them and with respect to other PhopGV previously reported. Thus, a high degree of conservation in sequence and gene organization was observed being the main differences small insertions and deletions located in some open reading frames (ORF) and other non-coding regions. Only four ORFs showed variability among all genomes, corresponding to PhopGV 027 (envelope fusion protein), 041 (metallo-proteinase), 046 and 092 (unknown function) ORFs. On the other hand, 24 ORFs were identical between TesoGV and TuabGV but they showed differences with respect to the PhopGV orthologs, while only five ORFs showed differences between TesoGV and TuabGV. When controlled bioassays of PhopGV, TuabGV and TesoGV were carried out, all isolates showed the highest performance against their original hosts, however TesoGV reduce the insecticidal activity against alternative host while PhopGV maintain high fitness in all. The proper biological interpretation of these variations may assist in understanding how baculoviruses support mutations that help them to increase their chances of success in nature. In this sense, they could be associated to the etiological cause of some phenotypical changes observed, thus transforming pangenomic studies into useful tools to nurture the field of functional genomics in *Baculoviridae*.

A new Group II alphabaculovirus isolated from *Spodoptera ornithogalli* **Vir04** **•184•**

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The yellow striped armyworm *Spodoptera ornithogalli* (Guenée) (Lepidoptera: Noctuidae) is a polyphytophagous insect widely distributed in America since Canada to Argentina. The larvae feed on various plants of agriculture importance such as alfalfa, tomato, tobacco and onion, among others. Particularly, *S. ornithogalli* can be a serious pest on transgenic cotton and ornamental flower crops in Colombia. In order to explore bioinsecticide agents to control populations of this insect, one field baculovirus isolate from *Spodoptera ornithogalli* larvae was characterized. Electron micrographs revealed the presence of Occlusion Bodies (OBs) with a mean diameter of 1.51 µm containing several rod-shaped nucleocapsids per virion. The virus genome was isolated and sequenced using Illumina technology. The phylogenetic tree based on translated proteins showed a closer relationships with alphabaculoviruses from insects belonging to Noctuidae family, *S. litura, S. exigua and S. frugiperda*. Based on the criterion for baculovirus species demarcation, nucleotide distances of concatenated polh, lef-8 and lef-9 genes were calculated by Kimura 2-parameter model. The nearest distance with respect to the other species [corresponding to *Spodoptera litura* nucleopolyhedrovirus (SpliNPV-II; GenBank NC_011616)] was greater than 0.05 indicating that SporMNPV is a new species belonging to the Group II of *Alphabaculovirus* genus of *Baculoviridae* family, thus enriching the available knowledge about *Spodoptera* spp. baculoviruses occurring in nature.

Characterization of baculovirus pathogenic to cassava hornworm (ErelGV) from Cruzeiro do Sul, Acre, Brazil **Vir05** **•185•**

William Sihler[1], ***Márcio Martinello Sanches***[1], *Rosana Falcão*[1], *Murilo Fanzolin*[2], *Joelma Lima Vidal Estrela*[2], *Marlinda Lobo de Souza*[1]

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Baculoviruses are rod-shaped enveloped viruses with circular double-stranded DNA, with the majority of species infecting insects of the order Lepidoptera. Due to its potential as pest control agents in agriculture and forests, they have been successfully used as bioinsecticides. The hornworm (*Erinnyis ello*) is an important pest of cassava and rubber tree with high capacity of migration. In this work it is presented the characterization of a baculovirus with occurrence in *Erinnyis ello* populations in area of small cassava farms in Cruzeiro do Sul, State of Acre, Brazil (Latitude -7,55086; Longitude -72,72570). The viral particles were purified from larvae with symptoms of infection through ultracentrifugation in sucrose gradient. After

treatment with uranyl acetate 2%, the particles were visualized using a JEOL 1011 transmission electron microscope. The virus with ovicylindirical occlusion body, which presented only one virion per envelope and size less than 0.5µm, was identified as member of genus *Betabaculovirus* (formerly *Granulovirus*). The viral DNA was extracted through phenol/chloroform cycles, digested with different restriction enzymes and submitted to agarose gel electrophoresis containing ethidium bromide. The analysis of restriction patterns obtained with the enzymes *Bam* HI and *Hind* III revealed a total of seven and three DNA fragments, respectively. As a result of the cleavage with *Pst* I, eight molar fragments and three submolar fragments were identified. Finally, after digestion with *Eco* RI 21 fragments were observed, with some submolar bands being also present. The comparison of the DNA restriction profile from ErelGV isolated in Acre, North Brazil, with the pattern described for ErelGV collected in Santa Catarina State, South Brazil, showed a very high similarity between them.

First report of iflavirus infection in *Spodoptera littoralis* (Lepidoptera:Noctuidae) **Vir06** **•186•**

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Iflaviruses are single-stranded RNA viruses with a cytoplasmic viral replication from the family of Iflaviridae (order Picornavirales) that primarily infect insects. Most iflaviruses cause sublethal infections and are difficult to be detected by symptomatic observations; while some could be lethal to the honeybee and silkworm hosts. Iflaviruses have been detected from *Halyomorpha halys*, *Heliconius erato*, *Lymantria dispar*, *Spodoptera exigua, Bombyx mori* and *Apis mellifera*; however, little is known on this family of viruses. In the current study, an iflavirus was identified through the occurrence of expression sequence tags (ESTs) from the larval midgut of *Spodoptera littoralis* (Lepidoptera:Noctuidae) and was denoted Spodoptera littoralis iflavirus 1 (SpliIV1). The identification was based on a cDNA encoding the polyprotein. BLAST analysis revealed an identity value of 97% with the polyprotein of Spodoptera exigua iflavirus 2. The SpliIV1 polyprotein cDNA is 4883 b in length, encoding a polypeptide protein of 1522 aminoacid with a predicted molecular weight of 171 kDa. The Spodoptera littoralis iflavirus 1 is the first iflavirus described in *S. littoralis*. However, more studies are required to understand the pathology and activity of the SpliIV1 in insect hosts.

A new virus infected mud vrab revealed by Cryo-EM **Vir07** **•187•**

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The mud crab (*Scylla serrata*), an economically important species, is cultivated commercially in many countries, including China, India and Australia. However, viral diseases have had serious negative economic effects on the mud crab, limiting its growth. Previously, the mud crab reovirus (MCRV) and mud crab dicistrovirus (MCDV) have been isolated from mud crabs that had obvious symptoms of sleeping disease (SD). Cryo-Electron Microscopy (Cryo-EM) single particle techniques have been applied to study the high-resolution structures of these viruses. Better than 4 angstrom three dimensional (3-D) structure of the MCDV and MCRV were obtained. Very surprising, we found a new virus in the two-dimensional (2-D) classifications in MCDV sample, although the size and shape in 2-D are very similar to that of MCDV. And therefore it is very difficult to found there are two species virus through the density gradient centrifugation and conventional electron microscopy techniques. Further studies on both 3-D structure, genome and protein analysis reveal that the new virus should be a new member of the *Nodaviridae*. We named the new virus as the mud crab nodavirus (MCNV). The structure of the MCNV shows a RNA-containing capsid shell with an inner diameter of approximately 227 Å and an outer diameter of about 300 Å. The most prominent structural feature is a total of 30 protrusions located on the icosahedral 2-fold axes, which is usually on 3-fold axes in other *Nodavirudae* members. These protrusions are approximately 36 Å tall and dimeric with two linking hinges. In contrast to the well-resolved capsid shell with icosahedral symmetry, the protrusions reveal highly flexible conformation as evidenced by the relatively weaker densities. This significant flexibility may be resulting from the long hinges connecting protrusions and capsid shell. The protrusions may play important roles in host reorganization.

Characterization of a persistent baculovirus infection established in an insect cell line **STU** **Vir08** **•188•**

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Baculoviruses such as Autographa californica nucleopolyhedrovirus (AcMNPV) are double-stranded DNA viruses that exclusively infect insects, leading to death of the larval host. Nevertheless, many insects harbour persistent, non-lethal virus infections throughout their life stages. Recently, a clonal culture of a persistent infection was achieved when infecting *Trichoplusia ni* cells (Hi5®) with a AcMNPV *p10* deletion mutant. This cell line (C20) has been maintained continuously for 6 years and constitutes an important model to study persistent virus infection *in vitro*. Furthermore, the C20 cell line also provides a mechanism to study the superinfection exclusion phenomenon, as it is largely refractory to infection with a second virus. This study aims to understand the balance between the host insect cell and the virus, which allows the persistent infection to be maintained. In addition, we intend to investigate the barrier(s) preventing baculovirus superinfection. Characterization of the C20 cell line revealed that the cells retain viability for longer than the parental cell line, being able to survive for 40 days before sub culture. The persistent infection is maintained at a very low level although the infectious virus titre fluctuates considerably within and between different cultures. Using a recombinant virus with eGFP fused to VP39, the baculovirus major capsid protein, confocal laser scanning microscopy was used to follow virus uptake and subsequent infection to detect the super infection blockage. This study, combined with scanning electron microscopy on early infection time points, suggested the secondary infection of C20 cells is blocked on the adsorption or uptake step. Further studies are examining this question using recombinant viruses with eGFP expression regulated by early and late promoters. Understanding the bio-logical relationships responsible for the maintenance of C20 cell line may help us, in the longer term, to understand the finely constructed relation-ship between virus and host that leads to either lytic or persistent infection.

Autographa californica multiple nucleopolyhedrovirus ME53 interacts with VP80, LEF5, EXON0, VP39 and GP64 **Vir09** **•189•**

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me53 is a conserved gene among the alpha- and betabaculoviruses, family *Baculoviridae*. Its deletion causes a 99.99% decrease in budded virus (BV) production *in vitro* and a slower spread of the virus. ME53 is a component of the nucleocapsid of both BV and occlusion derived virus (ODV). ME53 has two zinc fingers, lacks a nuclear localization signal (NLS), but does have a nuclear translocation sequence (NTS). It is expressed at both early and late time post-infection. Confocal fluorescence microscopy showed that at early times post-infection ME53 is found in the cytoplasm and translocates to foci in the cell membrane. At late times post infection, ME53 translocates to the nucleus accumulating at the peripheral region of the nucleus (in the ring zone), where ODV envelopment and embedding occur. That ME53 localizes to the membrane suggests that ME53 plays a role as a bridge between the envelope and nucleocapsid for efficient nucleocapsid envelopment and budding. We used the yeast two-hybrid system (Y2H) to identify ME53 binding protein partners. For that, *me53* and Y2H libraries were constructed from mRNAs from AcMNPV-infected Sf21 cells and used as bait and prey, respectively. Following library screening, VP80, P6.9, LEF5 and cellular receptor for activated protein kinase C (RACK1) were found as ME53-

interacting partners. These four genes were cloned as bait and prey and tested reciprocally with ME53-prey and ME53-bait in yeast, with positive results for VP80 and LEF5 but not P6.9. GP64, VP39 and Exon0 were also cloned as prey and bait and an interaction was found between them and ME53 using the Y2H system. The interaction between ME53 and VP80 was the strongest interaction in terms of selective media stringency among other interactions found in this study. These data suggest VP80 might be the protein that ME53 uses as a chaperone to translocate to the nucleus late post-infection and this strong interaction might facilitate the attachment of the nucleocapsid to foci in the cellular membrane for enhanced envelopment and budding allowing for faster release and spread of the virus.

Deletion of the *p94* (*ac134*) gene of the Autographa californica multiple nucleopolyhedovirus induces a delay in virus DNA replication, BV production, and insect mortality. **Vir10** **•190•**

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Homologs of p94 (ac134) are present in the genome of several alpha- and betabaculoviruses) and also in some bracoviruses. The disruption of the p94 gene presented no effect on the ability of AcMNPV to infect *S. frugiperda* and *T. ni* larvae. However, the transient expression of the bracovirus P94 is able to suppress the immune system and developmental processes of lepidopteran larvae. To understand the evolutionary history of *p94* gene, we constructed a phylogenetic tree of several homologs found in the Genbank. We found *p94* homologs in alpha- and betabaculoviruses, bracoviruses and Lepidoptera. Our phylogenetic analysis shows that baculovirus sequences grouped together forming a well-supported group with betabaculoviruses sequences as a sister group to alphabaculovirus. The bracoviruses *p94* homologs clustered together along with homologs from the monarch butterfly *D. plexippus*, confirming that horizontal gene transfer between bracovirus and Lepidoptera is likely common. To further investigate the biological impacts of this gene, we constructed three recombinant baculoviruses: vAc-pol-*p94*-del (p94 deleted), vAc-pol-*p94*-ha-rep (p94 repaired with an HA tag) and vAc-pol (parental virus). We found that the P94 was detected at 6 h post-infection (p.i.) in the cytoplasm of infected cells with maximum accumulation at 24 h p.i.. The deletion of the *p94* gene in the AcMNPV genome resulted in a delay in DNA replication, BV production and IE1 expression. OB production *in vitro* was also decreased. Although the *p94* gene of AcMNPV is not an essential gene, its presence is necessary for efficient viral DNA replication and establishment of viral infection.

AcMNPV-miR-6 down-regulates expression of host gene alg-2 **Vir11** **•191•**

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An Autographa californica multiple nucleopolyhedrovirus (AcMNPV) encoded miRNA, AcMNPV-miR-1 has been reported regulating viral own genes. However, AcMNPV encoded miRNA also regulate host genes. This study focused on AcMNPV-miR-6 targeting a host gene apoptosis linked gene-2 (alg-2) and the effects of this miRNA on viral infection. AcMNPV-miR-6 was expressed at 6 h.p.i. and reached the maximum expression at 24 h.p.i. detected by quantitative reverse transcription PCR (qRT-PCR). alg-2 was verified down-regulating by AcMNPV-miR-6 through dual-luciferase reporter assay. The expression levels of alg-2 mRNA and protein were decreased dramatically in AcMNPV-infected Sf9 cells at 24h.p.i. Administering AcMNPV-miR-6 mimic, the expressions of alg-2 mRNA and protein were decreased after transfected with alg-2 recombinant expression vectors in Sf9 and 293T cells, respectively. Constitutively overexpress AcMNPV-miR-6 recombinant viruses were constructed and mimic transient transfection was used in parallel to further explore the function of this miRNA. In the context of AcMNPV-miR-6 overexpression, the infectivity and production of budded virus were reduced, the polyhedron formation was delayed, but the viral DNA replication did not show obvious alteration. It suggests that AcMNPV-miR-6 can auto-regulate viral infectivity and prolong the life span of the host cells, thereby facilitates the viral proliferation.

***In vivo* transcriptome under suppression subtractive hybridization (SSH) of the *Betabaculovirus* TnGV in *Trichoplusia ni* larvae** **Vir12** **•192•**

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Five subtractive libraries under suppression conditions were constructed and sequenced obtained from *Trichoplusia ni* larvae infected at different times with the *Betabaculovirus* TnGV. At 24, 48, 72, 96 and 120 hours post-infection (h.p.i.), 35, 20, 7, 9 and 14 clones were obtained respectively. These were sent to be sequenced. At 24 h of the 35 clones, 10 sequences were showed no similarity with other proteins, 3 sequences showed similarity with hypothetical proteins, some of these sequences showed similarity with 2/3 actin complex proteins, heat shock proteins, factor elongation translation 2 isoform 1, elongation translation factor 2 isoform 2, ubiquitin E3 ligase, eukaryotic initiation factor 4A isoform 1 (elF4A), galactinol synthetase-2. At 48 h.p.i. within the 20 clones, sequences with chaperone similarity, eukaryotic initiation factor 4A isoform 1 (elF4A), copper center partial sequence, galactinol synthetase-2, cellulose synthetase, trans-criptional regulator of the TetR family were found. At 72 h.p.i. of the 7 clones sequenced, we identified 4 protein sequences that gave similarity to hypothetical proteins, and protein sequences with similarity to ubiquitin E3 ligase and RNA ligases. At 96 h.p.i. of the 9 clones sequenced, we found 2 sequences with similarity to hypothetical proteins, one with similarity to eukaryotic initiation factor 4A isoform 1 (elF4A), one to cytochrome oxidase III subunit, and another to that of cellulose synthetase. At 120 hpi, of the 14 clones obtained, one of the sequences did not show similarity to other proteins, 4 sequences gave similarity to hypothetical proteins, and sequences were found with similarity to the ubiquitin E3 ligase, to the partial center copper sequence, and the B1,3-glucanases. In some tissues in infected *T.ni* larvae, the expressed proteins are controlled by the *Betabaculovirus* TnGV, and this leads to a reduction of proteins and mRNAs of the cell. On the other hand, the host cell responds to the viral infection with the overexpression or repression of some point genes and the virus takes advantage of the machinery and proteins of the host cell for its own replication.

Functional assay of ORF105 (APSUP) from Lymantria xylina multiple nucleopolyhedrovirus (LyxyMNPV) **Vir13** **•193•**

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Baculoviral anti-apoptotic genes, *p35* and *iap* (inhibitor of apoptosis), play important roles in the initiation stage of viral infection. Recently, a new anti-apoptotic gene (apoptosis suppressor, *apsup*) was identified from Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV); herein, the *apsup* homologues gene (*Lyxy105*) was amplified and cloned from L. xylina MNPV (LyxyMNPV) and further studied. A 1,002 bp of PCR product was amplified by using gene specific PCR and sequenced. Comparison of nucleotide and putative amino acid sequences to 19 homologues genes, the *Lyxy105* showed high nucleotide identity (88%) and high amino acid identity (85 %) and similarity (88 %) to that of LdMNPV. Moreover, five conserved protein domains were identified. Functional analysis of *Lyxy105* was performed using an overexpression method in Sf9 cells. From our result, full-length LYXY105 could inhibit the *Drosophila* RPR protein (DRPR)-induced apoptosis. More data should be provided (*i.e.*, gene expression profile and anti-apoptotic activity of ActD-induced apoptosis, etc.) to further evaluate the full picture of the anti-apoptotic mechanism of LYXY105.

Construction of hyper baculovirus expression vector by the optimization of enhancer factors STU Vir14 •194•

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The baculovirus expression vector system (BEVS) is an effective and widely used system for the production of recombinant proteins in insect cells or larvae. However, the expression efficiency of recombinant proteins using the polyhedrin promoter could not acquire the protein yields observed for native polyhedrin. To overcome this limit, various enhancer factors have been developed by many researchers and enhanced successfully the production efficiency of recombinant. In this study, we tried to develop hyper expression vector by the optimal combination of previously reported various enhancer factors. The selected enhancer factors for optimal expression consists homologous region 5 (hr5), VP39 promoter and burst sequences. Enhanced green fluorescent protein (EGFP) was fusion expressed as recombinant protein for visual and numerical observations. Seven recombinant viruses were generated using these enhancer factors to compare expression efficiency. Each recombinant virus showed different expression levels each other, and the most of expression level was observed with higher than those of the previous vectors. The present study suggests a new option for higher expression of useful foreign recombinant protein using the BEVS.

The effects of transfection of bacmid DNA together with the helper plasmid on determination of defective virus multiplication capacity Vir15 •195•

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The Bac-to-Bac Baculovirus Expression System has been widely used in the studies of baculovirus gene functions because of the rapid and efficient way it provides to generate recombinant baculoviruses. The *Escherichia coli* strain DH10Bac from the system contains a baculovirus shuttle vector (the bacmid bMON14272) and a plasmid that encodes the transposase to provide the Tn7 transposition *in trans* (the helper plasmid pMON7124). After generating recombinant bacmid by transposition, the presence of the helper plasmid will not affect the transfection of the bacmid DNA. However, we noticed that when transfecting DNA in a quantified way, the existence of the helper plasmid may affect the determination of a defective-virus multiplication capacity. In the present study, we used an AcMNPV *orf34* (*ac34*)-knockout bacmid DNA as a backbone to generate different recombinant bacmids. After the transposition, the helper plasmid was kept in the DH10Bac cells or removed from the cells by electroporation of the bacmid DNA into the helper plasmid-free DH10B cells. To clarify the effects of the helper plasmid when determine the multiplication capacity of the viruses, we transfect the DNA mixtures (purified from DH10Bac cells with helper plasmid existed) or only the bacmid DNAs (purified from the DH10Bac cells free of helper plasmid) into Sf9 cells in a quantified way. After transfecting the quantified amount of DNA into Sf9 cells, we photographed the cells and measured the virus titers at different time points post transfection. The results were analyzed and discussed.

Induced plant defenses delay within-instar developmental resistance of fall armyworms, *Spodoptera frugiperda*, to the baculovirus *SfMNPV* Vir16 •196

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Resistance of larval lepidopterans to baculoviruses increases with each larval instar and can also increase over time within an instar. Most studies measure developmental resistance at incremental time points using insects that were feeding on a single defined food source, usually artificial diet. However, insects in the field are exposed to food sources that vary widely in nutritional and allelochemical composition. A few studies have shown that food quality (different host plant species or plant vs. artificial diet) can influence levels of developmental resistance. One of the most common variations in plant quality experienced by caterpillars in the field is differences in constitutive and/or induced plant defenses due to caterpillar feeding. We investigated the effects of induced plant defenses on developmental resistance in the fall armyworms, *Spodoptera frugiperda*. We fed newly molted fourth instars foliage from non-induced or induced soybean (*Glycine max*) plants for 2, 4, 6, 8 or 12 h prior to baculovirus (SfMNPV) challenge. Plants were induced with the phytohormone, jasmonic acid, which stimulates anti-herbivore defensive responses. We found that the mortality of SfMNPV-challenged larvae decreased significantly more over time (i.e., resistance increased over time) when larvae fed on non-induced than on JA-induced foliage before virus-challenge. This suppression of developmental resistance over time on JA-induced foliage was associated with slower larval weight gain and there was not a reduction in consumption of foliage. Results imply that plant-mediated larval growth inhibition can increase susceptibility to baculovirus. Moreover, our findings indicate that larval weight gain, not time post molt, was responsible for within-instar developmental resistance of fall armyworm to SfMNPV.

The hemolymph of *Mythimna separata* larvae infected with an entomopoxvirus kills parasitoid-derived cell STU Vir17 •197•

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When the gregarious endoparasitoid *Cotesia kariyai* parasitizes *Mythimna separata* larvae infected with Mythimna separata entomopoxvirus (MySEV), its embryos and larvae die inside the host. *C. kariyai* larvae die even in the virus-free hemolymph of M. separata larvae previously infected with MySEV, which we call VFP (virion-free plasma). Previous studies showed that a 28-kDa protein purified from the VFP was lethal to *C. kariyai*. This protein was named parasitoid killer toxin (PKT) and MySEV encodes pkt homologues. The VFP shows killing activity to some Microgastrinid parasitoids including *C. kariayai*, but not to other braconid parasitoids including *Meteorus pulchricornis*. However, it remains unclear whether the VFP affects not only at the individual level but also at the cellular level. In this context, understanding how the VFP acts on the parasitoid cell is important in terms of elucidating PKT mode of action. In this study, we examined the effect of the VFP on cultured cells established from *C. kariyai* (Ck1) and M. pulchricornis (Mp4). Ck1 showed unusual morphology, such as loss of pseudopod, blebbing and floating when exposed to the VFP. Moreover, the VFP suppressed Ck1 cell prolification completely and increased mortality of Ck1 significantly 24 hours after exposure. On the other hand, such deleterious effects have not been observed in Mp4. These results suggest that the VFP, containing PKT, specifically kills *C. kariyai* at the cellular level.

Effect of gut bacteria on Sindbis virus replication in the mosquito *Aedes aegypti* STU Vir18 •198•

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The *Aedes aegypti* mosquito is an important vector for several arboviruses that are a major public health concern around the world, including dengue virus, Zika virus, and chikungunya virus. *A. aegypti* is found in tropical and subtropical regions, with travelers enabling the spread of viruses to other regions that may not be endemic. We use Sindbis virus (SINV; *Togaviridae*) as a model arbovirus to help further understand viral replication within this species of mosquito. Past experiments by our group have consistently revealed a high degree of variability in SINV titer between individual mosquitoes when the mosquitoes are infected by the oral (bloodfeeding) route, with titers ranging from 10^2 to 10^6 infectious units per mosquito. In

contrast, mosquitoes that are infected by intrathoracic injection exhibit consistently high virus titers in the range of 10^4 to 10^6. The reasons for this difference between infection routes are unclear, but may be related to the fact that intrathoracic injection bypasses the midgut, while oral infection requires initial replication in the midgut before the virus can disseminate to other tissues. In this study, we wanted to determine whether resident gut microbes affect SINV replication via oral infection, and whether the gut microbiome could contribute to the extreme variability observed in virus titers by reducing virus replication in some individual mosquitoes. To test this, we treated mosquitoes with antibiotics prior to oral infection, in order to reduce the numbers of gut bacteria. We found that antibiotic treatment was successful in reducing the numbers of cultivatable gut bacteria. However, when antibiotic-treated mosquitoes were infected with SINV by the oral route, virus titers were similar to control mosquitoes that had not been treated with antibiotics. These results do not support the hypothesis that the presence of gut bacteria has a major influence on SINV replication in orally infected *A. aegypti* mosquitoes, in contrast to previous reports that gut bacteria reduce arbovirus replication.

Evaluation of Anticarsia MNPV and Pseudoplusia SNPV co-infection in insect cell culture **Vir20**

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It is well known that one particular insect cell line may be susceptible to different baculovirus. Therefore co-infection of a host cell with two distinct viruses can result in co-oclusion phenotype. This feature may be important to reduce costs of baculovirus *in vitro* production. The baculoviruses *Pseudoplusia includes single nucleopolyhedrovirus* (PsinSNPV) and *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) that infect two important soybean pests, *Chrysodeixis includens* and *Anticarsia gemmatalis* respectively, were tested regarding their infectivity to different cell lines. The cell lines IPLB-SF-21AE, Sf9 and BTI-Tn-5B1-4 were previously evaluated as productive to AgMNPV infection. In this work the susceptibility of these cell lines to single infection of PsinSNPV and mixed infection of PsinSNPV and AgMNPV was tested. The cells were seeded at a density of $1X10^6$ per $60mm^2$ dish. The viruses were obtained through hemolymph from infected larvae at 4 d.p.i. and allowed to adsorb to cells during 1 hour (P0). Infected cells were kept in TNMFH complete medium at 27^0C. Morphological analysis was initially monitored by light microscopy during five days. Then, the supernatants were collected for new infections (P1) using the same procedure. At 5 d.p.i. the supernatants were collected for the second passage (P2). The ultrastructure of the occlusion bodies was analyzed by Transmission Electron Microscopy (T.E.M.) for P0 passage. The amount of DNA obtained from Budded Viruses (BVs) of the three passages was monitored by real-time PCR (qPCR) using Sybr green system with primers designed to *gp64* of AgMNPV and *photolyase* of PsinSNPV. The experiment was repeated twice. The qPCR methodology presented the limit of detection 10^{-5} ng DNA for both viruses. The results demonstrated successful co-infection in these cells, but the amount of PsinSNPV tends to decrease in serial passages whereas the amount of AgMNPV tends to increase in the first passage and to stabilize in the second passage. Also, in PsinSNPV single infection experiments, the amount of BVs DNA has a tendency to reduce in further passages. Cell culture visualization by optical microscopy, in conjunction with the qPCR assays, indicates that infection with PsinSNPV is restricted to individual cells, resulting in low polyhedra production.
Support:FAP-DF

Production of porcine epidemic diarrhea virus (PEDV) VLP using the baculovirus expression vector system in *Bombyx mori* cells **STU Vir21**

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Porcine epidemic diarrhea virus (PEDV) causes viral diarrhea disease in pigs resulting in large economic loss to the swine industry in Asia. However, there is no effective vaccine to prevent PEDV. Viral particles of PEDV consist of a four structural proteins are Spike(S) protein, envelope(E) protein, glycosylated membrane(M) protein, and unglycosylated RNA-binding nucleocapsid(N) protein. The PEVD virion is assembled and released into the lumen of the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC), followed by secretion by exocytosis. The M and E proteins plays important in virus assembly and budding. The S protein mediated responsible for receptor binding and membrane fusion during viral infection and therefore the main target of neutralizing antibodies. Virus-like particles (VLPs) are consisted of one or more viral structural proteins, and their morphologies closely resemble those of the native virus. VLPs have no virulence and can elicit robust immune responses as compared with inactivated or live-attenuated virus vaccines. Thus, in this study, we tried two methods for VLP construction, one is traditional method and the other is chimeric VLP method using the influenza matrix protein. The PEDV four structural proteins (S, M, N and E) which were co-expressed in *Bombyx mori* 5 (Bm5) cells formed successfully VLPs. The chimeric VLP was also constructed with a matrix 1 protein from influenza virus and a modified spike protein that was generated by mutation or deletion of KxHxx motif in the cytoplasmic tail. Both methods could produce successfully PEDV VLPs with spike protein on their surfaces.

BACTERIA DIVISION POSTERS

Sex ratio distortion in tea pest *Homona magnanima*: A complicated association of host and endosymbiotic microbes **STU Bac01 ●199●**

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Endosymbiotic microbes are frequently detected from a variety of insects, and some of them interact with their host in some mutualistic or parasitic manners. For example, some microbes are known to manipulate host reproduction via male-killing or feminization. *Homona magnanima* (Tortricidae, Lepidoptera) is a serious tea pest, which widely distributed in East Asia. From field surveys across Japan, *Wolbachia, Spiroplasma* and RNA virus were detected from *H. magnanima*, and the latter two microbes caused sex-ratio distortion in their host. Although *Wolbachia*-infected Japanese host exhibited normal sex-ratio, we recently collected a *Wolbachia* infecting *H. magnanima* from Taiwan, which showed female biased sex-ratio. Here, we characterized both Japanese and Taiwanese *Wolbachia* strains to investigate factors correlated to the difference of phenotype in terms of sex-ratio distortion. First of all, we conducted phylogenetic analysis of *Wolbachia* by using *Wolbachia* MLST genes. As a result, Japanese host harbored three distinct *Wolbachia* strains (*w*Hm-a, -b and -c) and Taiwanese host harbored a *Wolbachia* strain (*w*Hm-t). Although *w*Hm-c and *w*Hm-t showed 100% similarity at MLST level, only *w*Hm-t caused sex-ratio distortion to the host. We next surveyed the density of *Wolbachia* (*w*Hm-a, -b, -c and -t) in their host to examine the correlation between density and the phenotype. The density of *w*Hm-t was significantly higher than that of any other *Wolbachia* strains. In particular, over 1000-fold difference in density was detected between *w*Hm-t and *w*Hm-c. We also observed the difference in density between two *w*Hm-t infecting host lines and named *w*Hm-t with high-density as 'W^{TH}' line and *w*Hm-t with low-density as 'W^{TL}' line. In terms of the phenotypes, W^{TH} line exhibited higher total mortality than W^{TL} line. Almost

all males of W^{TH} line were killed during embryonic stage like *Spiroplasma*-infected host, whereas males of W^{TL} line were killed during both embryonic and larval stage and exhibited same symptoms of RNA virus; such as white body color and abortion of larva. Given these results, the phenotype of sex-ratio distortion caused by *Wolbachia* is likely to depend on *Wolbachia* strain and its density, and *w*Hm-t can exhibit various phenotypes in terms of male-killing in *H. magnanima*.

