International Congress on Invertebrate Pathology and Microbial Control &

52nd Annual Meeting of the Society for Invertebrate Pathology &

17th Meeting of the IOBC-WPRS Working Group “Microbial and Nematode Control of Invertebrate Pests”

28th July - 1st August

Programme and Abstracts

https://congresos.adeituv.es/SIP-IOBC-2019
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Faculty advisor:
Patricia Stock
The International Organisation for Biological Control (IOBC) promotes research and development of environmentally safe methods of pest and disease control. Focus is laid on biological control and its implementation in integrated pest management (IPM). IOBC was established in 1955 as an organisation of institutional members as well as individual scientists working in all fields of biocontrol. Since then, it has become a well-known, non-profit organization, providing independent and professional advice on biological control and IPM to farmers and advisory services as well as to policy makers and governments.

One of IOBC’s missions is to promote international cooperation in research and development and to facilitate transfer of scientific knowledge into agricultural practice. This requires a regionalized organisation and close collaboration of all stakeholders. In order to achieve this goal, IOBC is organized in six regional sections, each of them running an array of specific working groups.

IOBC-WPRS (West Palearctic Regional Section) currently comprises 20 working groups (WGs) focussing on specific crops, pest organisms, or methods (see the list of WGs below). WGs usually consist of about 40 to more than 100 members, including scientists, students, and representatives of governmental institutions, advisory services and the biocontrol business. Meetings take place every second or third year to help exchange recent scientific findings, draw attention to newly emerging plant protection issues, or share experience from laboratory and field tests. Lively discussions and excellent networking opportunities contribute significantly to the popularity of IOBC-WPRS WG meetings.

The IOBC-WPRS WG “Microbial and Nematode Control of Invertebrate Pests” is active since 1985. It was founded by groups of scientists working on biocontrol of soil pests. Since then, the WG was growing constantly, broadened its scope and has become an international forum for insect and mollusc pathologists in general. Meanwhile, the WG has about 100-120 active members, who collaborate in six sub-groups (SGs), focussing on all important aspects of invertebrate pathology and control: (1) entomopathogenic fungi, (2) viruses, (3) bacteria, (4) entomoparasitic nematodes, (5) soil insect pests, and (6) slugs and snails.

The joint meeting with SIP in Valencia is the 17th meeting of this truly international and very active WG. It is currently embedded in a very favorable environment, with a world wide demand for reducing risks and application of chemical pesticides, and at the same time a growing interest in biological control. The main challenge of the WG remains nonetheless unchanged: to prove the potential of microbial and nematode control agents, to show the opportunities they provide in IPM, and to certify their safety to the user, the consumer and the environment.

IOBC-WPRS Executive Committee

From left to right:
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  Ahmed MAZIH, Institut Agronomique et Vétérinaire Hassan II, Maroc
- Guidelines for integrated production
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IOBC-WPRS Working Groups