Bacteria Collection of Invertebrates, one of the four Brazilian collections of the Global Net of Biological Resources: Challenges and expectations **Bac02** **•200•**

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In 2005, a project supported by Ministry of Science and Technology in Brazil was started in order to prepare Brazilian collections to act as National Biological Resource Centers. 16 collections were evaluated regarding compliances to the ISO 17025 standard requirements and four collections were selected aiming a Quality System (QS) implementation in order to prove their competence in doing technical and specialized services and in offering certified biological materials. The Embrapa Brazilian Invertebrate Bacteria Collection was one of the four collections selected by the project. Since then, the collection team and the Quality Management Center of Embrapa conducted the following activities to implement the ISO 17025: diagnosis of the actual situation of the collection regarding the Standard requirements; staff training; preparation of a Quality Plan to be used through the mapping process, preparation of QS documentation (Quality Manual, master list, 37 technical and 31 management procedures; 11 equipment instructions); implantation of the 5S program and disposal management; validation methods, quality control of the validation methods, physical infrastructure adequacy; equipment maintenance, calibration and qualification and external evaluations. During four years, the collection project has been externally evaluated three times in 2005, 2007 and 2009. The collection received respectively 34, 21 and 4 non-conformities. These results show the great progress of the collection activities within the QS. As a result of this effort, the collection, besides attending the ISO 17025 requirements, implemented the Standard for Biological Resource Center of the Organization for Economic Cooperation and Development (OECD). Thus, the collection can be part of the Network of Biological Resource Centers when Inmetro establishes a specific program for assessing conformity of Brazilian collections. To attend the OECD standard (NIT DICLA 061), all the information of the collection was inserted in a data bank and all strains were preserved under two methods (over 2600 strains on filter paper and lyophilized). These actions provided the collection to obtain three tests accredited by Cgcre/Inmetro in 2015 and have this accreditation maintained in 2017: identification, viability and purity test of *Bacillus thuringiensis* and *Lysinibacillus sphaericus*.

Bacillus thuringiensis israelensis* reference material production to be used in assessment tests accreditation of bioinsecticides toxicity for controling *Aedes aegypti **Bac03** **•201•**

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The difficulty in finding suppliers of certified reference materials is a limitation that laboratories with accredited tests face today. Embrapa was accredited for the toxicity determination of *B. thuringiensis* to control mosquitoes and due to this it was necessary to look for viable and non-costly alternatives to produce its own reference material. In 2016 Embrapa started to produce its own material. The *B. thuringiensis israelensis* strains was multiplied in a fermenter and lyophilized. The purity and viability of the material was then evaluated through seeding in Embrapa medium and the features analyzed through repeatability, reproducibility and sensitivity parameters. The material stability was also evaluated through LC 50 bioassays of 2nd instar *Aedes aegypti* larvae. The LC 50 results were calculated by Poloplus Probit program and the data was released on an average control chart previously built to evaluate the stability of the material over time. Other evaluated feature was the material stability by means of LC 50 bioassays using 2nd instar *Aedes aegypti* larvae. The purity and viability results presented a 100% satisfactory outcome and The LC 50 presented values between 0.203 and 4.37 ng/mL. These results were within the control limits, demonstrating the stability of the reference material data and the method suitability. So it is possible to assure our clients that Embrapa provides services with technical competence, ensuring the quality of its results.

Superior *in vivo* growth of *Bacillus thuringiensis* strains suggests a specialisation towards insect pathogenicity and single original *B. thuringiensis* clade **STU** **Bac04** **•202•**

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Bacillus thuringiensis (Bt) is a gram-positive bacterium, used as a biopesticide and source of Crystal (cry) toxin genes for transgenic crops. Bt strains have been identified by the presence of cry toxins, but genomic data has shown that Bt is not monophyletic, instead being distributed throughout the *Bacillus cereus* family. However, cry genes are often plasmid-borne and horizontal transfer rates are high in this family. It is therefore hypothesised that cry toxins evolved in a single 'Bt' clade, before being lost/transferred giving the current Bt distribution in the family. Additionally, it has been previously suggested that Bt is not an obligate insect pathogen. Taken together, Bt strains in the supposed 'Bt' clade are expected to show lower spore production in vitro and higher fitness in vivo suggesting that Bt originally evolved as a specialised insect pathogen. Spore production in media showed that 'Bt' clade strains produced fewer spores than 'Bc' clade ones, while in vivo competitions against a standard strain in *Plutella xylostella* showed that 'Bt' clade strains had higher relative fitness. 'Bt' clade strains also showed a negative relationship between spore production and in vivo fitness whereas 'Bc' ones showed a positive relationship, suggesting specialisation in the 'Bt' clade.

Genome sequence of mosquitocidal native *Bacillus thuringiensis* strain from Argentina **Bac05** **•203•**

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Bacterial insecticides have been used for the control of insects of public health importance. Mosquito-borne diseases are among the leading causes of mortality and morbidity in humans. For the control of these viral and parasitic diseases the continuous development of different management strategies of mosquito populations is essential. In a previous work, we characterized an Argentinian *Bacillus thuringiensis* strain (FCC 41) that exhibited mosquitocidal activity against *Aedes aegypti*, *Aedes* (*Ochlerotatus*) *albifasciatus*, *Culex pipiens* and *Culex apicinus*. In this work, we conducted the whole genome sequencing of *B. thuringiensis* native strain using next generation sequencing on an Illumina 1500 HiSeq. The paired short reads were assembled with SPADES, and a preliminary annotation was obtained using PROKKA. Furthermore, we identified Cry proteins coding sequences (CDS) using a HMMER search. With this approach we found 10 positive matches: these CDS were identified as two Cry4-like, two Cry19-like with their corresponding downstream Orf2 regions, a Cry24Ca with its Orf2, a Parasporin 3-like, and three contiguous small CDS within the same operon with homology to domains of several Cry proteins. The next step will be to clone and express these *cry*-like nucleotide sequences in heterologous systems to determine their specific toxicological activity and potential application against vector insects of public health importance. Besides, we will analyze Orf2-like regions in order to determine their function in the crystallization and/or stability of Cry toxins. *Supported by ANPCyT (PICT No 2013-0431 y PICT No 2015-0575) and Universidad Nacional de Mar del Plata (15/E692-EXA742/15).*

Toxicity of *Bacillus thuringiensis* Cry1Ac toxin in different *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations **Bac06** **•204•**

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Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), the diamondback moth (DBM), is a major insect pest of crucifers (Brassicaceae) worldwide. The most common bioinsecticides used to control *P. xylostella* are based on the entomopathogenic bacterium *Bacillus thuringiensis* (Bacillaceae) (Bt). Although many studies have focused on the action of Bt on various agricultural pests, such as DBM, many doubts still persist, particularly regarding the toxicity of Bt proteins. We analyzed the virulence of Cry1Ac protein in Brazilian populations and in a Hawaii population of *P. xylostella*. Bioassays of susceptibility in five Brazilian populations (PC, PA, PX, SBT, and BT) and one Hawaii population (NO_QA) of *P. xylostella* and *B. thuringiensis* Cry1Ac protein, estimating the virulence of the toxin, were performed. Also a complementation crossing between NO-QA and Bt was performed estimating the virulence of the toxin on the first and second generation. Seven concentrations were used to calculate the values of LC_{50}. Three replicates were performed, with each replicate being a petri dish containing 20 larvae, totaling 60 insects per concentration for each population. The PC, PA, PX, SBT, BT and NO_QA *P. xylostella* populations exhibited different levels of susceptibility to the Cry1Ac toxin. The PA, PX, and SBT populations showed LC_{50} values of 0.022, 0.016, and 0.058 µg/mL. The LC_{50} estimate for the BT and NO_QA populations was 1.26 and 1.51 µg/mL, while that for PC it was 0.001 µg/mL. The LC_{50} estimated for the NO_QA population was 1510 fold higher than PC *P. xylostella* population. In the complementation crossing the LC_{50} estimated for NO_QA male X Bt female crossing was 2.08 µg/mL in the first generation and 0.37 µg/mL in the second, while for NO_QA female X Bt male crossing was 1.73 µg/mL in the first generation and 0.25 µg/mL in the second. *Support: FAPESP*.

Factors involved in resistance to *Bacillus thuringiensis* of Brazilian *Plutella xylostella* populations. **Bac07** **•205•**

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Plutella xylostella is a major insect pest of cruciferous crops worldwide. Although controlled with both synthetic and biological insecticides it can rapidly evolve resistance to a variety of insecticides. The most common biopesticides used to control *P. xylostella* are based on the entomopathogenic bacterium *Bacillus thuringiensis*. Although many studies have been performed on *Bt*, the mode of action is still not fully understood. High levels of resistance to Cry1 toxins, due to reduced toxin binding, have been genetically linked to mutations or expression alterations of receptor genes. Resistance can also result from the overexpression of genes involved in protecting the insect against Bt toxins, genes that are normally expressed in response to exposure to the toxin. A wide diversity of genes are differentially expressed in the midgut of resistant insects, this suggests that a variety of cell processes may be involved in the preservation of resistance. Recent discoveries have shown that mutations in the gene encoding an ABCC2 transporter are responsible for resistance to Bt toxins in various different insect species. One group found no association between mutations in ABCC genes and resistance, they subsequently compared levels of expression for all five ABCC genes in BtR-1 locus in susceptible and resistant strains of *P. xylostella* using qPCR. Their analysis revealed that ABCC2 was significantly down-regulated in the resistant population as was the expression of another putative receptor ALP. They also found that MAP4K4 was constitutively up-regulated in larvae from the resistant strain compared to the susceptible strain. In our study we tested the hypothesis that the susceptibility of *P. xylostella* to *Bt* correlates with the level of expression of components of a putative stress-response regulon. In particular we were testing whether our resistant populations would show down-regulation of ALP, APN, ABCC2 genes and up-regulation of CDKAL1 and MAP4K4 genes; our research demonstrated that there were no patterns in their expression that associated with their resistance/susceptibility. We also investigated the DNA sequence of ABCC2 cDNA and found a frameshift mutation in the resistant population that could be responsible for the resistant phenotype. Support: FAPESP.

Laboratory resistance *Plutella xylostella* (L.) (Lepidoptera:Plutellidae) to Cry1Ac toxin and HD1 strain of *Bacillus thuringiensis* **Bac08** **•206•**

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The diamondback moth (DBM), *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is a key pest of crucifers. Although can be controlled with insecticides, *P. xylostella* can quickly develop resistance to insecticides, such as those of from *Bacillus thuringiensis*. We analyzed the virulence of Cry1Ac protein and HD1 strain in Brazilian populations of *P. xylostella*. Bioassays in two Brazilian populations (susceptible and resistant) of *P. xylostella* estimating the virulence of the Cry1Ac and HD1, were performed. The resistant population came from the susceptible and was selected in the laboratory with HD1 every generation. Seven concentrations were used to calculate the values of LC_{50}. Three replicates were performed, with each replicate being a petri dish containing 20 larvae, totaling 60 insects per concentration for each population. The *P. xylostella* populations exhibited different levels of susceptibility to the Cry1Ac toxin and HD1 strain. The HD1 strain showed LC_{50} values of 9.8×10^5 spores/mL to resistant population and 0.93×10^3 to susceptible population. The Cry1Ac protein showed LC_{50} values of 1.26 µg/mL to resistant population and 0.016 µg/mL to susceptible population. The LC_{50} estimated for the HD1 strain was 1054 fold higher and for Cry1Ac was 78.7 higher for resistant population than susceptible population. Support: FAPESP.

Study of the susceptibility of *Spodoptera frugiperda* (LEPIDOPTERA: Noctuidae) in corn cultures expressing the Cry1F toxin from *Bacillus thuringiensis* in Brazil **Bac09** **•207•**

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Brazil ranked second only to the United States in hectares planted to genetically modified crops in 2016. The use of the technology of genetically modified plants expressing toxins with activity obtained from *Bacillus thuringiensis* (Berliner) (Bt) emerged as an important tool for reducing the intensity of the application of agrochemicals and showed to be effective in controlling some of the major cotton pest. Recently corn producer in the Cerrado region reported that the control of *Spodoptera frugiperda* with Bt corn expressing Cry1Fa has decreased, forcing them to use chemicals to reduce the damage caused by this insect pest. Many putative Cry toxin receptors have since been reported, of which the best characterized are the aminopeptidase N (APN) receptors and alkaline-phosphatases (ALPs). The bioassay data reported here show that insects collected from Cry1Fa corn in the Cerrado region were resistant to Cry1Fa suggesting that resistance contributed to field failures of Cry1Fa corn to control *S. frugiperda*. This study the detection of the midgut receptors of the larvae of *S. frugiperda* (BBMV) was analyzed by determining the specific activity of ALP and APN by SDS-PAGE gel using the substrate for detecting the activity, and also western-blot

with ALP and APN antibodies of *Manduca sexta* (Lepidoptera). These data indicate that the levels of ALP receptors detection and the APN of some of the resistant populations showed low levels of ALP in all analysis, which is a low activity of this receptor when compared to the susceptible population. Demonstrating that the insect populations may be involved with ALP receptors, but not with APN, in these Brazilian populations.

Analysis of cross-resistance of resistant insect colonies from different species to Vip3Ca from *Bacillus thuringiensis* STU Bac10 •208•

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Bacillus thuringiensis (*Bt*) is a gram positive bacterium used in insect control because some strains produce insecticidal proteins. The Vip3 proteins are synthesized and secreted to the medium during the vegetative growth phase. The mode of action of Vip3 proteins parallels that of Cry proteins in that it involves activation by gut proteases, recognition and binding to midgut receptors, pore formation, and cell lysis, finally causing the death of the insect. We tested the susceptibility to the Vip3Ca protein of susceptible and Cry1-, Cry2-, and Vip3-resistant insect colonies from different insect species, to determine whether resistance to other insecticidal proteins could confer cross-resistance to Vip3Ca. As expected, the insect colonies resistant to Cry1 (from *Plodia interpunctella, Helicoverpa armigera* and *Trichoplusia ni*) or resistant to Cry2 (from *H. armigera and T. ni*) didn't show cross resistance to the Vip3Ca protein. However, insect colonies resistant to Vip3Aa or to Vip3Aa/Cry2Ab (from *H. armigera*) showed cross-resistance to the Vip3Ca protein. These results are in agreement with the published binding models where Vip3 proteins do not compete with Cry1 and Cry2 for binding sites, but different Vip3 proteins (Vip3A and Vip3Ca) compete for the same binding sites.

Modification of *a Bacillus thuringiensis* toxin to target soybean aphid (*Aphis glycines*) Bac11 •209•

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Current hemipteran pest management is heavily reliant on chemical pesticides. Management of this order of insects represents one of the greatest challenges in agricultural biotechnology. *Bacillus thuringiensis* (*Bt*)-based technologies have been widely employed for decades to manage populations of lepidopteran and coleopteran pests, but few *Bt* toxins have activity against hemipteran species. To overcome this limitation, we have applied an innovative strategy to enhance the activity of Bt toxins against hemipteran pests. Having isolated a peptide that binds aminopeptidase N in the gut of the pea aphid (*Acyrthosiphon pisum)*, we demonstrated that addition of GBP3.1 to the *Bt* Cyt2Aa toxin enhanced activity against the pea aphid and the green peach aphid (*Myzus persicae*) (Chougule *et al*., 2013). We applied this same approach to new hemipteran targets including the soybean aphid (*Aphis glycines*). Soybean aphid gut binding peptides were selected and the Cry4Aa toxin modified with these peptides for enhanced activity against soybean aphid. We used two approaches for isolation of soybean aphid gut binding peptides, an *in vivo* screen by feeding aphids on a phage display library and eluting phage bound to the gut epithelium, and *in vitro* selection for peptides that bound to recombinant soybean aphid gut proteins, aminopeptidase N and alkaline phosphatase. Five sites were selected for insertion of gut binding peptides into the Cry4Aa toxin. Our results show that the addition of gut binding peptide sequences to *Bt* toxins can enhance their activity against pests that are otherwise challenging to control.

Modification of a Bt toxin to target Asian citrus psyllid (*Diaphorina citri*, Hemiptera) Bac12 •210•

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The citrus industry is threatened by citrus greening, a disease caused by the bacterium *Candidatus liberibacter asiaticus* (CLas). CLas is vectored by the Asian citrus psyllid (ACP) and management of ACP has become a primary focus to limit disease spread. We previously demonstrated enhanced efficacy of a Bt toxin on addition of a peptide that binds to the gut of the target pest (*N. Chougule et al., 2013, PNAS*). Toward this end, *Bacillus thuringiensis* (Bt) isolates from the collection housed at USDA-ARS Beltsville, Maryland, were screened to identify strains with ACP toxicity. Five strains that show basal toxicity on feeding of adult ACP at 500 μg/ml of proteolytically activated toxin were identified, and the trypsin proteolytic profile of the toxic strains characterized. Individual Cry toxins were identified by MS/MS for one of the toxic strains. One of these Cry toxins was selected for modification with ACP gut-binding peptide to enhance toxicity against ACP. ACP gut-binding peptides were isolated by feeding adult psyllids on a loop-constrained heptapeptide (Ph.D.-C7C) library. Sequences encoding four of the selected peptides were cloned for production of mCherry-peptide fusion proteins. A peptide selected based on relative binding strength under both *in vivo* and *in vitro* conditions, was used to engineer the selected Bt toxin. Following engineering, the stability of the modified toxin was evaluated by trypsin digestion and the impact of modification on toxicity against ACP assessed.

Patterns of gene expression and histopathological effects in western corn rootworm (*Diabrotica virgifera virgifera*) neonates, challenged with Cry 34/35 Ab1 Bac13 •211•

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Western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) is a major corn pest in the United States, causing annual losses of over $1 billion. One approach to protect against crop loss by this insect is the use of transgenic corn hybrids expressing one or more crystal (Cry) proteins derived from Bacillus thuringiensis. Cry34Ab1 and Cry35Ab1 together comprise a binary insecticidal toxin with specific activity against WCR. Cry34/35Ab1 is a pore forming toxin, but the specific effects of Cry34/35Ab1 on WCR cells and tissues have not been fully characterized. In this presentation we integrate results from transcriptomics and histopathology for first instar WCR fed Cry34/35Ab1 proteins in diet based bioassays. High resolution histopathology revealed symptoms of intoxication for Cry34/35Ab1 including swelling and sloughing of enterocytes, constriction of midgut circular muscles, stem cell activation, and obstruction of the midgut lumen. With Next Generation Sequencing technologies, high-throughput RNA sequencing (RNA-seq) was conducted to examine gene expression in WCR neonates challenged with individual and combined Cry34Ab1 and Cry35Ab1 proteins. Results of three different statistical comparisons identified 114 and 1300 differentially expressed transcripts (DETs) in the Cry34Ab1 and Cry34/35Ab1 treatments, respectively, as compared to the control. The combined results of the histopathology and differential gene expression studies lay the groundwork for testable hypotheses aimed at extending the durability of Cry34/35Ab1 as an insect resistance trait.

The intracellular region of the *Bombyx mori* cadherin-like protein is not necessary to mediate cytotoxicity of Cry1A toxins STU Bac14 •212•

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The cadherin-like protein in lepidopteran insects, known as a receptor for *Bacillus thuringiensis* Cry1A toxins, is a single-pass membrane protein and divided into extracellular and intracellular regions. The extracellular region has been shown to be important for toxin binding and oligomerization, but the role of the intracellular region is still obscure, although the signal transduction hypothesis suggested its role in Cry1A intoxication. Recently the cadherin-like protein is also paid attention in the synergistic effect with the ABC transporter C2 (ABCC2) in Cry1A intoxication, but the mechanism is poorly understood. In the present study, we generated two mutants of the *Bombyx mori* cadherin-like protein (BtR175): BtR175-DEL, which completely lacks the intracellular region, and BtR175-GPI, which is fused with the glycosylphosphatidylinositol (GPI)- anchor signal of *B. mori* aminopeptidase N1 to express as a GPI-anchored protein. As well as wild-type BtR175, both the mutants conferred cultured cells the susceptibility for Cry1Aa, Ab, and Ac toxins. Furthermore, when both mutants were co-expressed with *B. mori* ABCC2, swollen cells were observed in lower toxin concentrations, compared to cells expressing BmABCC2 alone and namely both mutants preserve the synergistic effect with ABCC2. These results indicate that the role of the cadherin-like protein as a Cry toxin receptor, even in the synergism with ABCC2, depends solely on its extracellular region.

Critical amino acids for the insecticidal activity of Vip3Af from *Bacillus thuringiensis* Bac15 •213•

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Vip3 vegetative insecticidal proteins from *Bacillus thuringiensis* are an important tool for crop protection against caterpillar pests in IPM strategies. Despite already being implemented in Bt-crops, while there is wide consensus on their general mode of action, the details of their mode of action are not completely elucidated and their structure remains unknown. In this work the alanine scanning technique was performed on 558 out of the total of 788 amino acids of the Vip3Af1 protein. From the 558 residue substitutions, 19 impaired protein expression and 10 compromised the insecticidal activity against *Spodoptera frugiperda*. Substitutions that drastically reduced insecticidal activity mainly clustered in two regions of the protein sequence (amino acids 167-272 and amino acids 689-741). Most of the substitutions that impaired the activity to *S. frugiperda* behave likewise to *Agrotis segetum*, with few exceptions. The characterisation of the sensitivity to proteases of these 14 mutant proteins displaying decreased insecticidal activity revealed 6 different band patterns as evaluated by SDS-PAGE. The study of the intrinsic fluorescence of all selected mutants revealed only slight shifts in the emission peak, likely indicating only minor changes in the tertiary structure. An *in silico* modelled 3D structure of Vip3Af1 is proposed for the first time.

Construction of plasmid vector for dsRNA synthesis with *Bacillus thuringiensis* by using sporulation-dependent promoter STU Bac16 •214•

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RNA interference (RNAi) is a post-transcriptional gene regulation mechanism found in virtually all plants and animals including insects. RNAi has been considered as an alternative strategy to control agricultural pests whereby double-stranded RNA triggers a potent and specific inhibition of its homologous mRNA. Since double-stranded RNAs are required for various RNAi applications, there is a need for cost-effective methods for producing large quantities of high-quality dsRNA. The gram-positive bacterium *Bacillus thuringiensis* has insecticidal proteins produced during the stationary/sporulation phase of growth with expression of its genes being driven by sporulation dependent promoters. To develop a dsRNA mass-production method utilizing *B. thuringiensis*, the pHT1K-EGFP plasmid vector, which transcribes sense and anti-sense EGFP under the control of *cyt1*Aa sporulation-dependent promoter with STAB-SD sequence was constructed, and the transcription level was measured by Illumina RNA-seq. The sequencing result was calculated by the Kallisto program to demonstrate that the transcription level of EGFP was approximately 30 and 4,000 times higher than that of the reference genes, *mbl* and *rpoB,* respectively. Furthermore, qPCR analysis results were in good accordance with RNA-seq results. These results demonstrated the potential of *B. thuringiensis* as a new platform for dsRNA production.

Calcium-independent cellular PLA_2 prevents lipid peroxidation, which is detrimental to development and immunity of the Indian meal moth, *Plodia interpunctella* Bac17 •215•

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Reactive oxygen species (ROS) induces oxidation against various biomolecules including fatty acids. Calcium-independent cellular phospholipase A_2 ($iPLA_2$) has been known to function maintenance of fatty acids in phospholipids in mammals. An insect $iPLA_2$ (Pi-$iPLA_2$) was predicted from transciptomes of the Indian meal moth, *Plodia interpunctella*. It encodes 835 amino acid sequence, which possesses 5 ankyrin repeats in N terminal domain and papatin lipase domain in C terminal region, respectively. It was expressed in all developmental stages from egg to adult. In larval stage, it was expressed in all tested tissues of hemocytes, fat body, gut and epidermis. RNA interference (RNAi) against Pi-$iPLA_2$ was performed with specific double-stranded RNA (dsRNA) with more than 70% efficiency in reduction of its expression. Under this RNAi condition, *P. interpuctella* exhibited developmental retardation with significant accumulation of lipid peroxidation measured by the amount of melondialdehyde. The RNAi of Pi-PLA_2 also impaired cellular immune responses of *P. interpunctella*, in which hemocyte nodule formation was significantly decreased. The hazard effect of lipid peroxidation was simulated with treatment of chlorine dioxide (ClO_2), which is an insecticidal agent by generating ROS in insect cell and *P. interpunctella*. ClO_2 treatment increased lipid peroxidation in a dose-dependent manner in Sf9 cell line. However, the addition of an antioxidant (vitamin E), reduced the formation of lipid peroxidation. ClO_2 treatment also increased lipid peroxidation in the larval fat body of *P. interpunctella*. Furthermore, the larvae treated with RNAi against Pi-PLA_2 were significantly susceptible to ClO_2 treatment. These results suggest that Pi-$iPLA_2$ plays a crucial role in remodeling fatty acid composition of phospholipids. It also suggests that chlorine dioxide treatment causes the lipid peroxidation via ROS, which explains its insecticidal activity.

Selection of *Bacillus thuringiensis* strains against *Fusarium oxysporum* f.sp. *vasinfectum* Bac18 •216•

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Fusarium wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* on cotton is considered a major disease of this crop. The pathogen is difficult to control,

having high persistence in the soil due to its capacity to parasitize other crops, being saprophyte and able to produce chlamydospores which enable it to survive in the soil for long time without presence of the main host. The control of this pathogen is based on the use of varieties with some degree of resistance, use of certified seed and the use of fungicides for seed treatment. Once detected in the field, plant protection is based on the use of chemicals. In this context, it is justified the development of alternatives for the management of this pathogen in the field. Biological control stands out as a viable alternative for the control of this pathogen. *Bacillus* are known bioregulators and natural antagonists of various pathogens. Thus, the objective of this study was to select toxic strains of *Bacillus thuringiensis* (Bt) to *F. oxysporum* and adjust an *in vitro* method for implementation and evaluation of the tests for selection. It was possible to determine the best culture medium for direct confrontation test, proposing a grading scale to facilitate the selection of the best strain with the paired test, identified among the strains selected in the confrontation assays, those that can produce volatile metabolites and non-volatile metabolites toxic to *F. oxysporum* and test these selected strains to the control of the disease during seed germination in greenhouse assays. With this last assay, it was noted a potential protective effect of some Bt strains during cotton seed germination.

Dynamic distribution and colonization of inoculated *Bacillus thuringiensis* on the tissues of *Arabidopsis thaliana* **Bac19** **•217•**

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The dynamic distribution and colonization of *Bacillus thuringiensis* (Bt) in *Arabidopsis thaliana* (L.) tissues was studied using the transformed Bt HD73^{Cry-}GFP strain which expresses the green fluorescent protein (GFP) as a marker. After HD73^{Cry-}GFP inoculation, the distribution of the labeled Bt was recorded both internally and externally over the entire plant, including the phyllospere and rhizoshere using fluorescence and a confocal microscopy. The Bt HD73^{Cry-}GFP strain was able to stably colonize both internally and externally the roots, stems and leaves of *A. thaliana* under gnotobiotic and non-UV conditions. This work provides fundamental ecological information on the dynamic distribution, both internally and externally, of Bt HD73^{Cry-}GFP strain on *A. thaliana* tissues, our work provides basic information for a better understanding of Bt-plant interactions.

Promotion of plant growth by *Bacillus thuringiensis* in Brazil **Bac20** **•218•**

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The use of *Bacillus thuringiensis* (Bt) in pest control is already well known. This bacterium also has other important characteristics, such as the ability to promote plant growth, which should be further investigated. For this was used a Bt strain toxic to lepidopteran insects. The effect of the seeds being immersed in bacterial suspension (with and without chemical treatment) of 10 cotton genotypes was evaluated. The seeds were immersed in bacterial suspension with 10^8 CFU (spores) per mL and then seeded in trays for seedling production. The plant emergence velocity was evaluated and on the 15th days of the sowing, four genotypes (IMA 6035, IMA 8405 and IMA 5675 (not chemically treated) and IMA 2106 (chemically treated)) were harvested due to their most vigorous appearance. The height, root length, fresh and dry matter of the whole plant were recorded. The results indicate that seeds being immersed in bacterial suspension, with and without chemical treatment, can affect the emergence speed of the plants and that the IMA 6035 genotype with the seeds imposed under these conditions of bacterial suspension immersion presented the higher results for height (8%) and for plant dry matter (13%) compared with the treatment without the bacteria. In a second moment, the effect of the methods of inoculation of Bt was evaluated. For this assay, the IMA 1318 genotype and three inoculation methods were used: (i) Seeds being immersed in bacterial suspension with 10^8 CFU per mL (BS), (ii) BS (method (i)) + weekly applications of 1 mL of the bacterial suspension around the roots of the plants (with 10^7 CFU per mL), (iii) BS + addition of the bacterial pellet to the soil (with 10^7 CFU per mg). The evaluation occurred at the 35th days of the planting. The results showed that there was an increase in root dry matter yield provided by the inoculation methods for the analyzed genotype. A higher number of cells were observed inside the roots in the treatment (ii). For the IMA 1318 genotype, the root dry matter production increased regardless of the inoculation method of the bacteria.

The *Caenorhabditis elegans* CUB-like-domain containing protein F35E12.10 functions as a receptor for *Bacillus thuringiensis* Cry6Aa toxin **Bac21** **•219•**

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Plant-parasitic nematodes caused huge economic losses to agriculture per year, and nematicidal activity was exhibited in two major families of *Bacillus thuringiensis* crystal proteins, Cry5 and Cry6. Previous work showed that binding receptors were critical factors involved in processes of crystal protein intoxication. Now, the receptors of crystal protein Cry5Ba were identified as glycolipids in *Caenorhabditis elegans*. However, the receptors of Cry6 remain unknown. In this study, the CUB-like-domain containing protein F35E12.10, from *C. elegans* proteins released by phosphatidylinositol-specific phospholipase C (PI-PLC), was identified as a binding protein for Cry6Aa by affinity chromatography. F35E12.10 contained a predicted glycosylphosphatidylinositol (GPI) anchor site and was reported to locate in lipid rafts of intestinal cell surface. Western ligand blot and ELISA verified the binding interaction between Cry6Aa and F35E12.10 with high affinity and specificity. Bioassays showed that gene *f35e12.10* was implicated in Cry6Aa intoxication. Furthermore, *f35e12.10* mediated Cry6Aa uptake in *C. elegans* gut cells, suggesting that *f35e12.10* was required for interaction of Cry6Aa with intestinal cells. Finally, we observed that the mutation of gene *f35e12.10* did not decrease susceptibility to Cry5Ba in *C. elegans*. These results demonstrated that F35E12.10 is a functional receptor for Cry6Aa protein. To our knowledge, this is the first report about Cry6 protein binding receptors in *C. elegans*.

Physiological and histopathological effects of new proteins toxic to southern green stink bug (*Nezara viridula*) **Bac22** **•220•**

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A number of Hemipteran insect species, including stink bugs, are pests of major crops such as cotton and soybean. Stink bugs feed by extra-oral digestion. They pierce plant tissues, inject digestive enzymes, and suck nutrients from the tissues. New traits are required that will be capable of controlling Hemipterans when expressed in crop plants. Screening of diverse microbial strains by the Crop Science Division at Bayer has led to the identification of proteins that are toxic to the southern green stink bug. To begin to elucidate the mode of action of the proteins, insects were fed purified proteins, and their digestive systems were dissected and examined microscopically. Morphological and histological changes in different compartments of the digestive systems were observed, giving an indication of the location where the toxic effect is exerted.

Mining microbial metagenomes for novel insecticidal proteins **Poster – Bac23**

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Microbes are genetically highly diverse and have evolved a variety of strategies in order to compete for nutrients, including pathogenesis of insects. Insecticidal microbes and their toxin genes have been explored for agricultural uses for decades. Traditional methods to discover insecticidal bacteria and toxin genes are based on isolating bacteria on selective media, focusing primarily on the spore-forming bacteria *Bacillus thuringiensis*. Thus, the diversity and novelty of isolated bacteria is limited by what can form colonies on the selective media. An alternative approach to identifying toxin genes is based on nucleotide homology searches of all available sequenced genomes. However, most of the bacteria from soil microbial communities have not been cultured, and their genomes have not been sequenced. Our approach to identify novel insect toxins is based on mining soil metagenomes, where the genetic potential of the whole microbial community includes uncultured species. In addition, we use multiple enrichment strategies on natural microbial communities to increase the abundance of insecticidal bacteria. The metagenomes from the enriched communities are then assessed using the Second Genome discovery platform which includes statistical models trained on known insect toxins. The output from this analysis is a set of potentially novel insecticidal proteins that can be tested in vivo.

The analysis of *B.thuringiensis* tolerance to insect hemolymph and its effect on infection efficiency **Poster – Bac24**

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B.thuringiensis (Bt) is an impotent insect pathogenic bacteria and it infects insects by breaking the intestine and multiplication in the living host's hemolymph. Because its high and specific larvicidal activity, Bt is currently used as an insecticide. When Bt spore and crystal mixture was ingested by susceptible insects, the protoxins solubilized and activated proteolytically in the host midgut. The activated protein specific bind to midgut receptors, forming pores in the epithelial midgut and break down the epithelial cells. Then the Bt spores or germinated cells were go through the hole into host hemolymph. The Bt spores or cells multiplication in the hemolymph caused septicaemia and kill the insects. Although the Bt are armed with immune inhibitor A (InhA) metalloprotease, the insects had evolved strong immune system to combat with the bacteria infection. The ability of Bt break through immune system and multiply in hemolymph was important for Bt toxicity. However, there are still lack of data on compare Bt multiply ability in insect's hemolymph directly. In this report, we assayed the hemolymph (from *Protaetia brevitarsis*, removed cells) effects on Bt cells. The strain HBF-18 shown sensitive to hemolymph (influence rate: 0.06) and the strain G03 shown resistance to hemolymph (influence rate: 1.52) were selected for further analysis. The inject infection assay were performed on third instar larvae of *Protaetia brevitarsis*. The data shows, G03 have higher lethality than HBF-18 in lower injection dose while HBF-18 have stronger toxicity than G03 in higher injection dose. A time course of hemolymph effects on HBF-18 and G03 were subsequence performed. The data shows, most of the HBF-18 strain cells were died in earlier period (first two hour) but it quickly multiplies in later period (after six hour). Different from HBF-18, G03 shows resistance to hemolymph in earlier period, and have lower multiplies speed later period. In conclusion, we illustrated, although few strain sensitive to insect hemolymph, most strain were resistance to insect hemolymph and the distribution of hemolymph infect rate on Bt was approximately normal distribution. People can screen for high resistance Bt strain for pesticides development.

The purification and mechanism analysis of *Holotrichia oblita* toxic Vip1/Vip2 binary protein **Poster – Bac25**

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White grubs (larvae of Scarabaeidaes family) are serious agricultural pests worldwide. In China, *Holotrichia oblita* is one of main white grub pests and causing significant reductions of yield and great economic losses. White grubs are difficult to control, principally because of its soil living habits. In previously work, *Bacillus thuringiensis* (Bt) strain HBF-18 (CGMCC2070), containing two Bt genes (*vip1Ad1* and *vip2Ag1*), has been confirmed toxic to *H. oblita* larvae. The Vip1Ad1 and Vip2Ag1 protein is a binary toxin toxic to *H. oblita* larvae, and its toxicity is significant higher than those of Cry 8 proteins. The study of the mechanism of action of this Vip1Ad1/Vip2Ag1 binary toxin is important for their utilization in the control of *H. oblita*. In this study, we investigated the methods of extraction and purification of Vip1Ad1 and Vip2Ag1. The results showed that Vip1Ad1 and Vip2Ag1 are secretory proteins, and its inefficiency to extract these two proteins using ammonium sulfate precipitation method. But we have obtained purified Vip1Ad1 and Vip2Ag1 from fermentation broth after treated with Cross-Flow and then purified by ion-exchange and anion-exchange, respectively. Furthermore, we investigated the activation process of these two proteins. The results indicated that Vip1Ad1 (approximately 90 kDa) digested with Trypsin resulted in a protease-resistant core fragment of approximately 70 kDa (Vip1Ad1-T), but Vip2Ag1 could not be digested by both Trypsin and Chymotrypsin. What's more, we analyzed the binding characteristics of Vip1Ad1/Vip2Ag1 with brush border membrane vesicles (BBMV) in *H. oblita* larvae. The results showed that the protoxin of Vip1Ad1 and Vip2Ag1 could not binding with each other in solution; And, the Vip1Ad1, either protoxin or trypsinized, bound to BBMV from *H. oblita*, whereas the Vip2Ag1 alone did not; The binding of Vip2Ag1 was determined only when the BBMV were reacted with trypsinized Vip1Ad1, but not when they were reacted with Vip1Ad1 protoxin; In particular, the Vip2Ag1 bound to Vip1Ad1 oligomers-BBMV complex and the oligomers could generated both in solution and on the cell surface after Vip1Ad1 monomers bound with BBMV, but in either scenario, the Vip1Ad1 oligomers were generated only after proteolysis of Vip1Ad1 protoxin; And, the results of saturation binding assays demonstrated that Vip1Ad1 bound specifically to binding sites on the BBMV from *H. oblita* larvae. In addition, we identified Vip1Ad1-binding proteins in the midgut of *H. oblita* larvae. The results demonstrated that the Vip1Ad1 binding protein is not cholesterol and not GPI-anchored protein. Our work promotes the understanding of mechanism of Vip1/Vip2 binary toxin against *H. oblita* larvae.

A novel Type Six Secretion System in the entomopathogenic bacterium *Xenorhabdus bovienii* may impact insect virulence and bacterial competition **STU Bac26**

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Xenorhabdus bovienii is a Gram-negative bacterium that exhibits a dual lifestyle, behaving as a mutualist to at least Steinernema nematode species and as a pathogen to insects. *X. bovienii* displays a type VI secretion system (T6SS) in its genome that has yet to be studied. The T6SS is a one-step mechanism widely used by Gram-negative bacteria to transport effector proteins into target cells. T6SSs have been shown to play a key role in pathogenesis of host cells and in the competition with other bacteria. In this study, we used a homologous recombination approach to generate a *X. bovienii* mutant lacking hcp, a crucial structural gene of the T6SS, then conducted bioassays to assess the role of this secretion system. Preliminary results show a decrease in melanization and mortality in insects infected with the T6SS mutant compared to those infected with wild-type *X. bovienii*, suggesting a decrease in virulence in the mutant strain. Additionally, In vitro bacterial competition assays resulted in the wild-type strain outcompeting

the mutant strain. We speculate the T6SS may play a role in bacterial competition and insect virulence, which may contribute to the success of the mutualistic relationship between *X. bovienii* and *Steinernema nematodes*.

Evaluation of *Galleria mellonella* (Lepidoptera: Pyralidae) as a complementary model organism to study enteropathogenic *E. coli* STU Bac27

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Enteropathogenic *Escherichia coli* (EPEC) is a common diarrheal pathogen that affects approximately 0.8 million children per year in developing countries. EPEC uses a complex bacterial structure called the Type III Secretion System (T3SS) to inject bacterial effector proteins directly into intestinal epithelial cells, manipulating the cells' behavior and causing disease. Rabbits are a commonly used model to study EPEC. However, rabbits have several limitations as hosts including expense, specialized handling, ethical concerns and federal regulations regarding the use of laboratory vertebrates. Insects are potential complementary models for studying bacterial pathogenesis *in vivo.* Benefits of using insect hosts include low-cost, ability for mass infections, and analogous immune strategies to vertebrates. We hypothesize that larvae of the greater wax moth *Galleria mellonella* (GM) can serve as an effective model in studying the EPEC T3SS. In order to determine if EPEC can infect GM we injected EPEC into the body cavity (hemocoel) of the insects. We observed that live wild-type EPEC kills GM and that heat-killed EPEC does not kill, indicating that EPEC actively infects GM. To explore the mechanism of killing, we also considered EPEC mutant strains that lack different components of the T3SS. These mutants were defective in killing GM, indicating that EPEC kills these insects in a T3SS-dependent manner. We are currently adapting this model to test potential T3SS inhibitors and to study individual T3SS effectors. We are also attempting to model oral infections in insects to more closely represent human infection.

MICROSPORIDIA DIVISION POSTERS

The role of the decapping enzyme EOL-1 in *C. elegans* intracellular infection response Msp01 •221•

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Microsporidia comprise a phylum of obligate intracellular parasites most closely related to fungi. Although they infect a wide range of animal species and can cause lethal infections in immunocompromised patients, relatively little is known about the mechanisms of host defense against microsporidia. *Nematocida parisii* is a species of microsporidia that is a natural pathogen of *C. elegans*. *N. parisii* infects the *C. elegans* intestine, causing fusion of intestinal cells and ultimately lethality (Troemel et al, PLoS Biology 2008; Balla et al, Nature Microbiology 2016). Our lab has profiled the *C. elegans* transcriptional response to *N. parisii* (Bakowski et al, PLoS Pathogens 2014), with the goal of identifying features of the host response to microsporidia infection. One gene found to be induced by *N. parisii* infection was *eol-1*, a de-capping and exonuclease enzyme that has been reported to be involved in regulating pathogen-induced aversive olfactory learning (Shen et al, J. Neuroscience 2014). Our RNAseq data shows that *eol-1* is strongly up-regulated during *N. parisii* infection, beginning early at 8 hours post infection. In uninfected worms, we find that EOL-1 is expressed primarily in the spermatheca and URX neurons based on analysis of EOL-1 transcriptional and translational fusions. In addition, we found that EOL-1 expression is also induced in response to infection by the Orsay virus, another natural intracellular pathogen of *C. elegans* (Félix et al, PLoS Biol. 2011). Interestingly we find that in worms exposed to *N. parisii* or Orsay virus, *eol-1* expression is induced in the intestine, which is the tissue targeted by both pathogens. Our preliminary results from analysis of *eol-1* mutants suggest that *eol-1* plays a role in host response to intracellular infections.

Secretion of the peritrophic matrix in the honey bee, *Apis mellifera,* midgut is impaired by *Nosema ceranae* infection Msp02 •222•

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The honey bee (*Apis mellifera*) peritrophic matrix (PM) degrades after the bee's midgut becomes infected with the microsporidian *Nosema ceranae*. Consequently the PM becomes less functional in its roles as a substrate for digestive enzymes, as a barrier against ingested pathogens, and as a structure to move pollen grains through the midgut. We were able to observe PM structure and integrity by removing the midgut of a bee and clearing it in 10% KOH. The high chitin content of the PM provides it with a strong affinity for the fluorescent stain calcofluor white. Pollen grains also stain well with calcofluor white. By this method we could easily observe the structure of the PM, and its retention of pollen grains. We found that *N. ceranae* spores do not damage the PM directly, by releasing their polar tubes. Instead, PM secretion by midgut epithelial cells is impaired when these cells are infected.

The abundance and distribution of *Nosema ceranae* in honeybees in Mo'orea and Tahiti, French Polynesia. Msp03 •223•

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The global distribution and extent of infection by *Nosema ceranae* and *N. apis* in the European honeybee, *Apis mellifera,* is of increasing interest due to the widespread concern over the current decline of honeybees. Although *Nosemosis* has been found in *A. mellifera* across the globe, this disease has not yet been investigated in French Polynesia, where honey production, pollinator services, and the honeybee trade contribute substantially to the local economy. From October to November 2013, I collected 20-40 bees (*A. mellifera*) per hive from 21 hives across 14 locations around Mo'orea and Tahiti, French Polynesia. The midgut was excised from each bee and rinsed in DI water prior to examination under phase contrast microscopy at 400 magnification. All tools were sterilized with 5% bleach before dissection of each bee. Infection levels were categorized by spore intensity and remaining midgut tissues of each bee were stored in 0.01M EDTA at -20°C for DNA extraction. DNA was extracted from at least two infected bees from each hive using a modified Chelex method. I used a previously published duplex-PCR protocol based on species–specific primers for *N. ceranae* and *N. apis* to amplify the RNA polymerase II large subunit (RPB1). Visualization of the fragments on a 1.2% electrophoresis gel determined the presence of *N. ceranae* in all of the infected bees that were examined. Prevalence of infection with *N. ceranae* within hives ranged from 3% to 77% in Mo'orea and from 10% to 33% in Tahiti. Results were reported to the Department of Agriculture in Mo'orea, as well as to the local apiculturists in Mo'orea and Tahiti.

Anti-*Nosema* activity of entomopathogenic fungi cultural filtrates in honeybee nosemosis STU Msp04 •224•

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The population of managed honeybees has been dramatically declining the recent past in the worldwide. The one of most common disease of honeybees is nosemosis which is caused by *Nosema ceranae*. *Nosema ceranae* causes significant detriment to honey production and results in economic losses critically. In our knowledge, Fumagillin is the only antibiotic approved for control of nosemosis in honeybees. However, this antibiotic may have unintended effects on the honey bee host, ultimately contributing

to increased prevalence and pathogenicity of *N. ceranae*. To select fungal isolates with anti-*Nosema* activity against *N. ceranae*, in this study, entomopathogenic fungi cultural filtrates were prepared from 342 fungal isolates, and anti-*Nosema* activities were evaluated by *in vitro* polar tube germination assay method. As a result, six isolates, *Paecilomyces marquandii* 364, *Metarhizium anisopliae* 296, *Beauveria brongniartii* 183, *B. bassiana* 35 and 59, *Pochonia* sp. 60 were finally selected. These fungi cultural filtrates had bactericidal activity against *N. ceranae*. Safety and inhibition of nosemosis were further evaluated in honeybees. *Paecilomyces marquandii* 364 and *Pochonia* sp. 60 incrreased the survival rate of honeybees and decreased the production of *N. ceranae* spores. Fungi cultural filtrates of selected isolates have a good effect on inhibiting the nosemosis of honey bees.

Serendipitous Finding: Wild populations of Spruce Budworm are infected with both *Nosema fumiferanae* and a *Cystosporogenes* species **Msp05** **•225•**

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The most ubiquitous natural enemy found in close association with spruce budworm, *Choristoneura fumiferana* populations is the microsporidian *Nosema fumiferanae*. However during a recent survey, a *Cystosporogenes* species was isolated for the first time from wild spruce budworm populations. Detecting and distinguishing these natural enemies in 2nd stage *C. fumiferana* larvae by light microscopy to determine the health of the population for onward forest protection decisions is not feasible on an operational basis. We therefore developed a much more sensitive molecular platform, a multiplex real-time loop-mediated isothermal amplification (m-LAMP) assay to synchronously amplify, detect and distinguish *N. fumiferanae* and the *Cystosporogenes* spp. DNA in a single reaction using crude DNA without conventional extraction. The optimum amplification occurred within 30 min at 64 °C, and the amplified products were distinguished based on the different melting temperatures (*Tm* values) of the two specifically m-LAMP amplified products. Alternatively, the amplified products were detected by fluorescence, lateral flow dipstick and also confirmed by gel electrophoresis. The detection limit of (LOD) of the m-LAMP assay was determined to be 100 pg/µl and similar to that of multiplex PCR. The specificity of the m-LAMP assay was evaluated using 16 known microsporidia strains in the genera, *Nosema*, *Vairimorpha*, *Cystosporogenes* and *Endoreticulatus*. The m-LAMP showed 100% inclusivity and exclusivity. Additionally, the developed m-LAMP assay was validated using DNA extracts from spores recovered from pooled *C. fumiferana* larvae. In all cases, *Nosema fumiferanae* and the *Cystosporogenes* spp. were detected in pooled spores isolated from three different geographical populations of *C. fumiferana*. The developed m-LAMP assay is simple, rapid, sensitive, specific and suitable for direct detection of *N. fumiferanae* and a *Cystosporogenes* spp. in field populations of *C. fumiferana* without complex DNA extraction. Hence it has the capability to be used for on-site disease diagnosis and field surveys. Most importantly in order to avoid predetermination of a microsporidian species and resultant inaccurate data (confounded effects) generated in a bioassay when using spores isolated from pooled insects; it will be prudent to implement a similar assay to delineate microsporidia species present in pooled spores prior to bioassays.

Application of RNAi to the control of microsporidian parasite *Nosema ceranae* in honeybee (*Apis mellifera*) **Msp06**

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Honeybees are one of the most important insects in the world as a pollinator of numerous agricultural crops. Recently, however, honeybees have faced many diseases which threaten bee colony including a serious population decline phenomenon called CCD (colony collapse disruption). *Nosema ceranae* is a pathogen causing nosemosis, which is one of the most common diseases in honeybees. Microsporidian *N. ceranae* is an obligated intracellular parasites and depends on its host for energy and metabolic needs. Here we selected the several genes from mitosome of *N. ceranae* to develop RNAi for the control of *Nosema* species. Especially, TOM40 (translocase out membrane), FNR (Ferredoxin NADPH reductase) 1 and 2, Nar1 genes were chosen and their dsRNA were synthesized because their amino acid sequences show less homologous with those of the honeybees (*Apis mellifera*). After infection of *N. ceranae*, the honeybees were treated with dsRNA either by using single or combining two or more. The infection rate and specific gene silencing of *Nosema* were than analyzed. The targeted FNR1 and FNR2 genes were affected highly by dsRNAs, resulting in low productivity of *Nosema* spores. In addition, dsRNA FNR1 and FNR2 enhanced honeybee survival rate. To increase the effect of gene silencing, the effect of dsRNA complex particles with chitosan was investigated. Treatment of chitosan to dsRNA enhanced the effects for reducing spore productivity and bee survival rate more than the naked dsRNA treatment. To verify the function of FNR genes, additionally, the recombinant FNR proteins were overexpressed in insect cells using baculovirus expression system. The predicted FNR activity was confirmed through Cytochrome C oxidase activity assay, and the overexpression of the FNR increased the cell viability.

FUNGI DIVISION POSTERS

Studying the interaction among fungi involved in the multiple biological control of leaf-cutter ants **Fun02** **•227•**

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The use of multiple biological control agents simultaneously requires that the different agents do not affect negatively each other. With the aim to test this hypothesis with microorganisms used against leaf-cutter ants, we set up laboratory assays to evaluate the in vitro interactions among an entomopathogen, a mycopathogen and the fungi cultivar of leaf cutter ants. We first tested the two biocontrol agents against each other; i.e. the entomopathogen *Beauveria bassiana* with the mycopathogen T*richoderma lentiforme*. Second, we tested the effect of *B. bassiana* on one experiment and of *T. lentiforme* on another, against the ants' cultivar, *Leucoagaricus* sp. We measured their growth rate through time on PDA in co-ocurrence as well as alone (controls). We found no effect of the entomopathogen over *Leucoagaricus* sp. as well as of the *Leucoagaricus* sp. over the mycopathogen. We also found a negative effect of the mycopathogen over *Leucoagaricus* sp. which encourages the use of *T. lentiforme* as a potential biological control agent, as well as over the entomopathogen, in comparison to the controls. The second result warns regarding the simultaneous use of both controllers. The use of conidia in the application of these two agents could prevent the negative effect of the mycopathogen over the entomopathogen due to the fact that the germination should occur over different substrates in nature.

The importance of studying preservation methods of microorganisms used in the biological control of ants **Fun03** **•228•**

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Biological control methods rely on the use of live organisms against target pests. There are different types of biological control strategies; however with social insects such as ants, which formed supernumerary colonies with a reproductive queen highly protected, it is necessary to apply the biological control agents several times over long periods in the field. To fulfill this need appropriate methods for conserving the biological control agents are needed. In our case we studied common used laboratory methods to conserve fungi such as keeping cultivars in Petri dishes in the fridge at 4°C as well as storage of conidia in ultrafreezers at -80°C. Our biological control agents involved the

entomopatho-gens *Beauveria bassiana* and *Purpureocillium lilacinum* and the micopathogen *Trichoderma lentiforme* (ex *T. harzianum* complex). We grew these fungi on PDA media in order to obtain their conidia and prepare suspensions of known concentrations which were used, either for inoculating ants in the case of entomopathogens, or for doing Petri dishes challenges with the mycopathogen against *Leucoagaricus* sp., the leaf-cutter ants´ cultivar. We found that both methods were effective in maintaining their virulence for all the fungi except for *P. lilacinum* which conidia have lost its virulence using both preservation methods. Due to this result, we further evaluate the virulence when *P. lilacinum* was stored using a layer of mineral oil on top of PDA slant culture tubes kept in the fridge. Again, we did not detect virulence when expected although we observed an increased mortality at the end of the 15 days trial, and we recovered few ants with this fungus. Therefore, we have shown in this study that *B. bassiana* and *T. lentiforme* can be stored in ultrafreezers for at least 2 years and in the fridge for 6 months without losing their pathogenicity whereas for *P. lilacinum* further storage methods need to be tested to maintain its virulence, and we suggest that several passages through the host might be necessary in order to keep its virulence.

Intensive studies on the biodiversity of fungal entomopathogens of mosquitoes and other dipteran vectors of major human and animal diseases in central Brazil **Fun04** **•229•**

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This poster summarizes three years of work (2014–2017) in the central Brazilian states of Goiás and Tocantins to explore, to identify, to isolate and preserve (whenever possible), and to characterize the biodiversity of fungal entomo-pathogens (EPFs) affecting dipteran vectors of serious human and animal diseases. Not unexpectedly, the major emphasis was on pathogens affecting mosquitoes, but fungi affecting other arthropods were also collected from all sites visited. Major surprises among the results of these studies were that *Leptolegnia chapmanii* was–while little collected in the US and once in Argentina before now–occurred widely and commonly in central Brazil but without obviously producing its sexual stages on those Brazilian sites. Collections of *Pandora bullata* from calliphorid flies in a mountainous area of northern Goiás were extensive but this fungus, too, was found to be extremely reluctant to produce its resting spores that formed readily in previously known North American collections of this species. This poster will present the key highlights of this project including new species of fungi and oomycetes, and larvae (maggots) of *Musca domestica* were confirmed to be suspectible in laboratory studies to , and a new hypothesis about possible biogeographical effects on the types of spores produced by fungal entomo-pathogens, and a listing of the isolates of EPFs obtained during this study. This project was funded as CAPES Science Without Borders project PVE 71/2013, 149988.

Activity of *Culicinomyces* spp. (Hypocreales: Cordycipitaceae) against *Aedes aegypti* eggs, larvae and adults **STU** **Fun05** **•230•**

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Culicinomyces spp., that infect and kill aquatic nematoceran larvae after ingestion of conidia, need more attention in the effort to develop a mycoinsecti-cide against mosquito vectors of arboviruses, especially *Aedes aegypti*. In this study, larvicidal activity against *A. aegypti* of the majority of available *C. clavi-sporus* (ARSEF 372, 582, 644, 706, 964, 1260, 2478, 2479, 2480) and *C. bisporalis* isolates was tested. Ovicidal and adulticidal activities of the most promising isolates, ARSEF 644, 964 and 2479, were also investigated in *A. aegypti*. Third instar larvae treated with ARSEF 372, 644, 964 or 2479 at 10^6 conidia/ml died most rapidly (LT_{50} 1−1.3 days and LT_{90} 2.6−2.8 days). Values of LC_{50} and LC_{90} of these isolates varied between $2.1x10^5$–$3.6x10^5$ conidia/ml and $1.9x10^6$–$2.5x10^6$ conidia/ml, respectively. However, none of the selected isolates had any activity against eggs when treated directly with conidia ($5x10^6$ conidia/ml). Adults, which were fed with 10% sucrose solution added with 10^6 conidia/ml, were slightly susceptible to oral infection (25−46% mortality 10 days after feeding and 13% in the control without conidia). Results, made clear that highly virulent *C. clavi-sporus* isolates with potential for the control of *A. aegypti* larvae are available, and these isolates should be considered for the development of biological control of this important vector.

Adaptation of a simple method to detect entomopathogenic fungi from mosquito larvae **Fun06** **•231•**

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Aquatic immature mosquito stages, whether alive or dead, are delicate and not easy to handle in the field for monitoring fungal infections. The effectiveness of a new, simple technique to detect entomopathogenic fungi directly from larvae was checked for four fungal species with known larvicidal activity in *A. aegypti*. Larvae were exposed for 24 h to conidia of *Beauveria bassiana*, *Culicinomyces clavi-sporus*, *Metarhizium anisopliae* or *Tolypocladium cylindrosporum* and then set in Petri dishes on a soft water agar medium (WA) with chloramphenicol, thiabendazole, crystal violet and pH 5.5 and incubated at 25˚C. Mortality of larvae, and the presence of mycelium or conidia of the tested fungus, and of any saprobic fungi or bacteria on the larvae were recorded daily. All tested fungi except *C. clavisporus* developed mycelium and conidia on a considerable portion of dead larvae regardless of the initial conidial inoculum within 10 days; *C. clavisporus* required more time, and 15 days are recommended as a reasonable minimal period of incubation. Larvae exposed to *C. clavisporus*, whether infected or not, were able to survive for some time in the water film on the WA, and fungal growth on the larvae only started after their death. In the poster, the convenience of the method will be discussed.

Results of preliminary investigations on entomopathogenic fungi affecting important weevil and aphid forest pests in southern Chile **Fun07** **•232•**

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This poster summarizes seven months of collecting activities between November 2016 and May 2017 in an ongoing study on the biodiversity of pathogenic fungi affecting important weevil and aphid forest pests in the southern Chilean region of Los Lagos. In this work we identify, isolate and preserve (whenever possible) the fungal entomopathogens. Among the results of this study were that fungal entomopathogens species belonging to the genus *Lecanicillium* and *Neozygites* occurred widely and commonly in southern Chile on aphids associated to native, urban and monoculture plantations trees. Strains of *Isaria* were isolated from collections of *Gonipterus platensis* captured on the foliage of *Eucalyptus globulus* close to the city of Osorno in Los Lagos region. Fungi affecting other arthropods were also collected and isolated from all sites visited from the vegetation and soil

surfaces. These vegetation surveys were undertaken to help to expand the comparatively poor understanding of the biota of Chilean fungal entomopatho-gens. This poster will present the key highlights of this project including new reports of fungal entomopathogens, and a listing of the isolates obtained during this study. This project was funded by FONDECYT (Fondo Nacional de Desarrollo Científico y Tecnológico, Chile) project 11160555.

Pathogenicity of an isolate of *Beauveria bassiana* at *Hypsipyla grandella* collected in Brasilia, Brazil **Fun08** **•233•**

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One of the main obstacles to the establishment of mahogany plantations is the occurrence of *Hypsipyla grandella* Zeller, which attacks the terminal bud in new seedlings and trees, forming galleries. This study aimed to isolate, identify and verify the pathogenic ability of fungi associated with *H. grandella* in Brasilia, Brazil. For this, 200 fruit attacked by insects were collected and analyzed. A caterpillar with fungal infection symptom was found. Symptomatic caterpillar was placed in a humid chamber for increased formation of spores and then pure colonies were obtained from serial dilutions and successive subcultures of the fungus. Its molecular, morphological and pathogenic was made. The isolate was identified as *Beauveria bassiana* (Balsamo) Vuillemin, an entomopathogen widely used in biological control programs. Pure colonies were white, and the optical microscope showed unicellular conidia, conidiogenous cells with denticles conspicuous, featuring the species. As for the pathogenicity test, the isolated was effective, with 70% mortality of newly hatched caterpillar. This is the first report of an entomopathogenic fungus found on *H. grandella* in Distrito Federal, Brazil. The isolate was incorporated into the Coleção de Fungos de Invertebrados of Embrapa Recursos Genéticos e Biotecnologia.

Occurrence and ecological aspects of entomopathogenic fungi in maize agroecosystems of Central Mexico **Fun09** **•234•**

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Mexico is characterized for a wide variety of maize agroecosystems. Plant health is a key element in crop production. Soil dwelling pests in maize are commonly managed with chemical insecticides. However, the rhizosphere of crops is naturally inhabited by a broad range of beneficial microbes as entomopathogenic fungi (EPF). The conservation and use of this biodiversity is a promising strategy for agroecological soil pest management. In this sense, we examined the occurrence of native populations of *B. bassiana, M. anisopliae* and *Paecilomyces* spp in three contrasting maize agroecosystems: a) high input (improved maize varieties, intensive pesticide use); b) low input (native landraces, no pesticide use); c) intermediate (native landraces, moderate pesticide use), along three phenological crop stages (V6, flowering and senescence). The results showed 343 native isolates of EPF. The low input system registered the highest number (181), the intermediate system 145 and the high input system 17. *B. bassiana* was found in all agroecosystems while *M. anisopliae* only in the low input system. The ANOVA analysis and LSD test showed significant differences between systems and *B. bassiana* occurrence in relation to phenological stages. In a comple-mentary mesocosm experiment, we evaluated the interactions among *B. bassiana*, native populations of arbuscular mycorrhizal fungi (AMF) and the root feeding insect *Phyllophaga vetula*. Our results show that the combination of native AMF populations and the EPF *B. bassiana* induce tolerance in maize from root herbivory by *P. vetula* in terms of plant growth performance and nutrition.