- Benefits and risks of exotic biological agents
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- Integrated protection of olive crops
  Convenor: Paula BAPTISTA, Polytechnic Institute of Bragança, Portugal
- Integrated control of mite pests
  Convenor: George BROUFAS, Democritus University of Thrace, Greece
- Integrated protection in viticulture
  Convenor: Carlo DUSO, Università degli studi di Padova, Italy
- Induced resistance in plants against insects and diseases
  Convenor: Victor FLORS, Universitat Jaume I, Spain
- Landscape management for functional biodiversity
  Convenor: Bárbel GEROWITT, University of Rostock, Germany
- Integrated control in protected crops, temperate climate
  Convenor: Bruno GOBIN, Proefcentrum voor Sierteelt, Belgium
- Pheromones and other semio-chemicals in integrated production
  Convenor: Jürgen GROSS, JKI, Germany
- Integrated protection of fruit crops
  Convenor: Claudio IORIATTI, Fondazione Edmund Mach, Italy
- Integrated control in oilseed crops
  Convenor: Małgorzata Jędryczka, Polish Academy of Sciences, Poland
- Biological and integrated control of plant pathogens
  Convenor: Jürgen KÖHL, WUR, The Netherlands
- Integrated protection of citrus crops
  Convenor: María Teresa MARTÍNEZ FERRER, IRTA, Spain
- Integrated protection of field vegetables
  Convenor: Dr. Richard MEADOW, NMBU, Norway
- GMO’s in integrated plant production
  Convenor: Michael MEISSLE, Agroscope, Switzerland
- Integrated control in protected crops, mediterranean climate
  Convenor: Carmelo RAPISARDA, Università degli Studi Catania, Italy
- Integrated protection in oak forests
  Pino Angelo RUIU, Agris Sardegna, Italy
- Pesticides and beneficial organisms
  Guy SMAGGHE, Ghent University, Belgium
- Microbial and nematode control of invertebrate pests
  Convenor: Eustachio TARASCO, Università degli Studi di Bari, Italy
- Integrated protection of stored products
  Convenor: Pasquale TREMATERRA, University of Molise, Italy
- Integrated protection of date palms (Study Group)
  AbdulAziz M. A. MOHAMED, Arabian Gulf University, Bahrain
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Nematodes: Raquel Campos-Herrera, Vladmir Puza and Yolanda Bel

Viruses: Elizabeth Herniou, Miguel López-Ferber and Salvador Herrero
PLENARY SYMPOSIUM. Monday, 11:00 PL-1
Can insects develop resistance to fungal biocontrol agents?
Butt, T.M.1; Dubovskiy2, I.; Grizanova2, E.; Coates1, C.,

PLENARY SYMPOSIUM. Monday, 11:30 PL-2
Mechanisms of practical resistance to commercially relevant entomopathogenic bacteria
Jurat-Fuentes, J.L.
1

PLENARY SYMPOSIUM. Monday, 12:00 PL-3
Lessons told by nature: Resistance of codling moth against Cydia pomonella granulovirus and its implications for environmentally sound pest control
Jehle, J.A.; Fritsch, E.; Undorf-Spahn, K.; Fan, J.; Wennmann, J.T.

PLENARY SYMPOSIUM. Monday, 12:30 PL-4
Sand crickets and mole crickets are resistant to entomopathogenic nematodes and their bacteria
Lu, D.; Aryal, S.K.; Dillman, A. R.

SYMPOSIUM. Monday, 11:00-13:00 Auditorium 2
Resistance to microbial control agents
Organisers / Chairs: Raquel Campos-Herrera / Joel González-Cabrera

SYMPOSIUM. Monday, 11:30-13:00 Auditorium 2
Insolation, diversity and ecology
Chairs: Inmaculada Garrido-Jurado / Claudia López-Lastra

SYMPOSIUM. Monday, 14:30-16:30 Multispace AB
CONTRIBUTED PAPERS FUNGI 1

SYMPOSIUM. Monday, 15:00-16:00 Auditorium 2
The multiple layers of host-pathogen interactions
Organisers / Chairs: Umut Toprak / Salvador Herrero

SYMPOSIUM. Monday, 15:30-16:30 Auditorium 2
Fungal Epizootiology in Red mite, Dermanyssus gallinae of Chicken
So Eun P.1, Mi Rong L. 1, Sihyeon K. 1, Jong Cheol K. 1, Dongwei L. 1, Sehyeon B.1, Minsung J.1, Tae Young S.1, Leila G.1 and Jae Su K.1

SYMPOSIUM. Monday, 15:45-16:00 Auditorium 2
Diversity of the arthropod pathogenic fungus Metarhizium spp. in soils of three different land-use types
Fernandez-Bravo, M.1; Gschwend, F. 1; Widmer, F.. 1; Hug, A..2; Gubler, A.2; Enkerli, J. 1; Roversi, P.F.

SYMPOSIUM. Monday, 15:50-16:00 Auditorium 2
Occurrence and characterization of Metarhizium pingshaense infecting shoot borers, Conogethes punctiferalis
Senthilkumar, C. M.1; Jacob T. K.1; Devasahayam S.1; Geethu C.1; Harilaharan V.1

SYMPOSIUM. Monday, 16:10 Auditorium 2
Parasitic manipulation of insect behaviour
Yue Han1, Simone Gasque1, Hans M. Smid2, Monique M. van Oers1, Vera I.D. Ros1

CONTRIBUTED PAPERS. Monday, 14:30 F-1
Gene diversity-mediated characterization of biological features in entomopathogenic Beauveria bassiana
Gasmi, L.; Baek, S.; Kim, J. Cheol; Lee, M. R.; Kim, S. H.; Park, S. E.; Li, Dongwei; S., Tae Y.; Kim, J. S.Department of Agricultural Biology,

CONTRIBUTED PAPERS. Monday, 14:45 F-2
Isolation and characterization of microsatellites of the entomopathogenic fungus Metarhizium rileyi (Ascomycetes: Hypocreales)
Sosa-Gómez, D. R.1; Binneck, E.1

CONTRIBUTED PAPERS. Monday, 15:00 F-3
First Entomophthoral pathogen of leaf cutter ants: a possible new species of Conidiothelus
Goffrè, D. 1; Jensen, A. B.2; Lopez Lastra, C. 3; Valencia Carrasco, C. 1; Folgarait, P. J.

CONTRIBUTED PAPERS. Monday, 15:15-16:00 F-4 STU
Preliminary analysis of genetic variability of Metarhizium isolates from Parco del Ticino (Northern Italy).
Barzanti, G.P.1; Enkerli, J. 2; Benvenuti, C. 1; Marianelli, L. 1; Paoli, F.; Sabbatini Peverieri, G.; Mazza, G.; Bosio, G.; Venanzio, D.; Giacomello, E.2; Roversi, P.F.

CONTRIBUTED PAPERS. Monday, 15:30-16:00 F-5 STU
Diversity of the arthropod pathogenic fungus Metarhizium spp. in soils of three different land-use types
Fernandez-Bravo, M.1; Gschwend, F.; Widmer, F.; Hug, A.2; Gubler, A.2; Enkerli, J.1

SYMPOSIUM. Monday, 14:30-16:30 F-6 STU
Pathogens associated with invasive or introduced insects threaten the health and diversity of native species
Vilcinskas, Andreas1,2

SYMPOSIUM. Monday, 15:10 BVCS-3
What can we learn about Bacillus thuringiensis as a pathogen and its victims, from the targets of its toxins?
Heckel, DG.

SYMPOSIUM. Monday, 15:30 BVCS-4
Insect immunity as affected by stress factors
Pennacchio, F.
Defining the genomic drivers of evolution in the entomopathogenic Serratia spp.

CONTRIBUTED PAPERS. Monday, 14:30 MC-1

Bacteria and proteins

Chairs: Travis Glare / Monika Maurhofer

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Defining the genomic drivers of evolution in the entomopathogenic Serratia spp.

CONTRIBUTED PAPERS. Monday, 14:30 MC-1

Bacteria and proteins

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Defining the genomic drivers of evolution in the entomopathogenic Serratia spp.

CONTRIBUTED PAPERS. Monday, 14:30 MC-1

Bacteria and proteins

Chairs: Travis Glare / Monika Maurhofer

CONTRIBUTED PAPERS. Monday, 18:15 V-5 STU
Characterisation of the RNA virophage in Australian tephritid fruit flies
Sharpe, Stephen R.; Morrow, Jennifer L.; Brettell, Laura E.; Papanicolaou, Alexie; Chapman, Tony A.; Cook, James M.; Riegler, Markus

CONTRIBUTED PAPERS. Monday, 18:40 V-6 STU
Identification of new viruses specific of the honey bee mite Varroa destructor.

CONTRIBUTED PAPERS. Monday, 18:45 V-7 STU
Metatranscriptomics reveals novel RNA viruses of important rice insect-invertebrates in China
Haoran Wang; Shufen Chao; Guoqing Chen; Dawei Song; Guozhong Feng

SYMPOSIUM. Monday, 17:00 MFCS-1
Wireworm biology in Middle Europe – what are we facing?
Lehmhus, J.