Biologically-based control of *Varroa destructor* **STU** **Fun10** **•235•**

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The ectoparasitic mite, *Varroa destructor* and the pathogenic viruses that it transmits, are a significant cause of European honeybee, *Apis mellifera* (EHB), overwintering colony losses. Varroa has developed resistance to many of the selective synthetic pesticides that were previously used to control it, while the small number of alternative control measures are only partially effective. Therefore, there is a need for new control methods, including microbial control, underpinned by fundamental knowledge to ensure their sustainable use. Previous research has shown that Varroa is susceptible to a wide range of entomopathogenic fungi (EPF), which have potential therefore to be used as biocontrol agents. We have identified strains of fungi that infect varroa but do not kill EHB. However, there are knowledge gaps about how EPF interact with varroa and bees and this is an impediment to commercialisation and use. This project focuses on characterizing the virulence of different EPF against Varroa and bees using a series of laboratory bioassays. A key part of the work is to improve understanding of the effects of temperature upon the activity of candidate EPF strains, as the warm temperatures that are maintained in bee colonies can inhibit fungal activity. In addition, work is being done to investigate the effects of fungal treatment on transmission and activation of bee viruses and to determine whether fungal treatment has priming effects on EHB immune pathways associated with antiviral defenses.

Use of electrical penetration graphs (EPG) and quantitative PCR to evaluate the relationship between feeding behaviour and *Pandora neoaphidis* infection levels in green peach aphid, *Myzus persicae* **Fun11** **•236•**

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A real-time qPCR method was developed, validated, and used to quantity the fungal pathogen, *P. neoaphidis*, within aphids at different times during infection; colonization rate fitted the Gompertz model well (R^2=0.9356). Feeding behaviour of *P. neoaphidis*-infected and uninfected *M. persicae* were investigated, for the first time, using DC-electrical penetration graphs (DC-EPG) that characterized the waveforms made during different aphid stylet probing periods corresponding to epidermis penetration, salivation and ingestion. In the 6 hours following the 12-hour incubation period (to achieve infection), there were significant differences in the number of events of Np (non-probing) and C (stylet pathway) between infected and uninfected aphids. However, the difference between total duration of Np and C were not significantly different between infected and uninfected aphids. There were no significant differences in the number of events or total duration of E1 (phloem salivation) or E2 (phloem ingestion) between infected and uninfected aphids. There were significant differences in mean number of events and total duration of the pd waveform (intracellular punctures) in infected and uninfected aphids. In the 16 hours prior to death, the same differences in behaviour were observed but they were even more obvious. Furthermore, the total duration time of E2 was significantly greater in uninfected aphids than infected aphids, a change that had not been observed in the first 6 h observation period. In conclusion, qPCR quantification demonstrated 'molecular' colonization levels throughout infection, and EPG data analysis during the two periods (during early infection and then during late infection just prior to death) demonstrated the actual physical effects of fungal infection on feeding behaviour of *M. persicae*. These studies increase our understanding of the interaction between *P. neoaphidis* and its host aphid.

The antifungal peptide diapausin-1 from *Manduca sexta* binds to β -1, 3-glucan in fungal cell walls **Fun12** **•237•**

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Manduca sexta diapausin-1 is a 45 amino acid residue peptide expressed in the fat body after immune challenge, and it is also expressed in the fat body and midgut of naïve larvae at the prepupal stage. Diapausin-1 has activity against yeast and some tested ascomycete filamentous fungi. FITC-conjugated diapausin-1 was detected at the surface of *Saccaromyces. cerevisiae* cells, suggesting that the peptide may bind to fungal cell walls. To investigate the binding target(s) of this peptide, pull-down assays were performed, which demonstrated that diapausin-1 binds to zymosan, an isolated cell wall preparation from the yeast *S. cerevisiae.* Diapausin-1 binding to zymosan is consistent with its interaction with a component of the yeast cell wall, which may be a clue toward its mechanism of action in blocking fungal growth and survival. Diapausin-1 did not bind to bacterial cell wall peptidoglycan, consistent with its lack of activity against bacteria. Yeast cell walls have many components, including β-1, 3-glucan, mannans, and chitin, but the major structural polysaccharide is β-1, 3-glucan. We found that diapausin-1 binds to curdlan, an insoluble β-1, 3-glucan but not to starch, an α-1, 4-glucan used as a negative control. Surface plasmon resonance analysis of binding between diapausin-1 and β-1, 3-glucan yielded a dissociation constant of ~100 nM. Based on our current and previous results we hypothesize that the binding of diapausin-1 to β-1, 3-glucan in cell walls may directly affect the growth *S. cerevisiae* and filamentous fungi by interfering with cell wall growth or division. This hypothesis needs to be investigated further using additional methods

Effects of endophytic entomopathogenic fungi on soybean aphid, *Aphis glycines.* **Fun13** **•238•**

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Terrestrial plants can harbor endophytic fungi that may induce systemic changes in plant defenses that in turn affect interactions with herbivorous insects. We evaluated whether the application of entomopathogenic fungi *Beauveria bassiana* GHA and *Metarhizium brunneum* F52 to soybean seeds could establish them as endophytes and impact soybean aphid (*Aphis glycines* Matsumura). In studies conducted in growth chambers, were able to recover both fungi as endophytes in soybean stems but *B. bassiana* only from the leaves. Furthermore, we found that *M. brunneum* increased populations of *A. glycines*, whereas *B. bassiana* had no measurable effect. The present study confirms that some entomopathogenic fungi could be made endophytic in soybean, however these fungi may have a negative effect on the plants by increasing susceptibility of soybean to the aphid.

Insect growth regulatory and insecticidal activities of secondary metabolites from entomopathogenic fungi **STU** **Fun14** **•239•**

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It is increasingly recognized that the biodiversity in agroecosystems deliver significant ecosystem services to agricultural production such as biological control of pests. Entomopathogenic fungi have been widely studied for their potential as the effective biological control agents. They produce variety of secondary metabolites with insecticidal activities, and it is reasonable to assume that entomopathogenic fungi might produce secondary metabolites modulating juvenile hormone for their survival against defense mechanisms of host insect. In this study, *Beauveria* spp. and *Lecanicillium* spp. strains cultured on unpolished rice medium were extracted with acetone. Both extracts showed juvenile hormone antagonist (JHAN) activities in the yeast-two hybrid β-galactosidase assay and high insecticidal activity against *Aedes albopictus* and *Plutella xylostella.* In addition, to compare bioactivities of secondary metabolites from solid and liquid culture, the *Lecaniciilium* spp. strain cultured on unpolished rice medium or PDB (Potato Dextrose Broth) medium were serially extracted with acetone and ethyl acetate respectively. Both extracts showed JHAN activity and high insecticidal activity against *A. albopictus.* These results suggested that secondary metabolites of entomopathogenic fungi could be useful for development of novel IGR insecticides.

Solid culture of *Isaria javanica* and *I. fumosorosea* on grain substrates for enhanced conidial thermotolerance **STU** **Fun15** **•240•**

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Entomopathogenic fungi have high potential in controlling insect pests in agriculture and forest for pest management, however optimizing conidial production system with high productivity and stability still needs additional efforts for successful field application and industrialization. Although many virulent entomopathogenic fungal isolates received many interested in lab environment, very a few numbers of isolates reached agricultural fields as commercial products. Here in this work, we investigated the feasibility of grains substrates for conidial production, particularly focusing on conidial productivity and thermotolerance. *Isaria javanica* and *I. fumosorosea* conidia (provided by National Academy of Agricultural Science, Korea) were solid-cultured on ten cereal grains for 14 days in Petri dish conditions, and of the tested grains, millet, perilla seed and barley-based cultures showed high conidial production, 15.7 ± 8.4, 17.3 ± 5.2 and 13.5 ± 1.9 × 10^9 conida/g in *I. javanica* and 15.9 ± 0.6, 15.0 ± 0.5 and 15.0 ± 0.7 ×10^9 conida/g in *I. fumosorosea*, respectively. The two isolates had enhanced thermotolerance at 45℃ when cultured on millet, rice, sorghum, and perilla seed. This work suggests possible inexpensive grain substrates for conidial production with enhanced stability for high temperature.

Effect of culture media on storage stability of *Beauveria pseudobassiana* microsclerotia formulated as dry granules **Fun16** **•241•**

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Conidia are the traditional fungal propagules used as the active ingredient in commercial biopesticides. These aerial structures are usually produced in solid fermentation using large volumes of substrate, and requiring complex downstream processes to recover the propagules. Recently, resistant structures -microsclerotia (MS) - that can be easily produced, harvested and applied to the soil for pests and diseases control have been described for *Trichoderma harzianum* and some species of *Metarhizium* spp. Microsclerotia are small, compact, melanized structures of hardened fungal mycelia which allow extended persistence in the environment. Microsclerotia are produced by a New Zealand strain of *Beauveria pseudobassiana* which is under investigation for development as a potential bioproduct to control soil-dwelling scarab pests. The aim of this research was to study the storage stability of MS produced in liquid fermentation using two C:N ratios, formulated with diatomaceous earth (DE) and air dried to <3% moisture, to obtain granules with a final concentration of 10^5 MS/g. For comparison, conidia were produced on cooked rice and formulated with DE under the same conditions. Capacity to germinate of MS granules varied with C:N ratio and storage temperature. MS produced using a higher carbon source concentration retained 100% germination after six months of storage

at 4°C; when stored at 20°C, the germination rate was reduced to 68%. In contrast, MS produced in low carbon medium showed reduced germination (0 to 18%) after storage for more than 2 months at either temperature and had lost viability completely after six months. For surviving MS, conidia production seven days-post rehydration was similar for granules stored at either temperature and produced from both C:N ratios (>10^9 conidia/g). Conidia germination from formulated granules was also stable under refrigeration, but no germination was detected after 4 months at 20°C which could be related to the low moisture content in the formulation. The results demonstrate that MS produced with high carbon media are capable of surviving under low moisture, ambient temperature storage and able to produce around 1000 conidia/MS after germination; these attributes suggest that MS could be used as a new sustained release strategy to control soil-dwelling insects.

Beauveria bassiana produces microsclerotia-like propagules with active peroxisome biogenesis **Fun17** **•242•**

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Entomopathogenic fungi are able to produce several types of propagules such as aerial conidia in solid media, or blastospores, submerged conidia and micro-sclerotia in liquid cultures. Microsclerotia (MS) are usually melanized compact hyphal aggregates that are tolerant to dessication. As far as we know, there are no reports about the production of melanized microsclerotia by *Beauveria bassiana* isolates. The aim of this work was to describe microsclerotial growth in *B. bassiana* and to start with studies about the molecular and physiological mechanisms implicated in the production of these propagules. *B. bassiana* strain GHA was cultured in complete liquid medium for 4 days at 26ºC in the dark with vigorous agitation (250 rpm). Microsclerotia-like structures were separated by centrifugation and processed for microscopy observation and real time qPCR analysis. Under these culture conditions, *B. bassiana* was able to produce compact brownish aggregates that were able to germinate and produce viable conidia after desiccation. Optical microscope images showed similarity in form, structure and size with microsclerotia reported from other entomopathogens, and staining with 3,3-diaminobenzidine (DAB) revealed high peroxidase activity. Genes encoding for peroxisome biogenesis factors, named peroxins, showed high expression levels (up to 17-fold induction) for *Bbpex5*, *Bbpex7*, *Bbpex14/17* and *Bbpex19* genes in 4 days cultures compared with conidia used as starting inocula. Additional studies are being carried out to elucidate the relationship between microsclerotia formation and peroxisomal biogenesis, as same as stress tolerance, cell surface alterations and virulence against insect hosts.

Quantification of fungal growth and destruxin A during infection of _Galleria mellonella_ L. (Lepidoptera: Pyralidae) larvae by _Metarhizium brunneum_ Petch (Ascomycota; Hypocreales) **Fun18** **•243•**

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The entomopathogenic ascomycete *Metarhizium* sp. produce several secondary metabolites, but principally destruxin A, where lack of studies concerning production of this compound is most likely the biggest obstacle for the registration of new fungal strains. Although several studies focus on the production of destruxin A in culture media, only few studies examine destruxin A *in vivo* during host infection. For that in the current work, the dynamic of fungal growth and secretion of destruxin A was monitored *in vivo* during the infection process of *Galleria mellonella* used as an insect model by *M. brunneum* EAMa 01/58-Su or BIPESCO5 strains. Total mortality of sixth *G. mellonella* larvae that were immersed in a suspension of 1.0 x 10^8 conidia mL^{-1} of *M. brunneum* EAMa 01/58-Su or BIPESCO5 strains reached 85.5 % and 78.8 %, respectively, and the percentage of cadavers with fungal outgrowth was low at 12.2 % and 4.4 %, respectively. The average survival time of treated larvae was 5.5 days for both fungal strains. Destruxin A secretion was parallel to the fungal growth of EAMa 01/58-Su, but not coupled with that for BIPESCO5. EAMa 01/58-Su and BIPESCO5 strains secreted destruxin A from days 2 to 6 and from days 2 to 5 post treatment, respectively. For EAMa 01/58-Su and BIPESCO5, the maximum titer of destruxin A in the host was on day 4 at 0.369 and 0.06 µg/larva, respectively, and throughout the pathogenic process, the total production was 0.6 and 0.09 µg/larva, respectively. These results suggest that destruxin A could be virulence factor for EAMa 01/58-Su strain, whereas for BIPESCO5, the virulence could require the involvement of other factors as well as destruxin A during the infection process.

Infection of the tick _Rhipicephalus microplus_ treated with conidia or blastospores of _Metarhizium_ spp. and _Beauveria bassiana_ **Fun19** **•244•**

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The current study compared the virulence of conidia and blastospores of *Metarhizium robertsii* (IP 146), *Metarhizium anisopliae* s.l. (IP 363) and *Beauveria bassiana* (IP 361 and CG 307) against larva and engorged female of *Rhipicephalus microplus*; in addition, this study evaluated the development of both fungal propagules on the tick cuticle. *R. microplus* larvae were treated with Tween 80® 0.01% solution (control) or fungal suspensions at 10^6, 10^7 or 10^8 propagules mL^{-1}. In the groups treated with 10^6 propagules mL^{-1}, only the larvae treated with conidia of IP 361 presented mortality superior than that recorded in the control group at day 10 after treatment, 87% and 5% respectively. Engorged females were immersed in fungal suspension (10^7 propagules mL^{-1}) for 1 min, and their biological parameters monitored daily until the end of oviposition. Only females treated with blastospores suspensions of IP 146 or IP 361 presented lower nutrient and egg production indices in comparison to the control groups; the percent control was higher when females were treated with blastospores suspensions of IP 146 (98%) or IP 361 (92.9%) in comparison to the groups treated with conidial suspensions, 71% and 63.3%, respectively. Engorged females treated with IP 146 or IP 361, conidia or blastospores, were processed for scanning electron microscopy. Germination of blastospores of IP 146 and IP 361 were observed on the tick cuticle after 4 h incubation. No structures suggesting cuticle penetration were observed in blastospores of IP 146; however, evidences of blastospores penetration in natural openings were seen 96 h after treatment. Appressoria in blastospores of *B. bassiana* IP 361 were observed after 4 h of incubation, whereas germ tubes from conidia of both isolates were observed only 48 h after treatment. In conclusion, conidia were more virulent to *R. microplus* larvae than blastospores, whereas blastospores were more virulent against engorged females and developed faster than conidia on tick cuticle. Blastospores might be promising for use in biocontrol of ticks, since they were virulent against *R. microplus* and presented rapid development on its cuticle.

Secretome of the biocontrol fungus _Beauveria bassiana_ related to infection of the cattle tick _Rhipicephalus microplus_ **Fun20** **•245•**

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The biological control is considered a natural and ecological alternative tool to overcome the problems caused by the use of chemical methods in pest control. In addition, the biocontrol presents several advantages when compared to its chemical analogues, regarding to environmental impact, cost, handling, specificity and resistance development. Among microorganisms used in biocontrol, fungi stand out, including the filamentous fungus and arthropod pathogen *Beauveria bassiana*. This fungus is one of the most studied and applied worldwide. In this study, using proteomics and bioinformatics approaches, a total of 256 proteins were identified in *Rhipicephalus microplus* tick cuticle-induced secretome. Proteins involved in different important processes for the biocontrol activity, including degradation of the cuticle (proteases and lipolytic enzymes), defense/antioxidants (catalases, oxidoreductases) and effectors (glycoside hydrolases) were detected. The identification of possible molecular mechanisms involved in fungal infection on specific pests provides a better understanding of biocontrol activity and consequently helps to develop strategies to optimize this process. Moreover, these potential molecular markers, proteins, in a model that induces the infection system enables the oriented search for more efficient strains with greater virulence and specificity in the biological control of *R. microplus* tick.

Secretome of the biocontrol fungus *Metarhizium anisopliae* related to infection of the cattle tick *Rhipicephalus microplus* **Fun21** **•246•**

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The biological control is considered a natural and ecologic alternative to control pests avoiding the issues related to the use of chemical compounds. The biocontrol presents several benefits as the lower environmental impact, cost, handling, specificity, and the non-development of resistant pest strains. However, normally biopesticides need more time to kill and control pests when compared to chemical control. Among microorganisms used in biological control the fungus *Metarhizium anisopliae* is one of the most studied and applied to control several pests, including the cattle tick *Rhipicephalus microplus*. The understanding of the host infection process may contribute to optimize the biocontrol through the reduction of the time to kill the target pest. In this work, applying shotgun proteomics and an in vitro culture strategy to induce the infection system we identified more than 400 proteins in *M. anisopliae* secretome. Among these proteins, 133 were exclusively identified in the infection condition and 56 were found differentially regulated when compared to control. Several of these proteins are related to host cuticle digestion and penetration, fungal defense and modulators of host immune system. Besides, many of these proteins are being reported for the first time linked to *M. anisopliae* infection and 12 are shared in infection of insects and probably not connected to host specificity. The secreted protein profile identified here represents a contribution to the understanding of host-pathogen interaction and may help to optimize biocontrol strategies and to select fungal strains more specific and efficient to control the cattle tick *R. microplus*.

Isolation and characterization of *Bionectria ochroleuca as an* entomopathogenic fungi from edible Saturniid in Kenya **Fun22** **•248•**

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Traditionally, endophytic fungi are known to reside in living plant tissues and are asymptomatic to their host. However, the potential of this special niche of fungi to have other roles especially as an entomopathogen is increasingly gaining relevance. This study reports on *Bionectria* cf. *ochroleuca* as a potential candidate for microbial control of lepidopteran pests. This strain was isolated from *Bunaea alcinoe* (Stoll, 1780) cadaver and it had the characteristic pale yellow to white ascomata and two-celled hyaline ascospores associated with Bionectriaceous fungi. Conidia were obtained from two-week-old plates cultured on potato dextrose agar. The bio efficacy of the fungus was established on second instar *Galleria mellonella* larvae subjected to fungal concentrations of 1 x 10^2 to 1 x 10^9 conidia mL^{-1}. The treatment of fungus was undertaken both as contamination of artificial diet and onto the sterile soil. The treated insects were observed for mortality at 24h interval for 21 days. At a dose of 1 x 10^9 conidia mL^{-1}, highest mortality of only 56 % was recorded in the artificial diet bioassay as compared to 83.3 % in the sterile soil bioassay. Mycosis was observed in larvae subjected to sterile soil bioassay with mycosis rates of 38, 18 and 10% at concentrations of 1 x 10^5, 1 x 10^3 and 1 x 10^4 conidia mL^{-1}. This fungus was characterized using morphological features and through molecular analysis of its internally transcribed spacer (ITS) region. To our knowledge this is the first report of entomopathogenic fungi belonging to *Bionectria* isolated from edible saturniid caterpillars. Further studies on the efficacy of this entomopathogen to Saturniids and other lepidopteran pests needs to be undertaken.

Colonization of some horticultural crops with indigenous *Beauveria bassiana* as endophytic prophylactic protective agent **Fun23**

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Beauveria bassiana has been recovered as an endophytic colonist from several plant species and has been shown to protect plants against plant pathogens and insect pests worldwide. This research was conducted at the laboratories of National Center for Research and fields of Zadna International Company for Agricultural Production and Investment. The purpose was to to establish *Beauveria bassiana* inside some horticultural crops viz; okra,eggplant,mango, orange and grapevine so as to see the possibility of colonization of the plant parts. For the first time worldwide one of Sudanese indigenous isolate of *Beauveria bassiana* successfully colonized mango crop ,orange and okra crop. Whereas this the first study in Sudan to prove colonization of grapevine by *B.bassiana*. Ten to two weeks after inoculation; the fungus which had been sprayed on the plants was recovered in the leaves, stems and roots of all treated crops by 80-100% except roots in case of mango, grape and orange plants.

Establishment protocols for *Beauveria bassiana* in *Arachis hypogea,* potential for controling plant pests and diseases **Fun24**

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Beauveria bassiana is one of entomopathogenic fungi with the ability to colonize plants endophytically. These fungi have recently proven their efficacy as endophyte in protecting plants from plants pests and diseases. This study aimed to test four protocols to establish *B. bassiana* endophytically in the groundnut (*Arachis hypogea*), expecting subsequent evaluations of of this fungus as biocontrol agent against pests and diseases on groundnut. Plants were grown from surface-sterilized seeds for two weeks before receiving *B. bassiana* suspension at 1x10^9 conidia/ml applied either as a foliar spray or a soil drench or soaked seeds or coated to seeds. dividually surface sterilized, cut into five sections, and incubated in selective media up to the presence of the fungus on the surface of the plant part. The media is checked every 2-3 days to observe fungal outgrowth on plant sections and record the existence of *B. bassiana* to estimate the extent of its

endophytic colonization. Analyses of inoculation success comparing the occurrence of *B. bassiana* within a given plant part (*i.e.,* leaves, stems or roots) across treatments and controls. The percentage colonization of the fungus within one month for root stem and roots respectively was 93, 86, 26% for seed coating 100, 73, 33 for seed soaking. When drenched the percentage was 100, 86 and 73. When leaves were sprayed the results were 73, 100, and 100.

Baiting system-mediated collection and characterization of entomopathogenic fungi for library construction; JEF entomopathogenic fungal library **STU Fun25**

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Entomopathogenic fungi are facultative microorganisms and high potential in pest management. Some genera have been used as biological control agents worldwide. Construction of entomopathogenic fungal library could be a strong platform for the development of highly effective biopesticides. Herein this work, we have isolated entomopathogenic fungi using two different methods; selective agar medium-based isolation and *Tenebrio molitor* (mealworm) larvae-mediated baiting system. *The T. molitor*-mediated baiting tool showed much higher isolation efficiency than the selective agar medium including antibiotics and fungicide. The entomopathogenic fungal library, which was named as Jeonbuk (=Chonbuk) National University Entomopathogenic Fungi (JEF), was constructed and it had 350 isolates which were identified as 12 genera and 29 species. The fungal collection was grouped to two different categories based on the Internal Transcribed Spacer (ITS) sequence in a phylogenetic analysis. The morphology and physiological characteristics of the 17 species-representative isolates were characterized. Some of the species-representative isolates showed high virulence against *T. molitor*, *Riptortus pedestris* and *Plutella xylostella* in laboratory conditions. Overall the production of pathogenesis-related enzymes such as chitinase, Pr1 protease and lipase in LB medium was higher than in the SDB and PDB media. In the thermotolerance assay, *Purpureocillium lilacinum* isolate had higher thermotolerance than other isolates. This work reports the efficient construction of entomopathogenic fungal library using *T. molitor* and this library could be used as an attractive resource for research and development of fungal insecticides in pest management

NEMATODE DIVISION POSTERS

Mechanism of action of Bacillus methylotrophicus to control Meloidogyne incognita on cotton **Nem01 •249•**

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Cotton crop (*Gossypium hirsutum*) is affected by various diseases of economic importance, among them, the root galls caused by *Meloidogyne incognita* races 3 and 4 has such significance. The objective of this study was to study the interaction of *Bacillus methylotrophicus* with *M. incognita*. The first assay testing different biological products to control *M. incognita* allowed us to select the product Onix (*B. methylotrophicus*) to conduct the subsequent tests to understand the mechanism of action of the bacteria to control nematodes. For this evaluation it was adopted a method that enabled to visualize the pathogen inside the root, allowing monitoring nematode penetration in the roots and its development until beginning of reproduction. With the method of staining with acid fuchsin it was possible to verify that penetration o J2 decreased in early days after inoculation in plants treated with bacteria, but this difference has been lost over the course of days. Through adoption of a method of toluidine blue were noticeable abnormalities on the giant cells with vacuoles and thinner cell wall, and females presenting large vacuoles inside. The reactions observed in the histopathology assay may indicate the induction of plant resistance mediated by the *B. methylotrophicus*. This suggests further studies to understand this interaction.

Mechanism of action and evaluation of efficiency of products with *Bacillus* to control *Meloidogyne incognita* races 3 and 4 on cotton under greenhouse **Nem02 •250•**

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The root-knot nematode (*Meloidogyne incognita* races 3 and 4) of cotton, is an important pathogen of cotton crop that can cause damage higher than 40%. The objective of this study was to test the efficiency of commercial products with *Bacillus* to control *M. incognita* races 3 and 4 of cotton under greenhouse. In addition to biological products, a treatment with Carbofuran and an untreated control were included. After the application of the products, the plants were evaluated for 200 days. During the assay were evaluated plant height, fresh and dry shoot weight, fresh and dry root weight and the nematode reproduction factor. There was a positive response during initial growth of plants treated with biological agents in comparison with the control plants treated with and without chemical product. The products with *B. subtilis* (Rizos), *B. amyloliquefasciens* (NemaControl) and *B. subtilis* + *B. lincheniformis* (Nemix) showed better results for fresh shoot weigh when compared to the control plants with nematode. With respect to dry shoot weight, the treatments with active ingredients of *B. methylotrophicus* (Onix) and B. subtilis it was higher than in control, especially when treated with *B. methylotrophicus*. As for fresh root weight, only the treatment with *B. methylotropicus* provided weight statistically higher compared to the control. As far dry root weight is concerned, in plants without nematode, the treatment with *B. methylotrophicus* was better than the others treatments again. Same trend was observed for nematode reproduction factor. Therefore, there is evidence that the use of biological products can be effective to control *M. incognita*, becoming a tool to be used in the management to this important pathogen of cotton crop.

Susceptibility and viability of larval *Diabrotica virgifera virgifera* as a host for entomopathogenic nematodes. **Nem03 •251•**

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Seven populations of entomopathogenic nematodes were obtained and reared in mass for laboratory experiments. Populations included species of *Steinernema feltiae*, *S. riobrave*, *S. carpocapsae*, *S. diaprepesi*, *Heterorhabditis megidis*, *H. bacteriophora*, and a Missouri wild type population of *H. bacteriophora*. Larval third instar *Diabrotica virgifera virgifera* were exposed to either 60 or 120 nematodes for six days. Afterwards the larvae were dissected in order to determine the presence of nematode infection. Results indicated a significantly higher proportion of larvae were infected with species in the *Heterorhabditis* family and *S. diaprepesi* species than the other *Steinernema* families for both exposure rates. An additional experiment involved exposing third instar larvae to 60 nematodes of either *S. feltiae*, *H. megadis*, *H. bacteriophora*, or the wild type *H. bacteriophora* and allowing the nematodes to complete their lifecycles and emerge. Viability of the emerging nematodes was observed for up to nine weeks after leaving the host. After nine weeks nematode viability was found to be: *H. megadis* (87%), *H. bacteriophora* (95%), wild *H. bacteriophora* (89%), *S. feltiae* (86%). Results have shown that while the rate of infection may vary, all nematode species tested were capable of infecting larval *D. virgifera*. Additionally the viability of those nematodes able to emerge from *D. virgifera* hosts was not negatively impacted.

Efficacy of *Steinernema carpocapsae* formulated in sodium alginate capsules against *Phyllophaga vetula* in semi-field conditions STU Nem04 •252•

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To evaluate the capacity of the nematode *Steinernema carpocapsae* formulated in alginate capsules to control third instar larvae of *Phyllophaga vetula*, capsules of alginate containing each 700 IJ´s were elaborated. Prior to its elaboration by the ionic gelation process, the sodium alginate solution received 10% maize sap as a phago-stimulant, to which sodium benzoate had been added at concentrations of 1.5 and 3.0 g / L. To obtain the maize sap, 5 stems of maize seedlings were ground in a rotary juice extractor, applying the juice obtained to the sodium alginate solution. The Criollo Bolita white maize variety was planted the field in plastic bags of 40 x 40 cm filled with moist soil. Although in general it rained well, irrigation was applied when it was required. After two weeks two plants per bag were left and five larvae of *P. vetula* were applied per bag, discarding those that did not buried into the soil after 3 minutes. During plant growth, plant heights were recorded by duplicate every two weeks from the soil to each one of the top three leaves. At maize flowering the plants were uprooted to evaluate the presence of alive larvae and to determine plant and root dry weights. The data were processed in Excel and later analyzed with the SAS package to obtain the ANOVA and the Tukey test (α <0.05). All the treatments applied were different to the control (water suspension), but no differences were found in percent control (PC) between concentrations of sodium benzoate. Neither the final height of the maize nor the dry weight of the forage were statistically different; however, the root-forage ratio (PRF) was higher in treatments with capsules. The treatment where IJ´s were applied in aqueous suspension tended to produce the largest PC, as well as the highest PRF. The encapsulated nematodes had the same control efficacy as the nematodes applied in suspension, without giving significant differences between the levels of phago-stimulant. However, the root-foliage weight ratio was higher in treatments with alginate capsules.

Detecting and quantifying EPN in Virginia, USA, vineyards using qPCR Nem05 •253•

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For over a century, the grape root borer (GRB) has been known as an insidious threat to vineyard production in much of the eastern USA. While GRB has been detected in most vineyards sampled in Virginia, the degree of infestation varies widely among them. In addition, the soil factors that appear to promote GRB infestations are considered to be relatively unsuitable for entomopathogenic nematodes (EPN). Our research objectives were to identify and quantify populations of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* to determine whether variability in EPN may help explain GRB infestation patterns. These two EPN species are common and widely distributed, are considered important biological control agents of numerous crop pests, and are known to be virulent to grape root borer (GRB) larvae. Research was conducted over the 2016 growing season at 14 vineyards across Virginia. Using a qPCR approach to identify EPN presence, we found both EPN at varying densities across the 14 vineyards. Total seasonal grape root borer captures in pheromone traps also varied substantially among the 14 vineyards. This talk presents a model that integrates assessments of soil texture, EPN presence, and GRB abundance. In addition, we focus on next steps, including the suitability of different vineyards conditions for inundative EPN applications to treat existing GRB infestations.

Studying entomopathogenic nematodes in an undergraduate lab setting: the consequences of soil sterilization on EPN infection rates. Nem06 •254•

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Entomopathogenic nematodes (EPN) are intriguing organisms to use in a teaching context, as they integrate concepts of predation, parasitism, mutualism and symbioses. While the biology of EPN is complex, their life cycle is simple to understand, and their small size, short life cycles, and amenability to lab culture makes them suitable for original, student-derived research projects that are integrated within semester-long courses. This presentation details how we integrated this approach into a semester-long research project within a sophomore-level Ecology lab at Ferrum College. In collaboration with the faculty member, the student is asked to come up with a research question, write a proposal justifying the question and laying out the experimental protocol, conducting the research, and presenting the data; all of this is done in the context of a single semester-long project. The results we present here focus on the consequences of soil sterilization on EPN virulence, but the process is amenable to many different questions. Current plans describe a similar approach for freshman-level Biology courses at Ferrum College beginning in the fall of 2017; if such an active learning format can be scaled up to multiple sections it is possible that 100 or more students per semester may be exposed to EPN biology and the process of research. Beyond its value as a teaching tool that makes biological research real, this approach offers an unusual opportunity to crowd-source questions regarding EPN biology. We welcome suggestions for questions relating to EPN behavior or ecology that might be effectively answered in this context.

Occurrence of *Steinernema arenarium* in agricultural soil of School Farm of the State University of Londrina, Paraná, Brazil Nem07 •255•

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The entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) are cosmopolitan and can be found in soils of the most diverse ecosystems. Thus, the objective of this work was to isolate and identify EPNs from crop soils of farm school of the State University of Londrina, Paraná, Brazil. Eighteen soil samples were collected in cultivated areas with soybean and corn. In the laboratory, each sample was divided into three sub-samples (approximately 100g of soil) and each was placed in a 500-mL plastic vials. Then, each vial received five last instar *Galleria mellonella* (Lepidoptera: Pyralidae) caterpillars and were covered and stored, without light, in a climatic chamber at 25 ± 1ºC, for 72 hours. Dead caterpillars with symptoms of EPNs infection were transferred to Petri dish with a dry filter paper and stored under the same conditions for five days. After this period, the caterpillars were transferred to a *White trap* to collect the infective *juveniles*. The infective juveniles (IJs) were again inoculated in *G. mellonella* larvae for multiplication. Molecular identification was done from the amplification and sequencing of the rDNA ITS region using the primer pair SSU18A (AAAGATTAAGCCATGCATG) and SSU26R (CATTCTTGGCAAATGCTTTCG). Sequences were obtained from automated sequencer (ABI-PRISM 3500 XL) and later edited and aligned with the help of the MEGA v5.0 program. The BLAST application (NCBI) was used to compare the sequences obtained with the GenBank database, seeking information (genetic similarity) to identify the species. Of the 18 soil samples collected, one was positive for NEPs, and preliminary genetic analyzes identified the isolate as *Steinernema arenarium*. To the best of our knowledge, this is the first citation of the natural occurrence of this species in Brazil

Investigation of how the entomopathogenic nematode *Heterorhabditis bacteriophora* suppresses the host immune system STU Nem08 •256•

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Entomopathogenic nematodes (EPNs) are insect parasites often used in pest management. The infective juvenile (IJ), which is the only free-living stage and analogous to the dauer stage in *C. elegans*, is associated with pathogenic bacteria, which they carry in their gut. Developmentally arrested IJs invade insect hosts and initiate the parasitic part of their life style by releasing pathogenic bacteria from their gut. It is widely believed that the bacteria kills the insect hosts and then proliferates and supports nematode growth and reproduction. However, it has been shown that EPNs in the genus *Steinernema* contribute to pathogenesis through their secreted products, to manipulate host immunity and promote bacterial growth. We are testing this hypothesis in *Heterorhabditis bacteriophora*, an EPN that is widely used in both research and pest control, but that has not been previously shown to contribute to pathogenesis. We have adapted a method to obtain large amounts of bacteria-free IJs by culturing nematodes on a non-colonizing bacterial strain. We have developed a method to activate the IJs *in vitro* and are currently harvesting secreted protein products from activated IJs. Here we discuss the method of activation and discuss the quantity and identity of secreted protein products. The goals are to determine whether *H. bacteriophora* nematodes secrete active products to modulate host immune system and to characterize these components, focusing on the protein products.

DISEASES OF BENEFICIAL INVERTEBRATES DIVISION POSTERS

First confirmed report of a bacterial brood pathogen in stingless bees DBI01 •257•

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Susceptibility to brood pathogens in eusocial stingless bees (Meliponini), alternative pollinators to honey bees in subtropical and tropical regions of the world, is unknown. Brood losses in managed colonies of the Australian stingless bee *Tetragonula carbonaria* were studied over 20 months. Symptoms and bee behaviour were noted and recorded. We isolated a disease-causing bacterium, *Lysinibacillus sphaericus* (Firmicutes, Bacillaceae), from worker and queen larvae, brood cell provisions and honey stores, by using microbiological and molecular techniques. This is the first confirmed record of a brood pathogen in stingless bees. Subsequently, a *L. sphericus* strain with an identical 16S rRNA gene sequence has been isolated from a symptomatic *Austroplebeia australis* colony. Our findings warrant further research into the prevalence of *L. sphaericus* infections and possibly other brood pathogens across Meliponini.

Transcriptome sequencing and in silico cDNA library construction of *Varroa destructor*, a parasitic mite of honeybee DBI02 •258•

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Varroa destructor is a small ectoparasitic mite belonging to Acari which attacks Honeybee, *Apis mellifera. V. destructor* feeds on honeybee hemolymph, and often stays inside the capped brood cell to lay eggs on the larvae and pupae. This mite also harbors small RNA viruses such as deformed wing virus, and transmits these viruses in the infested beehive. To survey the genes of V. destructor, its total RNA was subjected to RNA-seq to construct in silico cDNA library. By using the Illumina HiSeq 2000 sequencing platform, 101 base paired-end sequencing generated 2 x 107,748,792 raw reads to obtain 2 x 8.3 Gbase of quality filtered nucleotide sequences. An in silico cDNA library was constructed by Trinity de novo assembler, and streamlined by using TransDecoder ORF finding, and CD-HIT clustering program. The final version of cDNA library contains 28,023 contigs with protein coding capability. This library was subjected to arthropoda reference sequences from EMBL database by BLASTX, and it returned 15,075 contigs which aligned with known sequences (e-value < 1e5). The contig sequences also reflect the metagenomic viral flora in the mites which harbors bee pathogens. These newly assembled gene sequences will provide valuable information for molecular biology of V. destructor and future pest control approaches such as using RNAi technology.

MICROBIAL CONTROL DIVISION POSTERS

***Serratia marcescens* as a bacterial pathogen of sunn pest, Eurygaster integriceps (Hemiptera: Scutelleridae)** MCn01 •259•

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Sunn pest, *Eurygaster integriceps,* is the most important pest of wheat and barley in the Middle East which threat food security. In order to find pathogenic bacteria with the potential to control sunn pest, a red-pigmented bacterial strain was isolated from diseased sunn pest collected from barley fields in Varamin, Iran. Bacterial isolate DNA was extracted using gram negative bacteria genomic DNA extraction kit (IBRC), then PCR was conducted with F27 and R1492 primers and finally PCR product was cloned in pTZR/T57 (Fermentas). Bacterial isolate was identified as *Serratia marcescens* by 16S rRNA gene sequencing and biochemical tests. The bioassay was conducted on hibernating adults using topical application. Bioassay results showed that the concentration of 10^8 CFU caused 60% mortality 3 days postexposure. Contact bioassay also led not only to change in sunn pest color to the pink-red, but also the insect cuticle seems to be degraded. Although *S. marcescens* cells could not use as a biological control due to their pathogenicity toward vertebrates, the produced toxic compounds could utilize in integrated sunn pest management.

Developing a novel virus biopesticide to target major lepidopteran pests MCn02 •260•

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The biopesticide market is estimated to be worth $2.3 billion[1], and expected to increase to $6.6 billion by 2020[2]. Baculoviruses are a category of biopesticides routinely used against lepidopteran pests, including Diamondback Moth (*Plutella xylostella*), Egyptian Cotton Leafworm (*Spodoptera littoralis*) and Codling Moth (*Cydia pomonella*). Biopesticides have many advantages over chemical pesti-cides: registration costs tend to be lower, a narrower host range reduces the scope for non-target effects, and they allow growers to meet consumer demands for reduced chemical inputs in food production. However, baculo-viruses are highly sensitive to UV degradation, with significant reductions in efficacy observed within 4-24 hrs

of application in full temperate sunlight[3]. As such, applications need to be precisely timed and repeated applications are often required to achieve control of multiple generations. A consortium of business and academic partners received funding from the UK's Agritech Catalyst fund, administered by Innovate UK, the UK's innovation agency, to investigate whether Exosect's proprietary technology Entostat® can be used to address this constraint. Entostat® is based on electrostatic micronized waxes, which delivers increased loading of active ingredients to the target surface. In addition to potentially improving delivery of virus onto leaf surfaces, it is hypothesized that by enveloping the virus in the wax, Entostat® can be used to extend the field life of the virus if the wax acts as a UV filter. Initial formulation work has confirmed that the envelopment process does not impair the efficacy of the virus. In fact, test insects (2nd-instar *Spodoptera littoralis*) have been shown to be significantly more susceptible to enveloped virus than unformulated material (LC50 of 1.8 x 10^4 OB/mL versus 3.5 x 10^4 OB/mL) in bioassays. The plans for testing UV stability will be discussed. *REFS: Global Industry Analysts, Inc., 2015; [2] Markets and Markets, 2015; [3] Ignoffo, Rice and McIntosh, 1989.*

Effect of optical brighteners on ChinNPV infectivity on *Chrysodeixis includens* caterpillars in bioassays and greenhouse **MCn03** **•261•**

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Virus-based insecticides have been used as biological pest control in several countries/regions around the world such as Australia, Brazil, China, India, and sub-Saharan Africa, mainly due to their specificity and safety to non-target organisms. However, their acceptance as bioinsecticides is limited because of their relatively slow action; in some cases, high doses are required, which implies higher productions costs. To circumvent these bottlenecks and improve *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV) performance, synergistic agents were studied. This work aimed to evaluate effective ChinNPV/brightener combinations to control soybean looper, *Chrysodeixis includens*. Therefore, three concentrations of optical brighteners (OP) [Fluorescent Brightener 28 (FB28); 4,4'-diamino-2,2'-stilbenedisulfonic acid and Calcofluor White Stain, ranging from 0.025% to 1.5%] in combination with ChinNPV 5.0 × 10^3 OB.mL^{-1} were incorporated into the artificial diet, without formalin and antibiotics, when the diet temperature reached 56°C. Early 3rd instar loopers feeding on the diet *ad libitum* were kept in trays in environmental chambers (26 ± 2°C, 70 ± 10%, 14-h photophase). In a greenhouse test, soybean plants were spread with: ChinNPV 1.5 × 10^{12} OB.ha^{-1}; ChinNPV 3.0 × 10^{12} OB.ha^{-1}; ChinNPV 1.5 × 10^{12} OB.ha^{-1} + FB28 (0.1%); and ChinNPV 3.0 × 10^{12} OB.ha^{-1} + FB28 (0.1%). Leaflets were collected immediately and 96 h after applications and offered to soybean loopers. Mortality was assessed daily until the 12th day. Data were submitted to the Competitive Risk Survival Analysis, following the Fine & Gray model, to compare curves of cumulative incidence of mortality. In lab bioassays, the addition of FB28 (0.1%) greatly increased the activity of ChinNPV, decreasing mean lethal time from 8.45 to 5.93 days and increasing mortality from 42% to 99%. In greenhouse, no significant differences were observed among the curves of cumulative incidence of mortality among the highest virus doses with or without OP. However, at the dose of ChinNPV 1.5 × 10^{12} OB.ha^{-1} a significant lower cumulative probability of death was observed. The most efficient brightener was FB28, which did not have any negative effects against *C. includens* larvae when used alone.

Effect of *Bacillus thuringiensis* products on larval survival and consumption of *Plutella xylostella* **MCn04** **•262•**

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Bacillus thuringiensis-based bio-insecticides stand out as biological agents against insect pests, mainly because they have specificity. These products can efficiently control *Plutella xylostella*, the main pest of Brassicaceae. The objective of the present work is to analyze the sublethal effects of *B. thuringiensis* (Agree®, Dipel® and Xentari®) based products, analyzing the leaf consumption by larvae as well as larval survival. The products concentrations that caused 25% mortality (CL25) in insects of two populations, susceptible-PS and resistant-PR, was tested. Autoclaved deionized water plus 0.5µl / mL of Triton-X100® was used as the control. In the bioassay, 10 *P. xylostella* second instar larvaes were used placed on leaf kale disks previously immersed in the different treatments. Each disc constituted a replication, being evaluated ten replications per treatment. For the larvae of the susceptible population, the highest leaf area consumed (13.8 cm^2) was observed in the control, compared with Dipel® (9.2 cm2) and Xentari® (9.8 cm2), but not differing from Agree® (12, 0 cm 2). For larvae of the resistant population there was no difference between the bioinseticides in relation to the foliar consumption. It should be pointed out that, in general, the insects of the resistant population always presented greater consumption of leaf area. About the larvae survival rate in the susceptible population, all treatments were different from the control, with survival percentages being, respectively, 26.0%, 38.0%, 26.0% and 54.0% for Agree®, Dipel®, Xentari® and control. However, in the larvae survival in the resistant population, Agree® (28.7%) differed from Xentari ® (52%), being similar to Dipel® (41.3%) and to the control (44.7%). The results indicate that leaf consumption and larval survival are biological characteristics showing sublethal effects of the bioinsecticides in the two populations of *P. xylostella*.

Characterization of newly isolated Iranian *Bacillus thuringiensis* strains based on gene content, proteomic analysis and insecticidal activity **MCn05** **•263•**

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Bacillus thuringiensis (Bt) is one of the most useful entomopathogens used nowadays for biological pest control. The identification of new Bt strains is a topical issue in order to discover novel Bt toxins to broaden the range of insecticidal activities and to manage better possible insect resistance problems. Seven Bt strains isolated from infected larvae and soils from Iran (IE-1, AzLp, IE-2, IP-2, IEp, RM and KhF strains), have been characterized. The spectrum of lepidopteran toxic activity of these Bt strains, was assessed against *Plodia interpunctella, Spodoptera exigua*, *Mamestra brassicae*, *Grapholita molesta*, *Ostrinia nubilalis* and *Helicoverpa armigera* neonate larvae. The studied Bt strains showed partial and total similarity in their protoxin and trypsinized protein profiles respectively, but they differed in their insecticidal activity spectrum. *G. molesta* and *O. nubilalis* were the most susceptible insects, and AzLp, IE-2 and IP-2 exhibited high levels of toxicity against *S. exigua* and *M. brassicae* compared to those of the reference strain, HD-1. LC-MS/MS analysis of solubilized crystal proteins was used to reveal the major proteins composing the parasporal crystal. In general, Cry1A family proteins were the most represented and abundant insecticidal crystal proteins in these strains. These results were compared with the ones obtained after PCR screening of Cry genes. As a final step in the strain characterization, the production of β-exotoxin was also checked and no type I β-exotoxin was detected in the studied strains. Results suggested that different complementary approaches are necessary for a robust characterization of new Bt strains, in order to understand their possible use as bioinsecticides. Due to the characteristics of AzLp, IE-2 and IP-2 strains, they could be considered as good candidates for lepidopteran pest control.

Field efficacy of *Bacillus thuringiensis* galleriae strain SDS-502 for the management of alfalfa weevil and the impact on *Bathyplectes* spp. parasitization rate **MCn06** **•264•**

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The alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), is considered as one of the most serious pests of alfalfa in US and worldwide. The damaging populations are frequently found in Northern Plains alfalfa. Chemicals are registered for control of alfalfa weevils in US. However, there is increasing pressure on conventional alfalfa growers to minimize the chemicals use for alfalfa weevil management because of negative impacts on non-target organisms including pollinators and natural enemies of alfalfa weevils. In addition, organic alfalfa production has increased over the years, especially in hay exported to Asia. Recently a new beetle-active *Bacillus thuringiensis* (Bt*) has been commercialized for use against a number of beetles, including the alfalfa weevil. Most commercial coleopteran Bts have a beetle-specific CRY3 family protein. This new Bt, *B. thuringiensis galleriae* strain SDS-502, has a novel toxic protein, CRY8Da. This study was, therefore, aimed to 1) evaluate the efficacy of this Bt strain against the *H. postica* larvae under Montana field conditions and 2) to determine of Bt impact on parasitization rate of *Bathyplectes* spp. against *H. postica* larvae. The field study was conducted at Montana two locations (Conrad and Valier) in 2016. The result indicated that this Bt strain provided 11 to 60 percent reduction of larval populations depending on the labeled lower and high rates. Interestingly, this Bt product has no negative impact on *Bathyplectes* spp. populations.

Entomopathogenic *Bacillus thuringiensis* as biological control agent for complex disease (tomato Fusarium wilt and root knot nematode) **MCn07** **•265•**

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In tomato production, the complex disease caused by Fusarium wilt (pathogen: *Fusarium oxysporum* f.sp. *lycopersici,* FOL) and root knot nematode (*Meloidogyne incognita,* RKN) is a severe problem, and it also causes huge economic loss. We already showed the plant growth promoting effect, biofilm formation ability, nematicidal effect of *Bacillus thuringiensis* strains. This study was estimated to use *B. thuringiensis* as an alternative agent to control the development of complex disease. Three strain; *B. thuringiensis japonensis* (BT17), *B. thuringiensis kurstaki* (BT18)*, B. thuringiensis CR371-H* (BT20) , Tomato (*Solanum lycopersicum*) cultivar Kyoryoku-Beiju(Takii Seed Co.) ,FOL and RKN were used for this experiment. Surface sterilized tomato seeds were grown at 25±1°C in a greenhouse. After 4-week cultivation, tomato seedlings were treated with vegetative spore at 10 ml/pot (3×10^8 cfu/ml). After 7 days, 5ml J2 suspensions were inoculated around plant roots (500 J2s/pot). After 7 days, the bacteria-nematode treated tomato seedlings were placed in the FOL-infested soil. 4 weeks after pathogen inoculation, symptom expressions of the complex disease were evaluated. 11 experiment groups were set: 3 BT strains（BT17、BT18、BT20) + FOL; 3 BT strains（BT17、BT18、BT20) + RKN; 3 BT strains (BT17、BT18、BT20)+ FOL + RKN; RKN control; FOL control; FOL+ RKN control; distilled water untreated control. As a result, *B. thuringiensis* strains could significantly suppress the symptoms of complex disease, decrease gall formation on the root, wilting score and internal symptom.

A molecular survey of the wireworm microbiome and its variation among habitats **MCn08** **•266•**

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Wireworms are a serious subterranean larval pest of agricultural crops for which entomopathogenic fungi are being developed and commercialized as pest control agents. How bacteria interact with fungal pathogens needs elucidation as interactions between microbes and their hosts can have major impacts on host behavior and fitness. DNA sequencing and computational biology have allowed for comprehensive genetic surveys of an organism's microbiome and can be done with relative ease, revealing taxa that might not be apparent from traditional isolation techniques such as plating. Identifying the bacteria associated with wireworms is the first step toward elucidating organismal relationships which may be invaluable for creating more effective pest control strategies. The bacterial microbiome from five separate wireworm (*Agriotes obscurus* and *A. lineatus*) populations (sites with different habitats) was sequenced, revealing 609 operational taxonomic units (OTU) among the total of individuals. The most common OTUs at each site exhibited a diverse range of bacterial groups, with the proportions of 40 genera being significantly different among the five collection sites. Principle coordinate analysis revealed clustering of two sites, indicating a difference in the composition of taxa and suggesting that habitat can influence a microbiome's members. The implications this may have on wireworm microbial control is under investigation, with symbiosis / disease resistance associated with certain taxa being an area of interest.

Feeding diel rhythm and insulin receptor expression of Maruca vitrata **MCn09** **•267•**

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The legume pod borer, *Maruca vitrata*, infests and causes serious economic damage on the Adzuki bean, *Vigna angularis*, but does not on other leguminous beans in Korea. However, a laboratory analysis of the larval growth of *M. vitrata* indicated that other beans are likely to be optimal hosts. For the purpose of the molecular interaction between host and growth in *M. vitrata*, this study aims to investigate an insulin signal of *M. vitrata*. Injection of a bovine insulin significantly changed growth rate along with feeding amount of 4th instar larvae. A transcriptome analysis using RNA-Seq provides genes predicted to be associated with insulin signal. Larval feeding rhythm was monitored every 3 h and indicated that most feeding occurred at early photophase. Starvation induced expression levels of 7 insulin signaling genes by up-regulating insulin receptor (Mv-InR) but down-regulating other 6 genes. Mv-InR encodes 1,213 amino acids and contains both functional sites of ATP-binding and tyrosine kinase. Expression of Mv-InR was confirmed in all developmental stages from egg to adults. RT-qPCR analysis showed that Mv-InR expression varied with feeding rhythm. The larvae fed with different leguminous diets showed variation in expression levels of Mv-InR. These results suggest that *M. vitrata* may infest other types of leguminous crops in Korea.

Molecular detection, characterization and biological evaluation of Red Palm Weevil (*Rhinchophorus ferrugineus*) associated fungal entomopathogen (*Metarhizium anisopliae*) from Pakistan **MCn10** **•268•**

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Red Palm weevil *(Rhinchophorus ferruginous)* is an important quarantine and destructive pest of date palm worldwide. It feeds inside the plant and early

detection is very difficult. Different strategies have been adopted including application of insecticides but the use of microbial pesticides has obtained a high level of significance. In the present work, laboratory trials were conducted to identify entomopathogens associated with red palm weevil and to investigate their effect against various life stages of this pest. Molecular markers and specific primers can be used that provide a means for the molecular identification, diversity and link to virulent isolates to screen different entomopathogens. Healthy and infected Red palm weevil adults and larvae were collected from different regions of Pakistan during a survey conducted 2016-2017. Some samples of fungal infected Red palm weevil were collected and brought to laboratory in 100% ethanol for their molecular identification. Fungal DNA was extracted from infected samples and amplified in PCR machine by using PCR-based RAPD markers. RFLP analysis with restriction enzymes and sequencing was also performed. Our results of molecular study showed that *Metarhizium anisopliae* was found on dead samples of Red palm weevil. We reared Red palm weevil in our laboratory on artificial diets. Bioassays were conducted by using adults and 2nd, 5th, 10th instars larvae of *Rhinchophorus ferrugineus* in order to know their pathogenicity. The data regarding various doses of *Metarhizium* and correspondent mortality (LD50/LC50) was recorded after every 24h till pupation, adult emergence and egg eclosion. The analysis was done by using Probit Analysis for all the bioassays carried out under laboratory condition (25±2°C and 75% r.h). We observed high mortality of Red palm weevil in laboratory trial with our native isolate of *Metarhizium anisopliae*. To our knowledge, this is the first report of molecular identification, characterization and biologic evaluation of native fungal entomopathogen (*Metarhizium anisopliae*) against Red Palm weevil from Pakistan.

Production of secondary metabolites from *Azadirachta indica* and their efficacy against Sf9 cell cultures **MCn11** **•269•**

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Secondary metabolites from neem (*Azadirachta indica* A. Juss) show a broad spectrum efficacy against insect pests including insecticidal, anti-feedant or insect repellent activities. To date, less is known about the mode of action of these active compounds and their production is still based on complex extraction procedures. Several studies have shown that plant cell cultures of *A. indica* can produce active secondary metabolites like azadirachtin A. In light of increasing insight into plant cell cultures from *A. indica* it can be hypothesized that the use of elicitor agents combined with an adjustment of the culture conditions are beneficial for the production of active neem compounds. Furthermore there is evidence that a rearrangement of the natural compound mixture can increase the efficacy against insect pests. The overall aim of this project is to develop a competitive process to produce high concentrations of bioinsecticidal compounds with either neem endophytes or neem plant cell cultures. Besides we want to shed some light into the mode of action of some neem compounds accompanying azadirachtin A. Here, we present data on production of active compounds with plant cell cultures as well as first results of novel compound mixtures tested against Sf9 cell cultures.First, we induced plant cell cultures from various plant tissues with a medium that allows a callus proliferation from more than 90 % of the explants. Furthermore we analyzed the mode of action of different neem ingredients against insect cell lines. We report production of azadirachtin A obtained with a novel high-throughput microbioreactor system (BioLector® Pro from m2p-labs GmbH) that enables 48 parallel batch cultivations in a volume under 1 ml. In addition to that we will describe how different plant hormones can be used as elicitor agents in neem cell cultures. Finally, we will present data on fluorescence and confocal laser scanning microscopy as well as FACS analysis of treated Sf9 cell cultures that will lay the basis to determine how active compounds influence the development of insect cells.

The project PICTA-KILL - Novel strategies for biological psyllid pest control **MCn12** **•270•**

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Psyllid pests are distributed all over the world and cause damage in crop plants. Novel defense strategies against these insect pests are of international interest. The use of entomopathogenic fungi as biocontrol agents for reducing psyllid pest populations represents a plant protection method of low risk for nature and humans as well. Being the vector of *Candidatus* Phytoplasma mali, the infectious agent of apple proliferation, the psyllid *Cacopsylla picta* is responsible for an annual economic loss of a three-digit-million range in Europe. Because there are no direct measures to combat apple proliferation, the vector itself has to be controlled in order to protect the plants. In Germany, there are no authorized plant protection products available, neither for organic farming nor for conventional farming. New insights into the scent preferences of *C. picta* offer innovative options for its control. Hence, the aim of this project is to develop formulations, which can be applied for Attract-and-Kill-strategies against *C. picta*. Furthermore, it will be examined if a combination with repellent agents supports the effect (Push-Pull-Kill- strategy). In laboratory and field trials new repellent, attractive and arresting substances, entomopathogenic microorganisms and other insecticides effective against *C. picta* as well as formulation materials and methods for these active ingredients will be screened. For the entomopathogenic microorganisms, cost efficient mass production processes will be developed. Appropriate formulations will be developed and tested in laboratory and field trials. This joint projects cooperates with the Eilenberg group of Copenhagen University on entomopathogenic fungi. First results of the recently started project on strain selection, cultivation, identification of semiochemicals and formulation will be presented.

Development of a biological tick control agent based on an innovative attract-and-kill strategy (BIOZEC) **MCn13** **•271•**

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Ticks are vectors for a multitude of pathogens, causing e.g., Lyme disease and tick-borne encephalitis in many parts of the Northern hemisphere. In Germany 8-10 million people suffer from tick bites every year whereby most bites are caused by *Ixodes ricinus*. At present, there is no individual control measure against ticks available. The overall aim of this project is the development of a novel biological control agent against ticks based on an innovative attract-and-kill strategy.The basis of this control agent is the attractive effect of carbon dioxide (CO2) combined with tick-specific attractants and substances causing aggregation in ticks (e.g., aggregation pheromones), slowly released into the vicinity by a specially tailored biopolymer bead. The attractive bead is combined with a kill component, an entomopathogenic fungus, isolated from indigenous dead ticks in Germany. The fungus infects the tick as it comes in direct contact with the bead and finally leads to death by growing through the cuticle into the tick's hemolymph. As the ticks are attracted by the beads, a high-dose application can be avoided. Furthermore, all components are biodegradable so that the environmental pollution is minimized. First experiments dealt with the encapsulation of baker's yeast in Ca-alginate beads to achieve a sustained release of CO_2 above ground. These beads significantly attracted *I. ricinus*

nymphs. Current investigations are focused on the selection and cultivation of virulent strains of entomopathogenic fungi from the genus *Metarhizium*, biomass encapsulation as well as drying and rehydration of beads. Moreover, a tick specific arrestant was successfully coated on the bead's surface which proved to have an aggregating effect for *I. ricinus*. This work will pave the way for novel tick control strategies based on an attract-and-kill approach.

The project ATTRACAP: Optimization of an attract-and-kill strategy for wireworm control in potato MCn14 •272•

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Wireworms, the polyphagous soil-dwelling larvae of click beetles (Coleoptera: Elateridae), are a major insect pest of worldwide relevance causing tremendous yield losses in several crop production systems, like potatoes, wheat and other grain crops, oil crops as well as vegetables. Within the last years wireworm damage in potato production substantially increased, both in conventional and organic production systems, resulting in existence-threatening yield losses for farmers. Currently, effective plant protection strategies are not available due to the progressive phasing-out of effective synthetic chemicals. Based on previous projects, an innovative and effective control strategy was developed, using solely biological components. A capsule releases carbon dioxide (CO_2) upon contact with the soil humidity, which attracts the wireworms towards these capsules, where they come into contact with an isolate of the entomopathogenic fungus *Metarhizium brunneum*, thus substantially enhancing the frequency of fungal infections of wireworms. The project aims at improving the efficacy levels of the in previous projects developed product candidate ATTRACAP®. The innovative formulation technology and resulting beads will be fine-tuned and tested and validated under varying field conditions. Apart from the academic partners, members of the project consortium comprise the company producing the product, and consultants which stay in contact with the farmers and will help with set-up of field experiments. As an outcome of the project an optimized product ATTRACAP® will be available, helping both conventional and organic farmers to maintain a sustainable potato production. This poster will present the first results of the recently started project including field trials 2017. Our formulation will pave the way towards novel Attract-and-Kill strategies in other pest problems.

Various roles of mite pathogenic fungi against *Tetranychus urticae* (two-spotted spider mite) MCn15 •273•

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The two-spotted spider mite, *Tetranychus urticae*, and gray mold disease caused by *Botrytis cinerea*, are important pest and plant disease, respectively, against fruit, vegetable and other plants in the world. To control these, many people have been relied on chemical methods for a long time. However, these are not always effective and their continuous use has resulted in resistance of pest and disease. Thus, many researchers have studied to overcome these problems. Recently, entomopathogenic fungi have been shown to have multiple roles as endophytes, phytopathogen antagonism, rhizosphere and plant growth promoter. Therefore, we performed to explore the various roles of mite pathogenic fungi. The antifungal activities of the selected fungi with high virulence to two-spotted spider mite were tested to explore the potential for the dual control of *B. cinerea* as well as *T. urticae*. Antifungal activities against the fungus *B. cinerea* were evaluated by dual culture assays using both aerial conidia and cultural filtrates of mite pathogenic fungi. As a result, two fungal isolates were selected considering both the virulence to mite and antifungal activity. To maximize the use of spores and cultural filtrates, the virulence to mite was further evaluated using the cultural filtrate, blastospore and aerial conidia. Consequently, the 2 fungal isolate selected in this study were confirmed to have diverse potential and would be used effectively for dual control agents against the two-spotted spider mite and plant diseases.

Diverse activities of entomopathogenic fungi with the virulence against *Myzus persicae* (green peach aphid) STU MCn16 •274•

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The green peach aphid, *Myzus persicae*, and *Botrytis cinerea*, causing gray mold, are major pest and phytopathogens, respectively, affecting vegetables and fruit crops in greenhouses and fields all over the world. To control this aphid and disease, farmers have depended on the use of chemical insecticides or fungicides, and their concentrated use over many years has led to the development of resistance. Recently, the potential of entomopathogenic fungi with multiple roles has been verified as endophytes in plants, phytopathogen antagonism, rhizosphere and plant growth promoter. Therefore, in this study, we tested these various roles of entomopathogenic fungi as an effective alternative to control simultaneously pest and plant disease. The antifungal activities of the selected fungi with high virulence to green peach aphid were investigated to explore the potential for the dual control both *M. persicae* and *B. cinerea*. Antifungal activities were evaluated to plant pathogenic fungi *B. cinerea* by dual culture assay on PDA media and 96 well plate using fungi cultural filtrates. Two fungal isolates were selected finally based on insecticidal and antifungal activities. For the usefulness of dual control, the virulence to aphid was further tested using the cultural filtrate, blastospore and aerial conidia of selected isolates. These fungal isolates selected in this study would be used effectively as the dual control agents of green peach aphid and gray mold.

A search for novel *Metarhizium* isolates for control of *Rhipicephalus microplus* ticks MCn17 •275•

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Inappropriate use of chemical acaricides for control *Rhipicephalus microplus* ticks (cattle ticks) cause both economic losses and human health risks due to meat, milk and environmental contamination. Control of *R. microplus* ticks with fungi is a promising choice as it involves low risk to human health and relatively low cost. Unfortunately, bioassays in the laboratory routinely demonstrate that entomo-pathogenic fungi (EPF) are considerably less virulent to ticks than to insects. Hence, to seek *Metarhizium* isolates with likely histories of infecting ticks, we conducted isolation of *Metarhizium* from sites where a cattle tick population occurred. *Metarhizium* from soil tend to be well adapted to this environment, sometimes as plant endophytes, and may be highly persistent in the field. Biological assays with fungi to control ticks are frequently performed with isolates previously deposited in fungal collections; thus, in most published studies, tick population and the fungal isolates to which they are exposed have no geographical or time congruence, which may explain a routinely low tick-control rates reported. Accordingly, the present study aimed to I) isolate *Metarhizium* fungi from soil samples collected close to the tick population to be tested (named FS); II) exam fungal isolates' heat tolerance to select highly tolerant strains, and III) assay these isolated fungi against FS tick larvae. The samples were examined as to their physical and chemical characteristics, and fungi were then isolated from this soil using CTC artificial selective medium (Fernandes et al. 2010). Heat tolerance tests were performed by exposing conidia to 42°C for four hours and relative germination was calculated after 24 h incubation; a well understood *M. robertsii* isolate (ARSEF 2575) was used as standard in thermotolerance tests. Nine isolates (named LCMS01 to LCMS09) were obtained from 100 soil samples. Isolate LCMS04 showed exceptionally high

thermotoler-ance (over 50% germination). Bioassay showed LCMS02, LCMS04 and LCM06 had the highest larval death 5 days after fungal exposure, reaching 91.9%, 85.3% and 95% average tick larval mortality. Accordingly, LCMS04 was selected for further studies as a promising new agent for control of cattle ticks in the field.

Enhancement of *Beauveria bassiana* virulence and efficacy to control *Diatraea saccharalis* in a sugarcane crop for *panela* production **Mcn18** **•276•**

***Juliana Gómez-Valderrama*[1], *Lorena García Riaño*[1], *Diana Marcela Monroy*[1], *Gustavo Adolfo Araque*[1], *Carlos Espinel*[1], *Laura Villamizar*[1,2]**

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The crop of sugarcane for *panela* production is of great importance in Latin America, Central America and India. *Panela* yield depends on sucrose content in sugarcane, characteristic seriously affected by the presence of the stem borers complex of the genus *Diatraea* sp., difficultly controlled by chemical insecticides. A Colombian isolate of *Beauveria bassiana* (Bv062) demonstrated high potential to develop a bioinsecticide for the control of *Diatraea* sp. The present work aimed to evaluate potential virulence enhancers for fungal conidia and determine efficacy of formulated conidia under laboratory and field conditions. Three potential enhancers (glucose, Trichoderma sp. enzymatic extract and sodium lignosulfonate) were mixed with Bv062 conidia and evaluated to control *D. saccharalis* larvae. Glucose and an enzymatic extract increased three times the insecticidal activity of the conidia. Conidia were formulated using two delivery systems: emulsifiable concentrate (EC) and dispersible granulate (WG). Mean lethal concentration estimated in a laboratory bioassay was $1x10^6$ con/mL for both prototypes and this value decreased to $4x10^5$ con/mL, when conidia enhanced with *Trichoderma* sp. extract. The EC formulation presented the highest in plant efficacy under semi-controlled conditions and was selected to be evaluated in the field at three different application rates. Significant differences were not detected between doses, with 93% efficacy obtained with the lowest dose ($1x10^{12}$ con/ha). Results demonstrated the high potential of the isolate Bv062 to control the predominant pest in sugarcane crops for *panela* production.

Selection of *Metarhizium* sp isolates based on thermotolerance, endophytic colonization and a gene expression assay **MCn19** **•277•**

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Entomopathogenic fungi can be endophytic-symbiotic with plant species, and can act against host plant pests. Their bi-functionality is characterized by the expression of different genes, and resistance to biotic, and abiotic stress factors. The aim of the present research was to study the thermotolerance, endophytic colonization of mung bean plants, and expression of the gene adhesin-like protein 2 (Mad2), in order to select Canadian isolates for field use. *Metarhizium* sp, isolates from soil of the South West region of British Columbia, Canada, were used for the thermotolerance experiment. Suspensions of 10^5 conidia/ ml of each isolate were placed in water-baths at 40°C, 45°C, and a control temperature of 25°C, for 2, 4, 8, and 12h. Germination was evaluated after a 48h incubation in the dark at 28 ± 1 °C. For the endophytic colonization trial and gene expression analyses, four isolates were used: ISH-CC-90, ISH-CC-277, ISH-CC-188, and ISH-CC-133. Mung bean seeds were treated by immersion in fungal suspensions of 10^6 conidia/ml of the respective isolates. Plants were treated with suspensions of 10^8 conidia/ml as a foliar spray, and a soil drench. The controls for both treatment applications were water. Two weeks later, plants were harvested and their leaves, stems, and roots were sampled to evaluate endophytic fungal colonization. After passage of the isolates through the plant, the relative expression of the gene Mad2 was profiled by qPCR, and compared with colonies cultivated in PDAY medium. Among the isolates submitted to the thermotolerance test, two showed more than 94% germination after 12h of heating at 40°C. For isolates exposed to 45°C for 2 hours, up to 73% germination was observed. All isolates tested for endophytic colonization were able to colonize all tissues analysed. The molecular results of the Mad2 gene expression are underway. Prior to field use it is essential to understand characteristics of new microbial agents and these results will be used in the selection of new isolates of *Metarhizium* sp., as well as contribute to the scientific knowledge about the ecology of this microorganism.

Lethality of the entomogenous fungus *Beauveria bassiana* Strain NI8 on *Lygus lineolaris* (Hemiptera: Miridae) and its possible impact on beneficial arthropods **MCn20** **•278•**

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Bioassays were conducted to examine the pathogenicity of the fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), strain NI8, against *Lygus lineolaris* (Palisot de Beauvois) and its impact on beneficial arthropods including *Apis mellifera* L., *Crysoperla rufrilabris* Burmeister*, Orius insidiosus* Say*, Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* (Pallas), *Coleomegilla maculata* De Geer, and field-collected Aranea spiders: Salticidae and Thomisidae. Insects were treated with four concentrations of NI8 (3.9×10^4, 2.3×10^5, 4.2×10^6 and 1.5×10^7 spores/mL) directly via topical spray. Median lethal concentration (LC_{50}), sporulation response (SR_{50}), and resistance ratio (RR_{50}) were estimated for all species except for the two groups of spiders. No significant differences in mortality (10-d after application) were observed among *L. lineolaris*, *A. mellifera* and *C. rufilabris*. All three species were highly affected when exposed to the highest concentrations of *B. bassiana* with 99.0%, 98.2%, and 90.0% mortality, respectively. Between 35 and 45% of the tested populations of *O. insidiosus*, *H. convergens*, field-collected crab spiders, and *C. maculata* were killed at 1.5×10^7 spores / mL; whereas only 22% and 27%, respectively, of the field-collected jumping spiders and *H. axyridis* were killed at 10-d with the same concentration. No significant differences were found between the LC_{50}s measured for *L. lineolaris* and *C. rufilabris* 10-d after application. Results suggested that *C. rufilabris* would be highly affected by the NI8 strain of *B. bassiana* when applied for control of *L. lineolaris.* In contrast, *B. bassiana* appeared to have lower effects on the other beneficial arthropods assayed at the concentrations targeted for *L. lineolaris* control.

Entomopathogens in conjunction with imidacloprid could be used to manage wireworms (Coleoptera: Elateridae) on spring wheat **MCn21** **•279•**

Frank B. Antwi[1], *Govinda Shrestha*[1], ***Gadi V.P. Reddy***[1], *Stefan T. Jaronski*[2]

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The soil-dwelling larvae of click beetles (wireworms) (Coleoptera: Elateridae) are serious pests of several agricultural crops worldwide. *Hypnoidus bicolor* and *Limonius californicus* are two major wireworm species damaging to spring wheat, particularly in the Golden Triangle, an important cereal-growing region in Montana. Wireworm damage to this field crop in the region is increasing, as currently available pesticides provide only partial control and no alternative options have been developed. We examined the effect of biopesticides alone, their mixtures or in conjunction with a conventional pesticide (imidacloprid) against wireworms. Biopesticides tested were: 1) spinosad *Saccharopolyspora spinosa*, (2) *Metarhizium brunneum*, (3) *M. brunneum* F52, (4) *Beauveria bassiana* GHA, (5) azadirachtin, (6) *B. bassiana* ANT-03 (7) pyrethrin, (8) *Chromobacterium subtsugae*, and (9) *Burkholderia* spp. strain A396. The efficacy of

biopesticides was based on crop stand protection, wireworm larval populations and grain yield. In the 2015 study, we found that entomo-pathogenic fungi alone or their combinations with imidacloprid had significant impacts on plant stand protection compared to water control. Applications of *B. bassiana* or a combination of *M. brunneum* F52 with imidacloprid protected wheat stand seedlings from wireworm damage better than water control at the Ledger location, while a combination of *B. bassiana* with *M. brunneum* F52 provided similar protection at the Valier location. Unexpectedly, wireworm larvae populations were found significantly higher on plots treated with *B. bassiana*, spinosad, *M. brunneum* F52 + spinosad, and *M. brunneum* compared to water control at 14 or 28 days post applications at the Ledger location, but without effects at the other location. We found significantly higher grain yield when plots treated with imidacloprid + *M. brunneum* F52 and *B. bassiana* + azadirachtin over water control at the Ledger location. In the 2016 study, there were no significant effects of treatments on studied parameters as compared to water control.

Performance of three Indian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and three commercial mycoinsecticides against three developmental stages of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae) **Mic22 •280•**

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Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), has been identified as one of the three most important agricultural pests in South East Asia. In India, it causes losses up to 80% on mango alone. In this study three isolates of *B. bassiana* and three commercial mycoinsecticides were screened against larva, pupa and adults of *B. dorsalis*. The isolates ITCC No. 6628 and B. NCIPM were found pathogenic to adult stage with LC_{50} $2.5x10^5$ and $7.5x10^6$ conidia /ml, respectively. While ITCC No. 6645 found effective against adult stage at 1.2x109 and 3rd larvae stage with LC_{50} $9x10^9$ conidia /ml. However, the three commercial mycoinsecticides prove their efficacy only on adult stages with 26.6%, 40% and 46.6% mortality for, Bio-power®, Bio-magic®, Bio-catch®, respectively. Significant differences between treatments and control were recorded accompanied by observation of mycosis on the cadavers.

Testing formulation improvements for application of the entomopathogenic fungus *Metarhizium brunneum* to control Asian longhorned beetles **MCn23 •281•**

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The Asian longhorned beetle (ALB) infestation in the US midwestern state of Ohio, first detected in 2011, is still under eradication by the USDA. In an ongoing effort by our lab group to assess the potential for biological control using insect-pathogenic fungi, in 2016 we tested a sprayable formulation of *Metarhizium brunneum* F52 microsclerotia within the Ohio ALB regulated zone, at East Fork State Park. Since ALB might be rare or absent, effectiveness was tested using adult beetles in our quarantine lab colony. Small wood veneer samples (attached to tree trunks with Velcro) were sprayed in June and then collected biweekly from June to August for use in bioassays with adult females. Additional samples were used for spore counts, since insect mortality depends on the density of infective spores produced by the microsclerotia. Spore production peaked at 4 weeks after spraying, resulting in a median ALB survival time (ST50) of 9.5 days after beetle exposure. The Ohio formulation included straw hydromulch and hydrogel to increase moisture retention. To test whether the gel provided a significant improvement over hydromulch alone, in a separate set of 4-week field trials in Ithaca, NY, three versions of the microsclerotial formulation were compared (straw, gel, or straw+gel). There were no significant differences in beetle mortality from quarantine bioassays for samples collected for July, August and September replicates. In the August trial, a mere 2-day exposure of ALB was effective in killing all beetles within 10-11 days. The area experienced a severe drought in June and samples in the field during that period were not effective. When rainfall was adequate, any of the three formulations showed potential for development as a spray application onto tree trunks for use during summer when adult beetles would be active.

Coating of maize seeds with entomopathogenic fungi promotes rhizosphere colonization, endophytic ability and provides biocontrol of a pest and pathogen **STU MCn24 •282•**

***F. Rivas*[1,2]**, *T.A. Jackson*[3], *N. Altier*[2], *M. Rostás*[1], *J. Hampton*[1], *T.R. Glare*[1]

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Entomopathogenic fungi were originally identified as pathogens of insects, but recently some species have also been described as plant root colonizers and potentially endophytes. These fungal – plant associations could lead to benefits to the plant such as plant growth promotion or increase systemic resistance to pathogens. We evaluated the impact of different New Zealand isolates of *Metarhizium anisopliae*, *M. robertsii*, *M. guizhouense*, *M. novozelandicum*, *Beauveria bassiana* and *Trichoderma harzianum*, on maize after applying them in a seed coating. The seeds were sown in potting mix in the presence of *Costelytra zealandica* (root feeding grass grub larvae) and *Fusarium graminearum* (maize pathogen). Rhizosphere colonization, measured by quantification of fungal colony forming units, was greatest ($P=0.019$) from roots treated with *M. robertsii* (F447), followed by *M. anisopliae* (F672) with *M. guizhouense* (BK41) the lowest. The presence of grass grub larvae significantly reduced rhizosphere colonisation for all isolates while *F. graminearum* had no effect. No differences were noted in endophytic ability among the isolates, however when larvae were present the endophytism rate was reduced while *F. graminearum* increased the endophytic colonisation by the entomopathogenic fungi ($P<0.05$). Seedling emergence and shoot length were unaffected by the biocontrol fungal strains alone, but *M. anisopliae* (A1080) and *M. guizhouense* (BK41) treatments produced increased shoot length in the presence of *F. graminearum*. Grass grub larvae reduced root dry weight in all maize plants, although this reduction differed depending on the fungal strain used for the seed coating treatment. Bare seeds, without a biocontrol fungal coating, were the most affected by *F. graminearum*, while *B. bassiana* (Bb21), *M. guizhouense* (F16) and *M. novozelandicum* (F99) reduced the damaged caused to the roots by this fungal pathogen. Biocontrol fungi differed in pathogenicity towards grass grub larvae; with *M. anisopliae* (F672) being the most pathogenic while *M. guizhouense* (BK41) and *M. robertsii* (F447) the least. This study showed that it is possible to deliver entomopathogenic fungi via a seed coating to provide biocontrol of both a fungal pathogen and an insect pest.

Evaluation of pyrethrum, extract of *Saccharopolyspora spinosa*, *Beauveria bassiana* and *Metarhizium anisopliae* for the control of *Chaetanaphothrips signipennis*, a pest of banana **Mcn25 •283•**

Alex Delgado[1], *Richard Hall*[1,2], *Daniel Navia*[1], *William Viera*[1], *Francisco Báez*[1], *Mirian Arias*[1], *and* ***Trevor A. Jackson***[3]

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The banana rust thrip, *Chaetanaphothrips signipennis,* is a significant pest of bananas in South America where it causes mancha roja (red ring disease) which leads to fruit discoloration and fruit rejection from the market. The problem is particularly significant for organic growers where chemical pesticides are prohibited. In this study we evaluated biological products and entomopatho-genic fungi isolated from Ecuadorian soils for control of thrips and prevention of fruit damage. A laboratory bioassay included the treatments of *Beauveria bassiana* (10^8 conidia per ml) isolated from soil of

organic banana orchard located in El Oro, *Metarhizium anisopliae* (10^8 conidia per ml) isolated from a pasture field located in Sucumbios, Phyriplus (Pyrethum) 2 ml/L, Spinosad (*Saccharopolyspora spinosa*) 1 ml/L, Phyriplus + *Spinosad* 2 ml/L+ 1 ml/L and a control. In the bioassay, Pyrethrum and Spinosad produced 100% thrip mortality within 24 h and fruit was completely free of red ring discolouration following treatment. The fungi *B. bassiana* and *M. anisopliae* produced 23% mortality, significantly greater than the control (7%), but there was no damage reduction with 63 % red ring recorded in the fungal treatments and 25% in the control. However, it is possible that using the high rate of 10^8 spores per ml killed the insects mechanically rather than through infective processes. Further assays were undertaken with seven strains of *B. bassiana* and ten strains of *M. anisopliae* using a maximum of 10^7 conidia per ml, a concentration likely to be maximal in the field and which would, if pathogenic, kill insects by infection. None produced significant mortality. Pyrethrum and Spinosad have also been tested in the field and adopted for commercial production with applications at bunch formation and at closure of the covering bag. The rejection rate of fruit due to mancha roja has dropped from 90 % to 8 %. It appears that the biological products, Spinosad and Phyriplus, are effective controls for mancha roja for organic farmers and, at this time, the entomopathogenic fungi cannot be recommended for use by producers.

Evaluation of biocontrol potential, plant growth promoting activities and physiological effects of bacterial endophytes against major pathogens of rice in Eastern Himalaya Region of India. **Poster – Mcn26**

Mohammed M. Abdelbaset Hassan[1], *Dipali Majumder*[2], *Dwipendra Thakuria*[2], *Krishnappa Rangappa*[3]

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Twenty bacterial endophytes obtained from. Out of 20 isolates tested by Gram staining, 4 were found as Gram+ve and remaining 16 as Gram-ve. Endophytes were tested for PGP attributes and, lytic enzymes and phosphate solubilization. 20 isolates were positive for IAA production (113.16 to 566.05 µg/ml), whereas none of the isolates were positive for phosphate solubilization and HCN production. One isolate (34WE) recorded positive for cellulase production. Three isolates (6WE, 11WE and 44E) were recorded for 100% germination. Rice seeds treated with isolate 34WE showed maximum shoot length (9.68cm) followed by 11WE (9.48 cm), which were statistically non-significant, whereas isolate 60E was recorded as best for root elongation (14.73 cm). Only one isolate i.e. 34WE was recorded as effective antagonist against P. grisea and X.oyzae pv. oryzae with inhibition of 20mm and 11mm respectively in dual culture assay. In-vivo study of endophytes for PGP attributes revealed that, rice seedlings treated with the isolates 8WE showed highest plant height (95.75 cm), followed by isolate 54E (93.75 cm) and 15WE (91.0 cm), which were found significant. All the isolates showed significant root length in comparison to control during in-vivo tests. Number of effective tillers/hill was recorded highest in case of isolate 60E (8.00) followed by isolate 19WE (7.00), which differed significantly. Number of filled grain/panicle was recorded highest in isolate 54E (335), which was statistically significant as compared to control (158.05). Rice seedlings treated with all bacterial endophytes showed significant seeds test weight in comparison to control during in-vivo evaluation. Out of 20 isolates inoculated into rice seedlings (CAUR1), only one isolate i.e. 26WE was observed as effective against the sheath blight pathogen.

WEDNESDAY, 8:00 – 10:00 pm

Fungi Division – Business meeting, *Village West, Building 2, Rooms 2A/2B*

Microsporidia Division – Business meeting / Workshop, *Village West, Building 2, Room 2C*

The Microsporidia as Eukaryotes **Microsporidia workshop** **•284•**

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We discuss, in plain terms, the relationship between phylogeny and taxonomy in the context of the Microsporidia as eukaryotes. The goal is not to make a statement about the position of the Microsporidia within the eukaryotes but to look at the data and to discuss the parameters we might weigh as new evidence and new analyses are presented. Factors to consider include: convergence, horizontal gene transfer, phylogenetic methods, long branch attraction, and apomorphic versus plesiomorphic character states. Controversial examples are taken from the vertebrates when considering the appropriateness of accepting paraphyletic taxa.

Virus Division – Business meeting and tribute to Michael R Strand's election to the US National Academy of Sciences. Organiser: Elisabeth Herniou. Vote of Thanks by Peter Krell. Response by Michael Strand

Village Tower West, 15th fl., 15B

THURSDAY, 8:00 – 10:00 am

Theater

BACTERIA DIVISION SYMPOSIUM

What is Bt? Current perspectives

Organizer and Moderator: Neil Crickmore

Challenges in understanding the biology of Bt **8:00** **•285•**

Neil Crickmore

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Despite the fact that *Bacillus thuringiensis* has been used successfully as a biopesticide for almost 80 years, and its toxin-encoding genes expressed in genetically modified crops for over 20 years, there is still much that we don't understand about this bacterium. Many of us have read, indeed written, papers that tell the world that Bt is a gram-positive, spore-forming, soil-dwelling, environmentally-friendly organism that produces toxins capable of forming pores in the gut of a target organism and thus killing it. Some of these we are sure about (it is gram-positive) but many of the other aspects remain controversial, or poorly understood. In this symposium we will look at various aspects of Bt and consider some of the broader topics related to its biology. Is Bt just *B. cereus* with a Cry toxin encoding plasmid? Why are the toxins on plasmids anyway? Where did the toxins come from, spore coat proteins or somewhere else? How are the toxins evolving? Can Bt target human cells? Can Bt even kill insects by itself? This first talk will introduce a number of these topics and will consider some of the difficulties encountered in defining many of the terms we commonly associate with Bt and its toxins.

Bacillus thuringiensis: A constellation of toxins **8:25** **•286•**

Daniel R. Zeigler

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Bacillus thuringiensis has been traditionally defined as a *B. cereus*-like organism that produces one or more parasporal crystals composed of proteins toxic to insects and other invertebrates. Nearly four decades of molecular analysis have unveiled a constellation of proteins with widely diverse histories that have become mingled in these organisms and are now co-evolving with them. Many proteins may co-exist in crystals, while others may be synthesized and secreted by vegetatively growing cells. Detailed analysis of the best-studied of these protein families—the three-domain Cry proteins—has revealed that they themselves are composites pieced together from domains with independent histories of their own. There is abundant evidence that homologous recombination between cry genes has resulted in Cry proteins with shuffled domains and therefore a wide array of finely tuned differences in structure and function. Genomic studies confirm that cry genes and other toxin genes are associated with mobile elements, especially megaplasmids. Plasmids are mobile elements. They can be cured, resulting in a crystal-free bacterium, or they can be mobilized in conjugation, resulting in a bacterium with a new combination of toxin genes. There are now numerous examples of cry genes in distantly related endospore-forming species from outside the family *Bacillaceae*. These observations challenge the traditional definition of *B. thuringiensis* and raise difficult questions about the phylogeny of the *B. cereus* species group.

A genomic perspective on the definition, evolution and phylogenetics of _Bacillus thuringiensis_ **9:00** **•287•**

***Ben Raymond*[1], *Guillaume Meric*[2], *Jinshui Zheng*[3], *Donghai Peng*[3], *Ben Pascoe*[2], *Ming Sun*[3], *Samuel K. Sheppard*[2]**

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Understanding how and why *Bacillus thuringiensis* is distinct from *Bacillus cereus,* an opportunistic pathogen of humans, is crucial when discussing whether *Bt* isolates make safe pesticides, and for studying the evolution of pathogenicity in the *B. cereus* group. Currently, using Cry toxins to define *Bt* imposes compli-cations because *cry* genes are typically carried on conjugative plasmids. Thus, diverse phylogenetic clades in the *B. cereus* group contain *cry* genes, while chromosomally similar strains can be either *Bt* or *B. cereus:* making *Bt* both polyphyletic and paraphyletic. Here I describe two population genomic studies that investigated the distribution of plasmids and virulence genes in the *B. cereus* group phylogeny. We found that strains carrying c*ry* genes can be found in all the major *B. cereus* clades. Surprisingly, parasporin genes and *cry* genes originally associated with *Lysinibacillus* and *Paenibacillus* spp. are common and widely distributed. However, the clade containing the classic biopesticidal isolates (clade 2 or the '*kurstaki*' clade), stands out for a number of reasons: *Bt* isolates in clade 2 carry more Cry toxins; toxins with clear invertebrate toxicity are conc-entrated in clade 2; and Cry toxins in clade 2 are also associated with a clade-specific plasmid. The tight association between virulence plasmids and bac-terial chromosomes appears to have evolved several times in the specialized pathogens in the *B. cereus* group. Strong directional selection in a pathogenic niche could also account for the reduced allelic diversity in *cry* positive isolates. The variation in carriage of virulence genes also confirms earlier work highlighting the consider-able ecological differences between the major clades in the *B. cereus* group. We could better incorporate phylogenetics into the definition of *Bt* by restricting this species name to Cry expressing isolates in clade 2. This definition would exclude several *Bt* isolates of dubious safety, *i.e.*, those closely related to *Bacillus anthracis.* An additional practical consequence would be that we would be able to use genes and plasmids unique to clade 2 to develop simple diagnostic tests for *Bt.*

General discussion **9:45**

The Forum

Microbial Control Division #4

Moderators: Roma Gwynn, Dietrich Stephan

Characterization of the efficacy and yield promoting effects of the nematopathogenic fungus _Purpureocillium lilacinum:_ Greenhouse evaluation of BioAct DC (_Purpureocillium lilacinum_ strain 251): understanding and predicting in-field efficacy and beneficial effects on yield **8:00** **•288•**

***Tracy K. Webb*[1], *Kristi R. Sanchez*[1], *Chun-Jou Hsiang*[1], *Kristen J. Rasmussen*[1], *Marc Rist*[2], *Malte Roemer*[2], *Punita Juneja*[1], *Matthew R. Tarver*[1], *Reed N. Royalty*[1]**

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BioAct DC*,* a novel liquid formulation of the fungus *Purpureocillium lilacinum* strain 251*,* has previously shown interesting efficacy in field tests. As is often the case with nematicides, the efficacy of BioAct DC was variable in these trials. However, a detailed cross-trial analysis of the trial results did show a consistent beneficial effect on crop yield. The subsequent development of *in vitro* plate and *in planta* bioassays (Sanchez et al.) was pivotal in understanding the fungal biology, plant-parasitic nematode infection process, and the effect of abiotic factors such as temperature on performance of *Purpureocillium lilacinum*. These lab assays also showed positive effects on growth of plants. In order to more fully understand the potential of BioAct DC as a useful nematode management tool for growers, it was necessary to transfer these lab results to the greenhouse. Bayer nematologists, greenhouse horticulturists, and biometricians worked together to develop an experimental design ideal for greenhouse evaluation of BioAct DC and other nematicide treatments. A latin-square design coupled with detailed measurements of environmental conditions allowed us to incorporate spatial effects into the experimental design. As a result, we created a bioassay method that has, with manageable replication, the statistical power necessary to differentiate among treatments. Our assays showed that data on dry root weight, total fruit weight, and egg counts reliably describe the activity of *P. lilacinum* and other bionematicides against *Meloidogyne javanica*. We demonstrated a clear dose-response with *P. lilacinum*. BioAct DC treatments outperformed competitive biological products, producing significantly higher yields and root weight in treated plants, and a decrease in root knot nematode reproduction. We also tested the potential of an integrated nematode management program featuring BioAct DC in combination with the chemical nematicide fluopyram (Velum Prime). By optimizing the experimental design of the greenhouse pot assays, we can now reliably predict and better understand the results of past and future field trials with *P. lilacinum* and other bionematicides.

Development of a laboratory testing cascade to predict the in-field activity of the nematopathogenic fungus _Purpureocillium lilacinum_ **8:15** **•289•**

***Kristi R. Sanchez*[1], *Marc Rist*[2], *Matthew R. Tarver*[1], *Reed N. Royalty*[1]**

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Nematopathogenicity of the fungus *Purpureocillium lilacinum* strain 251 has been well documented in the literature. Several commercial products have been introduced, but like many fungal-based biologic products, they have enjoyed limited commercial success due to inconsistent performance. In 2013, Bayer developed BioAct DC, a novel liquid formulation of *P. lilacinum*. The product showed promising efficacy in field trials, but activity was inconsistent. Our efforts to explain variable field performance with traditional laboratory and greenhouse nematicide bioassays were unsuccessful. To support Bayer's efforts to launch BioAct DC, we developed a cascade of progressively higher-tiered bioassays that characterize the

performance of nemapathogenic fungi. Our *in vitro* assay describes the pathogenicity to nematode eggs and motile J2 larvae. With this assay, we made detailed observations of spore attachment, germination, production of appresoria, and subsequent growth of hyphae on infected nematodes. *P. lilacinum* consistently infected and killed *Meloidogyne*, *Pratylenchus*, and *Heterodera* species. *P. lilacinus* attacked J2 larvae and eggs of phytophagous nematodes, while the free-living nematode *C. elegans* was not attacked. These results suggest properties associated with obligate parasitism. We also quantified the effects of temperature on parasitism with this assay. We also developed a small 'jar' test to characterize the activity of nematopathogens against *Meloidogyne* spp. *in planta*. This assay was easy to set up, and of short-duration (ca. 2-3 weeks). We found that counting egg masses was a faster and more statistically-robust measure of efficacy than the 'traditional' method of counting galls. Consistent suppression of egg mass production and positive effects on the growth of the host tomato were observed in response to *P. lilacinum*. BioAct DC provided superior control and plant growth promoting effects relative to other *P. lilacinum* formulations. Differences in efficacy between strain 251 and other *P. lilacinum* strains were observed. The bioassay also described a clear dose-response for treatments. Methods developed in this *in planta* 'jar' test were subsequently incorporated into greenhouse pot trials, where BioAct DC is also showing performance superior to other nematopathogens. Data from our lab tests are being used to both explain results from past field trials, and to design protocols for future testing.

Effect of entomopathogenic fungus, *Metarhizium anisopliae* on South American tomato leafminer, *Tuta absoluta* (Meyrick), in the field in Tanzania **8:30** **•290•**

Never Mwambela [2], *Thomas Dubois*[1], *Srinivasan Ramasamy*[1]

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Invasion of the tomato leafminer *Tuta absoluta* has become the major threat to tomato production in sub-Saharan Africa including Tanzania. Broad spectrum chemical pesticides are the primary mode of control locally, but mis- and overuse of these pesticides lead to development of resistance, environmental degradation and increasing production cost among smallholder farmers in Tanzania. The efficacy of alternative options, such as the use of biological control agents, has not yet been studied in sub-Saharan Africa. This study evaluated the efficacy of the commercially available biopesticide, *Metarhizium anisopliae* and pheromone lures for management of *T. absoluta* under field conditions in Tanzania during the rainy and dry seasons. *M. anisopliae* significantly increased growth and yield of tomato compared to untreated fields to a level that was on par with fields treated with pesticides, whereas pheromone traps were comparatively less effective in preventing damage. In fields treated with *M. anisopliae*, *T. absoluta* larval damage decreased by 79.3-85.2% across seasons whereas in fields treated with pheromone traps, damage decreased by 56.0-76.2% across seasons compared to untreated fields. This study demonstrates the biological control potential of *M. anisopliae* against *T. absoluta* on tomato in the highlands of Tanzania, and this biological control agent may be suitable for other regions of Africa, as well.

Successful mass production and application of *Metarhizium anisopliae* for rice pest control during the full growing season **8:45** **•291•**

Guoxiong Peng, Deyu Zeng, Mingsheng Hong, ***Yuxian Xia***

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Pest outbreaks cause significant losses in rice production worldwide. To control various rice pests including the rice stem borer, the planthopper, the leaf roller and others, several chemical insecticides have to be used repeatedly during the growing season. Current dependence of chemical insecticides to control rice insect pests causes increasing public concerns on human health and the environment. We show that a broad spectrum strain of the entomopathogenic fungus *Metarhizium anisopliae*, CQMa421, can infect all the major rice insect pests. We report on the results of a 5 year field trial study in different provinces in China that shows that an oil suspension of CQMa421 is effective in controlling the three major rice insect pests found, i.e., the stem borer, planthopper, and leaf roller. Although the efficacy of the CQMa420 oil suspension in terms of insect mortality to the three main insect pests were lower in the field trials as compared to use of chemical insecticides (Buprofezin, Chlorpyrifos, Thiamethoxam), overall rice yield in fungal versus chemical treated plots were not significantly different. The results were similar when the oil suspension of CQMa421 was applied repeatedly to control insect pests during the full growing season. Our results demonstrated that the broad-spectrum *M. anisopliae* strain CQMa421 can protect rice from major insect pests during the full growing season and that simple measurements of pest mortality may be a poor indicator of overall plant health and/or crop yield in agricultural applications. To produce enough of the fungal aerial conidia (and final formulated product) required for large-scale, economically feasible use, a solid-state fermenter was specially designed capable of producing 5 tons of formulated fungal spores within a month period. The successful mass production and application of *M. anisopliae* for rice pest control resolves a key technological hurdle in terms of production, application, and cost-effectiveness of mycoinsecticides for crop pest control.

Characterization of entomopathogenic fungi for microbial control of *Spodoptera exigua* **9:00** **•292•**

Ji hee Han*, Jeong Jun Kim, Sang Yeop Lee*

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Beet armyworm, *Spodoptera exigua* is one of the most destructive polyphagous pests for many economically important crops but this pest is difficult to control using chemical insecticides because of the development of insecticide resistance. Various control agents may use for eco-friendly beet armyworm managements, Entomopathogenic fungus can be used alternative to chemical control agent. In this study, we investigated characteristic of *Paecilomyces fumosoroseus* which is high virulent isolate against *S. exigua*. *P. fumosoroseus* caused 100% mortality 6 days after treatment at 1×10^4 conidia/ml against 2nd instar larvae and effectively controlled the larvae at 20 ~ 30℃ and 45 ~ 100% RH. Control efficacy of *P. fumosoroseus* against 4th-5th instar larvae was 96.7 ± 2.1% and 36.7 ± 8.4% and LT_{50} was 4.4 and 6.7 days. This isolate was also found to repel *S. exigua*. Adult of the beet armyworm avoided oviposition at Chinese cabbage treated with *P. fumosoroseus* compare to control. This result may be used to control and prevent the infestation of moth in crop production.

Comparison of different formulations of *Beauveria brongniartii* for control of white grubs of *Melolontha melolontha* in apple orchards **9:15** **•293•**

Maximilian Paluch, Joschua Göttmann, ***Dietrich Stephan***

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White grubs of the common cockchafer, *Melolontha melolontha* can cause dramatic damage in apple orchards. Because no pesticides are registered to control white grubs in Germany new control methods are needed. Although *Beauveria brongniartii* is known to control white grubs, up to now no product is registered. This may be due to the high production and registration costs and the complicated application technique. To reduce the production and formulation costs we developed a liquid fermentation system for the *B. brongniartii* strain BIPESCO2 and coated submerged spores in a fluid bed dryer on millet as carrier. After 68 hours of fermentation time we got the maximum yield of $6.3\text{x}10^8$ submerged spores per ml and a dry weight of 4.9 mg per ml. 15 ml of a fungal suspension containing 2 % of biomass (dry weight) was used for coating 100 g millet. For quality control the millet was incubated on water agar and the fungal outgrowth was evaluated. When the coated millet was stored for at least 42 days at 20 °C, the fungus was growing on 100 % of the coated millet grain. No contamination was visible. Up to $1\text{x}10^6$ conidia per millet grain were produced on water-agar after 11 days. In a first bioassay all white grubs treated with the coated millet died within nine

weeks. In a field trial at an organic apple orchard three different formulations were compared. Before application soil samples were taken and based on the *Galleria* and *Tenebrio* bait method and using selective media, the occurrence of entomopathogenic fungi was determined. The commercial product Melocont and the coated millet were applied with an application rate of 50 kg per ha by slitting the turf and adding the granule underneath of the turf. The application is based on conventional direct sowing machines. Additionally, for one treatment a liquid suspension (7,5 l/ha with a concentration of around $1x10^6$ submerged spores/ml was poured in the slits. Six weeks after application again the occurrence of entomopathogenic fungi was evaluated. First results of this ongoing field trial will be presented and discussed.

Combined effect of *Beauveria bassiana* and *Heterorhabditis bacteriophora* against *Rhynchophorus ferrugineus* (Olivier) **9:30** **•294•**

Waqas Wakil[1], *Muhammad Yasin*[1,2]

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The interaction between entomopathogenic nematode *Heterorhabditis bacterio-phora* and entomopathogenic fungi *Beauveria bassiana* against fourth-instar of red palm weevil (*Rhynchophorus ferrugineus*) was studied in the laboratory experiment. *Heterorhabditis bacteriophora* was added simultaneously or 1, 2 and 3 weeks after application of *B. bassiana*. The results showed that integrated application of *B. bassiana* with *H. bacteriophora* increased larval mortality either in an additive or synergistic manners. To achieve stronger additive and synergistic interactions, larvae had to be exposed at least 2 or 3 weeks before the nematode application. In combined treatments increase in mortality, decrease in pupation, adult emergence and egg hatching was found with a direct relation to the application interval. Our study suggested that the combined use of *B. bassiana* with *H. bacteriophora* may offer an effective integrated approach for *R. ferrugineus* control.

Infection of invasive red palm weevil by endophytic *Beauveria bassiana* colonizing date palm in combination with *Bacillus thuringiensis* **9:45** **•295•**

Muhammad Yasin[1,2], *Waqas Wakil*[2*]

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The effect of *Beauveria bassiana* inoculated in date palm petioles alone and then with integrated application of *Bacillus thuringiensis* var. *kurstaki* (*Bt-k*) was evaluated against second-instar larvae of *Rhynchophorus ferrugineus* in laboratory studies. Both the alone and combine treatments were applied against *R. ferrugineus* larvae to monitor larval mortality, pupation, adult emergence and egg eclosion after treatment. Mortality was low in sole treatments, while in combined treatments increased mortality while decreased pupation, adult emergence and egg eclosion were found. Synergistic effect (CTF≥20) on the mortality was observed when larvae were exposed to simultaneous application of *B. bassiana* with 40 µg ml^{-1} of *Bt-k*. It can be surmised from the findings that endophytic colonization of *B. bassiana* in combination with *Bt-k* has potential as an effective strategy to control *R. ferrugineus*.

Roosevelt Room

DISEASES OF BENEFICIAL INVERTEBRATES SYMPOSIUM

The Pathobiome Concept: An emerging view of microbes and diseases

Organizers and Moderators: David Bass, Helen Hesketh

The pathobiome concept: An emerging view of microbes and disease **8:00** **•296•**

David Bass[1,2], *Ronny van Aerle*[1] , *Lydia Doherty*[1], *Jamie Bojko*[1], *Dominique Chaput*[3], *Grant Stentiford*[1]

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Recent studies have shown that pathogens, parasites, and related lineages are highly diverse in environmental and organismal matrices. While some of these are recognized disease causing agents, the role of others is less clear – they may be asymptomatic or contribute directly or indirectly to a cumulative effect on host health. The pathobiome idea is starting to replace a one-pathogen-one-disease paradigm, based on the concepts of a host organism being the focus of a diverse community of microbial symbionts, multiple infections being the norm rather than exception, and synergistic effects occurring between infecting microbes. This talk will present the results of recent studies investigating microbial diversity associated with a range of invertebrate and plant hosts, and review broader findings supporting the pathobiome concept, a further case study being the microbiome associated with disease outbreaks in aquaculture pond systems.

Characterization of pathobiomes using high-throughput sequencing: Challenges and opportunities **8:30** **•297•**

Ronny van Aerle[1]

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Recently, it has been shown that many pathogens are part of complex microbial communities, collectively known as the pathobiome, in which they interact with other micro-organisms (e.g., bacteria, viruses, fungi, protozoa) to influence or drive disease processes in the host organism. In order to understand the role of the pathobiome in the disease process, it is important to study the diversity of organisms in this community. Recent advances in high-throughput sequencing technologies and the development of comprehensive bioinformatic tools facilitated the analysis of species diversity within pathobiomes based on sequence information. This presentation will provide an overview of the current approaches used in the characterization of pathobiomes, including metagenetics (e.g., 16S/18S rRNA amplicon sequencing) and metagenomics (shotgun DNA sequencing of environmental samples). The importance of study design (e.g., inclusion of relevant controls, sequencing coverage/depth) as well as challenges (e.g., limited sequence availability in public reference databases, false positives) and opportunities (e.g., single molecule sequencing, functional metagenomics) will be presented.

Effects of the gut microbiota on susceptibility to pathogens in social bees **9:00** **•298•**

Nancy A. Moran[1]

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Honey bees and bumble bees have a characteristic gut microbiota that has coevolved with them for millions of years. In honey bees, the adult gut is dominated by 9 bacterial species, each of which can be present as multiple

strains with distinct capabilities. Early evidence for a protective effect of the gut microbiota came from bumble bee studies showing that the microbiota protected against trypanosomatid parasites. Later studies have shown that the gut microbiota of honey bees has a protective effect against opportunistic Gram negative bacterial pathogens. The routes of protection are not well understood, but colonization by the entire gut microbiota or by single strains of the core species results in upregulation of antimicrobial peptides of the host. There may also be direct interactions within the gut that enable the core microbiota species to kill opportunistic pathogens or to block their entry from the gut into bee cells or the body cavity. Additionally, the gut microbiota in bees causes a major shift in the physicochemical conditions within the gut, likely making the environment more hostile to invasive opportunists. Use of antibiotics in bee colonies, for prophylaxis against larval pathogens, may result in higher adult mortality due to disruption of the adult gut microbiota. Both honey bees and bumble bees are beneficial insects that appear to be undergoing increasing rates of mortality. Preservation of honey bee and bumble bee health will likely be enhanced by practices that help to sustain a healthy gut microbiota.

Competitive interactions and disease suppression in soil microbiomes **9:30** **•299•**

JP Dundore-Arias*, Linda L. Kinkel*

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Plants live in intimate association with diverse microbes, and these associations are essential for plant performance and survival. Our work explores the interacting roles of plants and microbial species interactions in determining the pathogen-suppressive capacity and composition of soil microbiomes, and the consequences for plant productivity. Using culture-based approaches we have found that plant community diversity plays a critical role in determining the likelihood of antagonistic arms race coevolution vs. niche differentiation among sympatric soil populations, with significant implications for plant disease suppression. Moreover, amplicon sequencing of rhizosphere communities associated with different plant hosts provide insights into non-cultured taxa associated with disease suppression. This presentation will cover what we have learned about the factors that contribute to determining the pathogen-suppressive and plant growth-promoting potential of indigenous soil microbes, and the possibility to create tailored approaches targeting species interactions to enhance plant health and productivity.

10:00–10:30 am Coffee Break

THURSDAY, 10:30 am – 12:30 pm

Theater

Society for Invertebrate Pathology
Annual Business Meeting

J. Jehle, President, SIP

THURSDAY, 12:30 – 1:30 pm

IMPORTANT: All posters must be removed before noon today.

Awards Committee member lunch *(Green Room)*
Monique van Oers, Chair

THURSDAY, 1:30 – 3:30 pm

Theater

FUNGI, NEMATODES, AND MICROSPORIDIA DIVISIONS CROSS-DIVISIONAL SYMPOSIUM

Ecology of Invertebrate Pathogens

Organizers: David Shapiro-Ilan, Ann E. Hajek

Interaction networks of invertebrate pathogenic fungi: Ecological consequences of multifunctional lifestyles and community diversity **1:30** **•300•**

Nicolai V. Meyling[1]*, Ann E. Hajek*[2]

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Two main taxonomic groups include invertebrate pathogenic fungi, Entomophthorales and Hypocreales. These fungi interact with many hosts, are widespread in terrestrial ecosystems and are causing significant ecological effects. Recent studies have revealed an extraordinary diversity within hypocrealean invertebrate pathogenic fungi regarding both their genomic variability and ability to exploit hosts from different kingdoms as resources. The traditional view of fungal species as a single entity is therefore challenged as is the perception of the entomopathogenic hypocrealean fungi as solely acting as pathogens in nature. We give examples of recent knowledge of resource utilization and community diversity within the main genera *Metarhizium* and *Beauveria* to emphasize the complex interaction networks that these fungi may engage in. We discuss the significance of these interaction networks in an ecological context to predict the potential effects of the interactions as well as the possible causes for the complex community structure of the invertebrate pathogenic fungi observed in various ecosystems.

Dispersal, dispersion and disruption: where and why entomopathogenic nematodes move **1:50** **•301•**

Edwin Lewis[1]*, Ivan Hiltpold*[2]*, David Shapiro-Ilan*[3]

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Entomopathogenic nematodes (EPNs) are unusual as pathogens because of how much they move. Infective juveniles move to maximize the probability of encountering hosts. The strategies EPNs employ to find hosts have a great impact on which host species they ultimately infect. Many studies describe and measure EPN infective stages' movement toward hosts, or toward plants that are infested with potential hosts, but what stimulates and governs movement when hosts are absent? EPNs are active without being directly stimulated by host cues and their population structure does not depend entirely on host population structure. Thus, an intriguing question is: what makes an obligate parasite with no function other than to find a host move, other than the presence of a host? Dispersal of EPN IJs as they leave the cadaver is driven by conditions in the cadaver and the presence of semiochemicals, including ascaroside pheromones, at the end of an infection. In addition to stimulating dispersal from the host, these substances also influence infection behavior, tolerance to environmental extremes and lifespan of the IJs. Distribution of IJs in the soil is known to be patchy, but what is the advantage to staying in groups? Group dynamics studies suggest that there is a cost to being alone. For EPNs, that cost is the inability of a single IJ to successfully infect a host (assuming success is defined as producing offspring). Thus, not only do EPN IJ populations have a clumped distribution in the field, but they move *en masse* in the absence of a host as well. A disturbance can be defined as the interruption of a settled and peaceful condition. Soil conditions affect all aspects of EPN existence, especially dispersal and dispersion. Thus, layered on top of complex behaviors, physiology and infection dynamics are the temporally and

spatially variable conditions within the soil environment. Peace and happiness for EPNs in an unforgiving world remains a challenging goal.

The ecology of microsporidia - a balancing act between minimizing host damage and maximizing pathogen reproduction and transmission **2:10** **•302•**

Leellen Solter[1]*, Gernot Hoch*[2]

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The Phylum Microsporidia, a monophyletic taxon of eukaryotic unicellular pathogens that appear to be ubiquitous in invertebrate populations, possess a suite of unique genetic and biological characteristics that determine interactions with their hosts. Similar to pathogens in other taxonomic groups, interactions between each microsporidian species and its host(s) can vary considerably from those of other microsporidian-host relationships, but the overall trend is a general chronicity that maximizes pathogen production, vertical transmission or both. Typically, microsporidia do not cause detectable harm to the cells of their hosts until sporulation begins. When sporulation is completed, horizontally transmitted species often cause high mortality in their hosts. Vertically transmitted species tend to be less virulent. Characteristics that best describe microsporidia include an uncertain taxonomic placement related to Fungi; obligate intercellular development; absence of many eukaryotic features, notably functional mitochondria; interactions with host mitochondria in infected cells that suggest direct energy (ATP) uptake; lack of plant and fungal hosts; and possession of a unique invasion apparatus consisting of a polar filament through which the infective form is injected from the spore into a host cell. Microsporidian life cycles range from simple to highly complex and, in some cases, include obligate intermediate hosts. As well, pathogen-host population dynamics and impacts on host populations are highly diverse. Here, we highlight some of the unique characteristics of microsporidia that govern the ecology of these primary pathogens of invertebrate animals.

The hidden ecology of bacterial entomopathogens **2:30** **•303•**

Trevor A. Jackson[1]*, Colin Berry*[2] *and Maureen O'Callaghan*[1]

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This presentation will review the ecology of bacterial entomopathogens which are frequently reported from dead and diseased insects and include well known species, such as *Bacillus thuringiensis*, and more recently discovered pathogens like *Yersinia entomophaga*. Unlike other pathogen groups, bacteria are rarely obligate pathogens and often exist in commensal forms with an ecology outside the insect host. In spite of this, entomopathogens often show a high degree of host specificity suggesting an evolutionary advantage from the close host association. The pathogenicity genes of entomopatho-genic bacteria can be chromosomal or borne on transferable elements (plasmids) suggesting a gene flow (transfer) between pathogenic and non-pathogenic forms. During infections, entomopathogenic bacteria use the insect host as an energy source to multiply and once an epizootic has developed can become dominant forms in the microbiota. To take advantage of the relationship with the insect host, bacteria must have a mechanism for transmission between hosts and survival through periods with absence of susceptible hosts. This can be achieved through resistant stages (spores), or survival and multiplication in the host's environment. Vertical transmission through all stages of the insect life cycle is suggested for some species. Despite their abundance, the ecology of most species of bacterial entomopathogens is not well understood and they constitute a hidden resource for further study and exploitation. Bacterial entomopathogens are an important resource for pest management and knowledge of their ecology will aid the search for new forms/genes as well as guide strategies for their use and management after application.

Ecology of insect viruses: how viruses adapt to their insect host population **2:50** **•304•**

Madoka Nakai[1]***,*** *Trevor Williams* [2]

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Insect viruses cause both overt disease and covert infection in their hosts. One key characteristic that determines the ecology of insect viruses is the presence or absence of an occlusion body (OB). The OB is particularly important in the transmission of viruses that infect insects in terrestrial habitats. The best studied insect viruses from an ecological standpoint are baculoviruses because they can be used as biological insecticides against agricultural and forest pests. Parameters affecting the efficacy of baculoviruses as natural biological control agents are well documented involving infectivity, speed of kill, persistence in the environment and as covert infections in the host population and the range of host species that are infected naturally in a particular ecosystem. Other perspectives of baculovirus ecology include the study of cyclic population dynamics of some forest pests including the Gypsy moth (Lymantria dispar) or the Western tent caterpillar (Malacosoma californicum pluviale). In non-occluded insect viruses transmission often ocurrs directly from infected host to susceptible host through mating and cannibalism, or indirectly via an insect vector. Ascoviruses are unusual insect viruses that are transmitted via endoparasitoids. Overall the transmission, persistence and dispersal of insect viruses are modulated through host factors such as insect feeding and dispersal behavior, and by biotic and abiotic factors, however, these should not be viewed in isolation but rather as a set of interacting and interdependent processes.

Ecology of emerging infectious diseases of invertebrates **3:10** **•305•**

Colleen A. Burge*, Natalie D. Rivlin, Amanda Shore-Maggio*

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Infectious diseases are important natural drivers within ecosystems. However, epidemics of infectious disease have become more frequent, causing concern about the long-term impacts on ecosystem function, economic growth, and food security. Of particular concern are emerging infectious diseases (EIDs), defined as those which have rapidly increased in incidence or geographic range, recently moved into new host populations, and/or recently been discovered or are associated with a newly evolved pathogen(s). Even though invertebrates represent the greatest diversity of animals in both marine and terrestrial ecosystems, EIDs of invertebrates have been poorly defined and are not well understood, in part from a lack of baseline data, unresolved or complex etiologies, lack of specific diagnostic tools, and inherent difficulty of monitoring for disease outbreaks. The best information on invertebrate disease ecology, human influence on disease emergence, and best practices for disease management/prevention come from investigations of commercially important invertebrates (e.g., oysters, shrimp, and bees). More effort is being made to better understand diseases of ecologically important species (e.g. corals, sea stars). Disease emergence is often defined by the disease triad (host-pathogen-environment relationship) and how direct anthropogenic change and climate change shift the underlying homeostasis. Several case studies will be used to discuss EIDs of invertebrates in terrestrial and marine systems, specifically highlighting the basic ecology and etiology of the disease, the human activities or environmental covariates of disease emergence and/or transmission, and attempted or preventative measures. Given the high impact of invertebrate EIDs, we need to be ready to detect and to respond to the next invertebrate EID.

The Forum

MICROBIAL CONTROL DIVISION SYMPOSIUM

Biopesticides III: Beyond entomopathogenicity - Reliable tools or just a novelty?

Organizers amd Moderators: Roma Gwynn, Travis Glare, Michael Brownbridge

We increasingly see papers showing effects of microbial inoculants on plant performance (improved stress tolerance, better growth, etc.), and evidence of effects of endophytes and epiphytes on plant genes and expression of compounds in response to stress (abiotic and biotic). Entomopathogenic fungi have long been considered as biocontrol agents based on their ability to directly infect and kill arthropods. More recently, non-lethal effects which complement crop protection efforts have been documented and their status as (part-time?) endophytes demonstrated. Effects of 'non-entomopathogens' on insect and other pests are also well recognized for true endophytes such as Epichloë, but not so much for many 'newer' endophytic associations (are all entomopathogenic fungi really endophytic?). To take full advantage of the benefits these organisms can bring to crop protection efforts we need to be able to demonstrate the type of plant responses that can be expected from these associations, the level to which these are induced, and the consistency of these responses (on a commercial scale). In addition, cost-effective ways of deploying these technologies must be found, with attendant regulatory pathways defined and end-user buy-in confirmed before they can be fully commercialized. 'Biopesticides III' builds on prior Symposia at SIP, and brings together a mix of industry and academia to consider the end-game for these plant-microbe associations.

Biopesticides III - The final chapter? 1:30 •306•

Michael Brownbridge

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Biopesticides are increasingly considered as replacements for chemical pesticides that have come under regulatory scrutiny, as essential components of resistance management, and as components of IPM systems where functional compati-bility with other biological control agents is essential. In the first Biopesticides Symposium, we considered the prevalence of insect-pathogenic fungi in nature, aspects related to improving the consistency of their performance, and how other characteristics shown by these organisms, e.g., as endophytes, may contribute to pest management. In the second chapter, we explored growing and evolving opportunities to use microbial biocontrol agents in plant protection. While these approaches are based on insect pathogens, they use their multiple ecologies, bioactives or new formulation and delivery systems to more fully exploit these beneficial microbes. In the third session of this series, we will focus on non-lethal effects of 'insect pathogens' which complement crop protection and assess their status as endophytes. Effects of 'non-entomopathogens' on insect and other pests are well recognized for true endophytes such as *Epichloë*, but not so much for entomopathogenic fungi such as *Beauveria* and *Meta-rhizium*. To take full advantage of these organisms in pest management we need to be able to demonstrate the type of plant responses that can be expected from these associations, the level to which these are induced, their impact on pest (and non-target) organisms, and the consistency of these responses on a commercial scale. In addition, cost-effective ways of deploying these technologies must be found, with attendant regulatory pathways defined and end-user buy-in confirmed before they can be fully commercialized.

Plant associations of *Metarhizium* and *Beauveria* 2:00 •307•

***Travis R. Glare**, Federico Rivas, Aimee C. McKinnon, Maya Raad, Maria E. Moran-Diez, Mchael Rostás*

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Entomopathogenic fungi in the genera *Metarhizium* and *Beauveria* have been well studied as direct mortality agents against invertebrate pests. Many products are based on strains from these genera. The discovery of the ability by species of both genera to colonise plants has suggested exciting possibilities for further exploitation in pest control. A plethora of publications have indicated endophytic occurrence and other studies have shown root colonisation abilities. What is the true ecology of these insect pathogens and what is the potential for commercial and practical use of the ability to closely associate with plants? It seems that not all species or strains within the genera share plant association abilities, but there also does not appear to be specialisation of strains within species. Are some species truly endophytes or are these largely opportunistic associations? The crucial aspect of these plant associations, whether they confer benefits to the plant in terms of pest and disease resistance and plant growth promotion, also appear to be variable. We will explore the evidence that is accumulating on plant associated entomopathogens from *Beauveria* and *Metarhizium*, including some of our own data. The response of plants to colonisation provides fascinating insights into resistance mechanisms. Is it now possible to develop crops with stable communities of associated entomopathogens?

Beauveria bassiana strain ATCC 74040 – does the understanding of its endophytic activity affect its use as a biocontrol agent? 2:30 •308•

***Edith Ladurner**[1], Massimo Benuzzi[1], Sergio Franceschini[2], Francesco Greco[2]*

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Beauveria bassiana strain ATCC 74040, the active ingredient of the formulated plant protection product Naturalis®, has been shown to be able to endo-phytically colonize some plant species, in particular grapevine and tomato. Furthermore, evidence exists that the microbial control agent, once endo-phytically established, can contribute to negatively affecting the survival and/or development of key insect pests of grapevine and tomato, respectively grape mealybug (*Planococcus ficus*) and tomato leaf miner (*Tuta absoluta*). In short, the likelihood exists that the endophytic activity of the fungal strain could be exploited to complement crop protection. The potential of the findings of the above-mentioned studies in affecting the use of *B. bassiana* strain ATCC 74040 as a biocontrol agent is discussed in consideration of several important aspects, such as application rates tested in the studies on endophytic activity of the antagonist *versus* authorized application rates of the microbial control agent, observed percentages and consistency of endophytic colonization, efficacy observed in the above-mentioned studies *versus* efficacy considered acceptable for the purposes of registration of a plant protection product and by growers, etc.. An in-depth analysis of the possible effects and repercussions of the observed endophytic activity of *B. bassiana* strain ATCC 74040 on its use as a plant protection product is provided.

Approaches for the commercialisation of plant colonising microorganism: the importance of educating the gatekeepers 3:00 •309•

Roma Gwynn

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Microbials developed for crop protection for farmers and growers (for both for insects and diseases) have so far been based on well-known species and the interactions have been more or less binary, e.g., entomopathogenic fungi directly colonising and killing the host. Established commercial development pathways are in place for these technologies, including appropriate regulatory frameworks or approaches. Whereas, plant colonising microorganisms are complex, they have a wide range of interactions that are influenced not only by the interaction with the pests and diseases but also by the plant which in turn, is influenced by its biotic and abiotic environment. Capturing the net of all these effects and using them to develop a product that can be sold is largely unknown but probably equally complex. Applied research into plant colonising microorganisms for pest and disease management is moving forwards quickly but will bringing plant colonising microorganism onto the market be simple? Once it is established that the strain can be produced reliably on a large enough scale, commercialisation is a combination of does the product 'work', how to use it to obtain consistent benefits and how can it be regulated. In particular, how can their effectiveness be demonstrated and how can this be explained to end users (agronomists, crop consultants, sales personnel and farmers). It will be important to know what benefits the microbes deliver, whether they are always measureable and whether they can be demonstrated commercially. Sitting alongside this for commercial development companies, is that they need to know what the regulatory pathways are for plant colonising

microorganisms. This presentation will consider the commercialisation processes for plant colonising micro-organisms and how this will influence the ease of availability and adoption of these new technologies.

Plant microbe interactions, microbial solutions for invertebrate pests, and crop yield: Current and future focus of the crop protection industry **3:30** **•310•**

***Reed N. Royalty**, Dilara Ally, Magalie Guilhabert*

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Many microbial-based pest and disease control products have been introduced into the agricultural marketplace. Although some of these products have been successful commercially, they are justifiably perceived as being less efficacious than alternative solutions. For this reason, there has traditionally been minimal R&D investment by the crop protection industry in microbial solutions, relative to the level of investment in synthetic chemistry and genetically-modified crops. However, due to the recent commercial success of microbial seed treatments, and to regulatory pressure on and public concerns about chemical pesticides and genetically-modified crops, the agrichemical industry is now investing significantly in microbial solutions. Increased investment is occurring both in major agrichemical companies and in smaller 'start up' firms. Research and development on microbial solutions differs from research on chemical and genetically modified plant solutions in that the research often focuses on the interactions between microbes, plants, the targeted pest, and the environment. This broader 'ecological' approach is viewed by many companies as having a greater likelihood of technical success than simply focusing on controlling a particular pest with a particular microbe. In this presentation we present our perspective on recent research activities by the agrichemical industry on endophytic and rhizospheric-competent microbes.

Roosevelt Room

Virus Division #5

Moderators: Roma Gwynn, Dietrich Stephan

New viruses exclusive to the honey bee pathogen mite *Varroa destructor* **1:30** **•311•**

***Nor Chejanovsky**[1], Sofia Levin[1,2], Noa Sela[1],*

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Varroa destructor, the endoparasitic mite of *Apis mellifera* carries and/or promotes replication of viruses pathogenic to the honey bee like the Deformed wing virus, the Acute bee paralysis virus, the Israeli acute bee paralysis virus and the Kashmir bee virus. However, viruses exclusively associated with *Varroa* and not present in *A. mellifera* were not found. To search for viruses associated with- or infecting *V. destructor* we performed next generation sequencing (NGS) of RNA extracted from Varroa mites and honey bees in *Varroa*-infested colonies. Comparative bioinformatic analysis of the two separate contig-assemblies generated from the sequences' reads, annotated using Blastx, enabled identification of new viruses unique to *Varroa* and absent in *A. mellifera*: an Iflavirus and a virus with homology to Ixodes scapularis associated virus 2, that we named Varroa destructor virus 2 (VDV-2) and 3(VDV-3), respectively. Furthermore, we confirmed these findings sequencing the mite- and honey bee-viromes and in by Reverse transcription polymerase chain reaction in separate mites and honey bees randomly sampled. The genomes of VDV-2 and VDV-3 bear 9576 nucleotides and 4202 nucleotides, respectively. Phylogenetic analysis of VDV-3 suggests that it belongs to a new group of viruses. Our results open venues for investigating the pathogenicity of these new *V. destructor* viruses.

A microcarrier-based process for production of the *Oryctes* nudivirus in spinner-flask bioreactors **1:45** **•312•**

***Charlotte Pushparajan**[1], Juan Daniel Claus[2], Gabriel Alberto Visnovsky[3]*

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The coconut rhinoceros beetle, an economically important palm pest, is effectively controlled by application of its natural pathogen, *Oryctes* nudivirus (OrNV). Production of OrNV can be performed *in vivo* by infecting larvae. *In vivo* production has several disadvantages, primarily, lack of sterility and inconsistency in virus titers produced. *In vitro* production of OrNV in the susceptible and permissive DSIR-HA-1179 host insect cell line, is an attractive alternative to produce pure, sterile virus at a standardized titer. Among other factors, the feasibility of producing viral biological control agents in insect cell cultures in bioreactors rests on the ability to grow and subsequently infect host cells in suspension culture. The anchorage-dependent nature of the DSIR-HA-1179 cell line precludes its growth in free suspension, but microcarriers which provide a surface area on which cells can grow attached to, can be used to cultivate them within a suspension-like environment and provide a starting point to scale-up the system. This study evaluated OrNV production in DSIR-HA-1179 microcarrier cultures in spinner flask bioreactors. Cells were grown in TC-100 culture medium supplemented with 10% fetal bovine serum on three types of microcarrier, Cytodex-1, Cytodex-3 and Cultispher-G, in 60ml spinner flasks. Cells attached to Cytodex-1 and Cytodex-3 with efficiencies of 99.6% and 98.5% respectively, but failed to attach to Cultispher-G. Maximum cell density was dependent on bead type and concentration and cell-to-bead ratio. With a bead concentration of 1 mg/ml and cell-to-bead ratio of 30, a maximum cell density of 1.7×10^6 cells/ml was reached on Cytodex-1 and 1.2×10^6 cells/ml on Cytodex-3 microcarriers. Since it supported higher cell yields, Cytodex-1 was chosen to study the kinetics of OrNV production in this system. Infecting cells during the early exponential growth phase at a multiplicity of infection of 1 led to an OrNV yield of 1.3×10^8 $TCID_{50}$/ml and cell-specific yield of 177 $TCID_{50}$/cell, which was an improvement over volumetric (6.5×10^7 $TCID_{50}$/ml) and cell-specific yields (102 $TCID_{50}$/cell) obtained in infections in attached T-flask cultures. Future work will focus on optimization and scaling-up of OrNV production in the microcarrier system in stirred tank and airlift bioreactors.

Vago*-like gene, *BtSVC* is involved in antiviral immune responses and antimicrobial peptides (AMPs) expression in bumblebee *Bombus terrestris **2:00** **•313•**

***Haidong Wang**, Guy Smagghe and Ivan Meeus*

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The single von Willebrand factor C (SVCs) proteins are a recently discovered arthropods protein family with their functionalities poorly characterized. Recent study has shown that Vago, a member of SVCs can act as an antiviral modulator in *Drosophila melanogaster* and *Culex* mosquitos. Within hymenopteran species, we noticed a reduced repertoire of SVC protein family members compared to flies and mosquitos. Especially in most bee species, there is just one SVC which function is not clear. Therefore we asked if this SVC in bumblebee *Bombus terrestris* is an immune modulator. More specifically, we tested its antiviral function in relation to Israeli acute paralysis virus (IAPV). Our results showed that silencing *BtSVC* lead to increased viral titers in fat bodies. Silencing *Dicer-2*, a key player of the siRNA pathway resulted in a lower expression of *BtSVC* and increased viral titers. Interestingly, we also found that silencing *BtSVC* can lower the expression of four antimicrobial peptides (AMP)-coding genes. These results proved that *BtSVC* has a *vago*-like function and plays a role in the antiviral immunity of bees. Furthermore, the interaction of *BtSVC* with AMPs related pathway also shows its potential to be an immune modulator in the insect innate immune system.

Pesticide enhance the level of chronic bee paralysis virus **2:15** **•314•**

*Beibei Li[1,2], Shuai Deng[1,2], Fei Li[1,2], Qingyun Diao[1,2], **Chunsheng Hou**[1,2]*

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Honey bee plays a vital role in pollination for plants and crops, especially for wild plants in mountain area in China. However, there are increasing reports about the large number of honey bee dead or unable to forage in many apiaries. Although a variety of emergent microbial pathogens were thought to be impact to honeybee colony losses, little is known about the interaction between viruses and environmental stressors, such as pesticides. To understand better the causes, we collected samples from several provinces and to screen the 8 common viruses and imidacloprid. The results shown that samples were widespread infected by CBPV as well as imidacloprid from environment. To confirm whether imidacloprid can elevate the level of CBPV under the natural condition, we infected healthy bee samples with crude CBPV at low level and then feed the imidacloprid, and found that imidacloprid can enhance the level of CBPV of honeybee. These indicated that pesticides from environment have impacts on honey bee healthy in a long term. This work was funded by the National Natural Science Foundation of China (31572471) and the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2017-IAR).

Shinobi tetravirus and Kunoichi rhabdovirus, latent viruses in mosquito cultured cell line, suppress multiplication of arboviruses **2:30** **•315•**

***Ryosuke Fujita**[1,2], Fumihiro Kato[2], Shigeru Tajima[2], Masayuki Saijo[2], Haruhiko Isawa[2], Kyoko Sawabe[2]*

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Viruses in blood-sucking insects can be categorized in following 3 groups; insecti-cidal viruses, human/animal-pathogenic viruses so called arthropod-borne viruses (arboviruses), and other viruses without any symptoms in infected hosts. Recent technical developments such as next generation sequence analysis enable us to identify viruses without morphological changes in infected cells. We could successfully identify and isolate two viruses which latently infect to mosquito *Aedes albopictus* cultured cell line C6/36 obtained from a cell bank of JCRB. Shinobi tetravirus (STV) was a novel single-strand, positive sense RNA virus, and Kunoichi rhabdovirus (KRV) was a novel virus categolized in a family Rhabdo-viridae. These viruses were not found in the C6/36 cells obtained from a different cell bank. Using these cell lines, we compared growth of Zika virus (ZIKV), Dengue virus (DENV), Japanese encephalitis virus (JEV), Sindbis virus (SINV), and Getah virus (GETV). As a result, the growth of flaviviruses (ZIKV, DENV, and JEV) were dramatically suppressed in JCRB C6/36 cell lines whereas the differences of growth kinetics of alphaviruses (SINV and GETV) were not so significant. We cloned STV and KRV from JCRB C6/36 cell line, then re-infected them to another C6/36 cell line, resulting in reproduction of latent infection of each virus. ZIKV growth was also suppressed in STV and/or KRV re-infected C6/36 cells. We also determined the response of innate immune signaling pathway in viral infected C6/36 cells and found that IMD pathway was up-regulated by STV and/or KRV latent infection, although the contribution of IMD pathway to suppression of ZIKV was still obscure. This is the first demonstration that latently infected rhabdovirus and/or tetravirus suppressed flavivirus replication in mosquito cells.

Origin of supplementary capsid proteins in different AAV serotypes produced with the baculovirus/insect cell expression system **2:45** **•316•**

*Lionel Galibert[1,2], Adrien Savy[1,3], Yohann Dickx[1], Delphine Bonnin[1], Bérangère Bertin[1], Isidore Mushimiyimana[4], Aurélien Jacob[1,5], Marjorie Boutin-Fontaine[1], Monique M. van Oers[6], Philippe Moullier[7], Fulvio Mavilio[1], **Otto-W. Merten**[1]*

[1]Généthon, Evry, France; [2]FinVector Vision Therapies, Kuopio, Finland; [3]Synpromics Ltd., Edinburgh, U.K.; [4]University of Eastern Finland, A.I. Virtanen Institute for Molecular Sciences, Kuopio, Finland; [5]TIGET, Ospedale San Raffaele – Milano, Milan, Italy; [6]Wageningen University, Laboratory of Virology, Wageningen, The Netherlands; [7]INSERM UMR 1089, Thérapie génique translationnelle des maladies neuromusculaires et de la rétine, Nantes, France
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The baculovirus/insect cell expression system is a common scalable production system for adeno-associated viral (AAV) vectors which are of great interest for *in vivo* gene therapy of rare genetic disorders. The generation and analysis of several AAV serotypes produced with this system has shown that, in addition to the three structural proteins (vp1, vp2, vp3) supplementary viral peptides could often be revealed by Western analysis. Moreover, for several serotypes, the expression level of vp1 was well below the expected level (which normally should be equivalent to that of vp2). More detailed analysis demonstrated that, in particular for AAV8, a part of these supplementary peptides observed in SDS-PAGE/Western blot was due to cleavage of vp1/vp2 by baculoviral cathepsin. The origin of other peptides was probably due to the codon-optimization performed during the development of the dual baculovirus system for the generation of AAV vectors (Smith et al. (2009) Mol. Ther. 17, 1888). Concerning the cleavage by cathepsin activity, we could show that the addition of the specific inhibitor E64 prevented the cleavage of vp1/vp2. A similar susceptibility to cathepsin cleavage was observed for AAV serotypes 1 and 6. The cleavage site was identified as L(189)-G(190)-E(191) (AAV8 amino acid numbering) and it could also be shown that the replacement of L by I or E by Q (as established for serotypes 2 and 9/10, for instance) led to resistance towards cleavage by cathepsin. Since it is not feasible to use E64 at a large scale, two strategies have been evaluated for alleviating this 'cleavage' problem: (i) mutation of single amino acids to render the cleavage site 'non-cleavable', and (ii) use of a cathepsin knock-out baculovirus. Whereas the first strategy led only to a shift of the problem to hidden downstream cleavage sites, the second approach completely alleviated this problem, resulting in AAV preparations with a higher (about 3x) *in vivo* potency in mice than when using the non-modified baculovirus system. The paper will present details on the generation of supplementary peptides as seen in Western blot analysis either caused or not by baculoviral cathepsin activity.

A challenge of constructing insect/mammalian shuttle vector using two constitutive virus-based promoters **3:00** **•317•**

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It is quite often that a gene is required to be stably or transiently expressed in one of the baculovirus host cells such as Sf9, using the constitutive promoter OpIE2. In addition, a transient expression in mammalian cells is commonly driven by CMV promoter. However, two separate vectors would have been constructed if the same gene is required to be expressed in both types of cell lines (insect and mammalian cells). Here we are trying to construct a shuttle vector that can support the constitutive expression of transgenes in two cell lines of different species (Sf9 and Hela cells) using two of the aforementioned constitutive promoters. It was reported previously that the presence of ATGs in the second promoter of a shuttle vector eliminates its candidacy from being used. Accordingly, a truncated version of the OpIE2 includes only one ATG was cloned downstream of a wild type CMV promoter and upstream of the gene under test: enhanced green fluorescent protein (EGFP). The remained ATG was subjected to all possible nine single point mutations. Each of the mutated copies resulted in different expression level of the gene under test, in both sf9 and Hela cell lines. Although the nine mutated OpIE2 have no ATG remained, each mutated clone derived a variable expression level of EGFP in Sf9 and each has a negative, yet variable, impact on the gene expression in Hela cells. Further experiments are needed to decipher the reason for this variable expression patterns in both Sf9 and Hela cells.

Using recombinant baculovirus AcMNPV as a safe drug finding platform **3:15** **•318•**

Tzong-Yuan Wu[1], *Chun-Chung Chen*[1], *Ming-Kun Liu*[2]

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Previous studies have found that the addition of HDACis could significantly increase the baculovirus-mediated exogenous gene expression in mammalian cells. Based on this phenomena, we hypothesize that baculovirus could be designed to screen candidate drugs for HDACis. In this study, we test the hypothesis, using baculovirus-transduced cells and histone (acethyl Lys5, Lys8, Lys12, Lys16) antibody to identify a compounds that can enhance baculovirus-mediated gene expression in U2OS cells via stabilizing the hyperacetylation state of histone and implied that Bacmam could be a drug screening platform for HDACis. Besides, we also incorporated alphavirus structure proteins and replicon components into the Bacmam and used these recombinant baculoviruses to screen compounds that can interfere the entry or replication of alphavirus.

Inhibition of melanization by serpin-5 and -9 promotes nucleopolyhedrovirus infection in *Helicoverpa armigera* **3:30**

Chuanfei Yuan[1], *Longsheng Xing*[2], ***Manli Wang***[1], *Xi Wang*[1] *Mengyi Yin*[1], *Qianran Wang*[1], *Zhihong Hu*[1], *Zhen Zou*[2]

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Melanization, an important insect defense mechanism, is mediated by clip-domain serine protease (cSP) cascades and is regulated by serpins. Here we show that proteolytic activation of prophenoloxidase (PPO) and PO-catalyzed melanization kill the nucleopolyhedrovirus (NPV) *in vitro*. Our quantitative proteomics revealed that NPV infection of the cotton bollworm, *Helicoverpa armigera*, reduced levels of most cascade members in the host hemolymph and PO activity. By contrast, serpin-9 and serpin-5 were sequentially upregulated after the viral infection. The *H. armigera* serpin-5 and serpin-9 regulate melanization by directly inhibiting their target proteases cSP4 and cSP6, respectively and cSP6 activates PPO purified from hemolymph. Furthermore, serpin-5/9-depleted insects exhibited high PO activities and showed resistance to NPV infection. Together, our results characterize a part of the melanization cascade in *H. armigera*, and suggest that natural insect virus NPV has evolved a distinct strategy to suppress the host immune system.

3:00–3:30 pm Refreshments Break

THURSDAY, 6:30 pm – 12:30 am

Estancia La Jolla Hotel

6:30 Cocktail Hour
7:30 Banquet
Awards Ceremony
Live Band and Dancing

The Estancia La Jolla Hotel is a 15 minute walk from the Village (same distance as Price Center).

A shuttle will circle between the Village and the banquet starting at 6:15 from the Village.

Return shuttles from the banquet to the Village will run as needed, starting at about 9 pm.
The last bus leaves the banquet site at 12:30 am.

We look forward to seeing you at the 51st Annual Meeting of SIP!

12–16 August 2018
Gold Coast, Australia

Contact: Caroline Hauxwell
(*caroline.hauxwell@qut.edu.au*)

INDEX OF AUTHORS

Presenting authors and the abstracts they are presenting are listed in ***BOLDFACE ITALICS***.

Papers lacking appended numbers can be found in the program by the Division oral paper session or by the Division poster number. Divisions abbreviations are the following **Bac** (Bacteria), **DBI** (Diseases of Beneficial Invertebrates), **Fun** (Fungi), **Mcr** (Microbial Control), **Msp** (Microsporidia), **Nem** (Nematodes), and **Vir** (Virus).

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ADDITIONAL, LATE RECEIVED ABSTRACTS FOR POSTER PRESENTATION and OTHER PERTINENT CHANGES IN THE PROGRAM

MONDAY am

SIP Founders' Jubilee Celebration *(Theater)*

Micrsporidia research through the ages

Ann Cali[1]

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Since the establishment of the SIP, a primary interest has been the discovery of potential biological control agents and pathogens of beneficial insects. The Microsporidia are part of this mission and the related studies of safety, epidemiology, organism survival, and impact data are necessary. An example was the development of the microsporidium *Paranosem*a (*Nosema) locustae.* It is now a commercially available biological for the control of rangeland grasshoppers. While others have studied microsporidia for control of mosquitoes, lepidopterans, fire ants, beetles, honey bees etc. Microsporidia are obligate intracellular pathogens and an amazing amount of detail has been observed with the tools of the first 100 years. Approximately sixty years ago, Huger published the first electron micrographs (EM) of spores revealing their features. Subsequently, microsporidial EM studies have made huge advancements. Details of lifecycles and the host/parasite interface could be discerned, differences between organisms became apparent and the descriptions of genera were being modified. For example, at our first Microsporidia division meeting in 1970, EM of microsporidial development of three species of *Nosema* revealed their similarities and differences, which were sufficiently significant to separate them into two genera and *Encephalitozoon* was made a microsporidial genus. By 1976, Vavra and Sprague published "Biology of the Microsporidia" with EMs illustrating the presence and absence of cell organelles and the presence of new structures. In 1977, their second volume reviewed the known 46 genera. Publications of both morphology and taxonomy followed, but it was molecular techniques that became an integral part of biosystematic studies in the Microsporidia. The complete genome of *Encephalitozoon cuniculi* was published containing 2.9Mbases, one of the smallest known for a eukaryote. The small subunit rDNA became the standard for organismal comparison. This information lead to massive reclassification of the Microsporidia. They were considered very primitive, and since they lacked mitochondria, Cavalier-Smith (1987) moved them from Protozoa to the kingdom Archezoa. They were reconsidered because of features such as Golgi enzymes and microtubule sequence data to be Kingdom Protista but close to Fungi some consider them to belong in the Fungal kingdom. This is still a debate. Since 1985, several human infections reported especially *Enterocytozoon bieneusi*. It or similar identified in fish, pigs, and Arthropods.

TUESDAY am

Microbial Control Contributed Papers #1

(Marshall Room)

10:00 Mohammed I. Elbashir, P. Bishwajeet, K. Shankarganesh, P. Sharma. Performance of three Indian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and three commercial mycoinsecticides against three developmental stages of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae)
Paper #280; MOVED from poster MCn22

TUESDAY am

DBI Contributed Papers #1 *(Marshall Room)*

10:45 STU Nina S. Lottsfeldt, Mariah E. Weavil-Abueg, Lisa M. Crosson, Carolyn S. Friedman. *Candidatus Xenohaliotis californiensis* viability and infectious dose in *Haliotis rufescens*
THIS IS A JUDGED STUDENT PRESENTATION.

WEDNESDAY pm

POSTERS *(West Ballroom)*

FUNGI DIVISION POSTERS

Roles of two photolyases in sustaining UV-B resistance and photoreactivation of *Beauveria bassiana* **STU Fun26**

Ding-Yi Wang*, Sheng-Hua Ying, Ming-Guang Feng*

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Solar UV irradiation exerts a harmful effect on applied fungal biocontrol agents. Photolyases are enzymes that can repair DNA damage caused by UV irradiation. This study seeks to characterize functions of the CPD photolyase Phr1 and the 6-4 photolyase Phr2 in Beauveria bassiana, a filamentous fungal insect pathogen. Deletion of phr1 resulted in a loss of ~40% UV-B resistance and of >90% photoreactivation in conidia. Deletion of phr2 led to a significantly less loss of either UV-B resistance or photoreactivation. Short blue (450 nm) and violet (480 nm) lights were evidently optimal for the photoreactivation of both Phr1 and Phr2. Interestingly, the ∆phr2 mutant displayed a DNA repair activity increased with the time (0-5 h) of exposure to white light, greater tolerance to the cell wall stressor calcofluor white and increased yields of submerged blastospores and aerial conidia. Overexpression of phr2 in B. bassiana resulted in reversed changes in the aforementioned phenotypes. Conclusively, Phr1 and Phr2 played important, but differential, roles in the fungal response to harmful UV irradiation and hence in sustaining the fungal biocontrol potential against arthropod pests.

Daylight length-dependent translocation of VIVID photoreceptor in cells and its regulatory role in conidiation and virulence of *Beauveria bassiana* **STU Fun27**

Sen-Miao Tong*, Sheng-Hua Ying, Ming-Guang Feng*

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The fungal insect pathogen *Beauveria bassiana* has the blue-light photo-receptor VIVID (VVD) but lacks a pigmentation pattern to trace its light responses. Here we show that the fungal VVD is transcriptionally expressed, and linked to other blue/red photoreceptors, in a light-dependent manner. GFP-tagged VVD fusion protein was localized to periphery, cytoplasm and vacuoles of hyphal cells in light/dark (L:D) cycles of 24:0 and 16:8 and aggregated in cytoplasm with shortening daylight until transfer into nuclei in full darkness. Deletion of *VVD* reduced conidiation capacity in L:D 12:12 cycle of 450/480 nm blue light (91%) more than yellow-to-red (540–760 nm) and

white lights (~70%). The conidiation defect worsened with shortened daylight in different L:D cycles of white light, coinciding well with drastic repression of key activator genes in central development pathway. Intriguingly, the deletion mutant displayed blocked secretion of cuticle-degrading Pr1 proteases, retarded dimorphic transition *in vivo* or *in vitro*, and hence a lethal action twice longer than those for control strains against *Galleria mellonella* regardless of the infection passing or bypassing insect cuticle. Conclusively, VVD sustains conidiation level in a daylight length-dependent manner and acts as a vital virulence factor in *B. bassiana*.

Transcriptomic analysis unveils the potential pathways regulated by autophagy-related protein 1 (BbATG1) during conidiation of *Beauveria bassiana* **Fun28**

Wei-Xia Dong, Ming-Guang Feng, ***Sheng-Hua Ying***

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Conidiation promotes fungal dispersal and survival in the environment, and is a determinant for the biocontrol potential of *Beauveria bassiana*. Autophagy-related protein 1 (BbATG1), a serine/threonine protein kinase, plays an important role in autophagic process and development of *B. bassiana*. To explore the down-stream targets regulated by *BbATG1* under menadione stress, a comparative trancriptome was applied on the wild-type and the Δ*BbATG1* mutant strains during conidiation. Transcriptomic analysis indicated that the conidiation-related genes regulated by *BbATG1* were significantly enriched in the functional catalogs of metabolism, protein fate, cell rescue, and transportation. Conclusively, these results indicate that *BbATG1* contributes to conidial development of *B. bassiana* by mediating the potential pathway of metabolism and protein fate. These findings highlight the potential genetic network involved in fungal conidiation, and provide the new clues for improving condiation potency of entomopathogenic fungi.

Ethanol dehydrogenase I contributes to growth and sporulation under low oxygen condition via detoxification of acetaldehyde in *Metarhizium acridum* **Fun29**

Erhao Zhang[1,2], ***Yueqing Cao[1,2]****, Yuxian Xia[1,2]*

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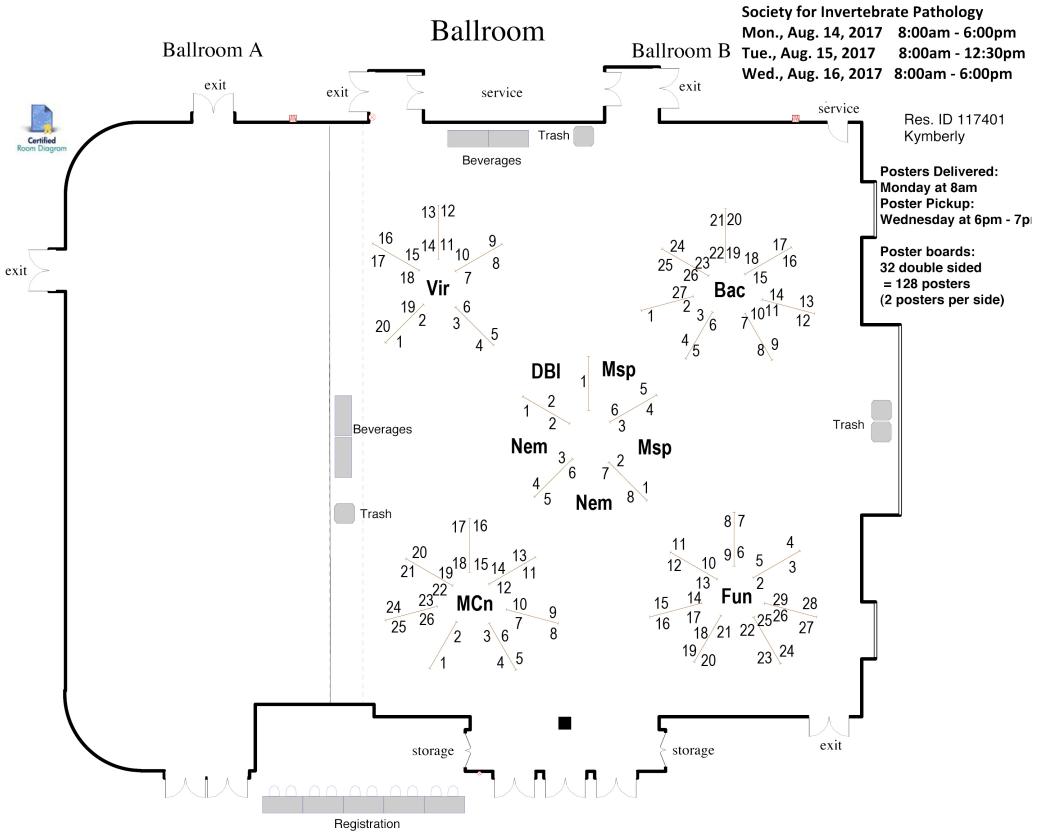
The entomopathogenic fungi encounter hypoxic conditions in nature and in culture. Alcohol dehydrogenases (ADHs) are a group of oxidoreductases that occur in many organisms. Here we demonstrate that an alcohol dehydrogenase I, *MaADH1*, in the locust-specific fungal pathogen, *Metarhizium acridum*, functions in acetaldehyde detoxification mechanism under hypoxic conditions in growth and sporulation. The *MaADH1* was highly expressed in sporulation and had much higher expression under hypoxic conditions. Compared with a wild-type strain, the Δ*MaADH1* deletion mutant showed inhibited growth and sporulation under hypoxic conditions, but no impairment under normal conditions. Δ*MaADH1* mutant produced significant decreased alcohol, but significant increased acetaldehyde compared to wild type. Though alcohol and acetaldehyde could inhibited growth and sporulation of *M. acridum, M. acridum* was much more sensitive to exogenous acetaldehyde than alcohol. These results indicate that the *MaADH1* plays an important roles in growth and sporulation under hypoxic stress by detoxification of acetaldehyde in *M. acridum*.

MICROBIAL CONTROL DIVISION POSTERS

MCn22 Mohammed I. Elbashir, P. Bishwajeet, K. Shankarganesh, P. Sharma. Performance of three Indian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and three commercial mycoinsecticides against three developmental stages of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae)
Paper #280 in program; *rescheduled for oral presentation in Microbial Control #1 (Tuesday am, 10:00; Marshall Room)*



WEDNESDAY – 16 August

8:00 10:00	Bacteria Division #3 (*Theater*)
	***Microsporidia Division Symposium*: The past and future frontiers in Microsporidiology (Retrospective look at Microsporidia research from the first meeting 50 years ago)** (*The Forum*)
	Microbial Control Division #2 (*Roosevelt Room*)
10:00 – 10:30	*Coffee break*
10:30 – 12:30	Virus Division #3 (*Theater*)
	Microbial Control Division #3 (*Roosevelt Room*)
12:30 – 1:30	*Lunch*
	Nematode Division business meeting (*Roosevelt Room*)
	Student Worshop on Science Communication (*The Forum*)
1:30 – 3:30	Virus Division #3 (*Theater*)
	Bacteria Division #4 (*The Forum*)
	Fungi Division #3 (*Roosevelt Room*)
	Microsporidia Division #3 (*Marshall Room*)
3:30 – 4:00	*Refreshments break*
4:00 – 6:00	POSTERS (*West Ballroom*)
8:00 – 10:00	**Fungus Division** business meeting (*Village West, Bldg. 2, Rooms 2A/2B*)
	Microsporidia Division business meeting/workshop (*Village West, Bldg. 2, Rom. 2C*)
	Virus Division business meeting and tribute to Michael R. Strand's election to the US National Academy of Sciences (*Village Tower West, 15th fl., Rm. 15B*)

THURSDAY – 17 August

8:00 – 10:00	***Bacteria Division Symposium*: What is Bt? Current perspectives** (*Theater*)
	Microbial Control Division #4 (*the Forum*)
	***Diseases of Beneficial Invertebrates Division Symposium*: The Pathobiobiome Concept: An emerging view of microbes and diseases** (*Roosevelt Room*)
10:00 – 10:30	*Coffee break*
10:30 – 12:30	**SOCIETY FOR INVERTEBRATE PATHOLOGY business meeting**
12:30 – 1:30	*Lunch*
	Awards Committee (*Green Room*)
1:30 – 3:30	***Fungi/Nematodes/Microsporidia X-Divisional Symposium*: Ecology of Invertebrate Pathogens** (*Theater*)
	***Microbial Control Division Symposium*: Biopesticides III: Beyond entomopathogenicity – Reliable tools or just a novelty?** (*The Forum*)
	Virus Division #5 (*Roosevelt Room*)
3:30 – 4:00	*Refreshments break*
6:30 – 12:30	**SIP BANQUET** (***Estancia La Jolla Hotel***) Cocktail Hour Banquet Awards Ceremony Dancing

The support of these
organizations for the
2017 Annual Meeting
and Golden Jubilee Celebration
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is gratefully acknowledged.