SYMPOSIUM. Monday, 17:30 MFCS-2
Attract & kill: an effective control strategy targeting wireworms in potato, but why are results not consistent?
Vidai, S.; Laurenz, S.; Patel, P.; Beitzien-Heineke, W.

SYMPOSIUM. Monday, 18:00 MFCS-3
Wireworm biocontrol – an open field of opportunity in biology and agriculture
Kabalu, T.

SYMPOSIUM. Monday, 18:30 MFCS-4
Microbial control of wireworms in cover crops – is this the road to success?
Grabenweger, G.; Eckard, S.; Reinbacher, L.; Rogge, S.

CONTRIBUTED PAPERS. Monday, 17:00 B-1 STU
Insights into the in vivo crystallization pathway and mechanism of toxicity of Cry1AA, a naturally crystalline mosquitocidal toxin

CONTRIBUTED PAPERS. Monday, 17:15 B-2 STU
The ABC transporter C2 acts as a Cry1A receptor independently of its ATP binding site II in Spodoptera exigua

CONTRIBUTED PAPERS. Monday, 17:30 B-3 STU
Silencing ABC and Cadherin genes in Leptinotarsa decemlineata (Coleoptera:Chrysomelidae) treated with Bacillus thuringiensis ssp. tenebrionis Cry 5-endotoxin

CONTRIBUTED PAPERS. Monday, 17:45 B-4 STU
Receptor analysis of Cry1Ca toxin expressed on Sf9 cells
Adegawa, S.; Sato, R.

CONTRIBUTED PAPERS. Monday, 18:00 B-5
The Bacillus thuringiensis Cry37Aa protein is not necessary to mediate toxicity and binding of Cry23Aa protein on Cylas puncticollis Khorramnejad, A.; Prentice, K.; Vera-Velasco, N.M.; Smagghe, G.; Hernández-Martínez, P.; Escriche, B.

CONTRIBUTED PAPERS. Monday, 18:15 B-6 STU
New Cry7 protein active against Leptinotarsa decemlineata (Coleoptera: chrysomelidae)
Domínguez, M.; Villanueva, M.; Fernández, A.; Caballero, P.

CONTRIBUTED PAPERS. Monday, 18:30 B-7
ABC transporter-mediated resistance mechanism to Bacillus thuringiensis Cry1AC toxin in diamondback moth
Guo, Z.; Zhang, Y.

CONTRIBUTED PAPERS. Monday, 18:45 B-8 STU
Novel method for determining the insecticidal crystal protein composition of Bacillus thuringiensis insecticides
Caballero, J.; Jiménez-Moreno, N.; Orea, I.; Williams, T.; Fernández, A.; Villanueva, M.; Ferré, J.; Caballero, P. & Ancín-Azpilicueta, C.

CONTRIBUTED PAPERS. Monday, 19:00 N-9
Diabrotica v. virgifera management using genetically improved strains of Heterorhabditis bacteriophora
Ehlers, R.-U.; Melina, C.; Vandenvossche, B.; Dörfler, V.; Barg, M.; Toepfer, S.

CONTRIBUTED PAPERS. Monday, 19:15 N-10 STU
Potential microbial control of xylophagous pests with entomopathogenic nematodes and fungi
El Khoury, Y.; Noujeim, E.; Ravlić, J.; Oreste, M.; Addante, R.; Nemer, N.; Tarasco, E.

CONTRIBUTED PAPERS. Monday, 19:30 N-11
The potential use of native entomopathogenic nematodes isolated in the Italian areas infested by Popillia japonica (coleoptera: scarabaeidae)
Tomini, G.; Paoli, F.; Simoncini, S.; Strangi, A.; Cutino, I.; Benvenuti, C.; Mazza, G.; Bosio, G.; Venanzio, D.; Giacometto, E.; Tarasco, E.; Sabbatini Peverieri, G.; Roversi, P.F.; Marianelli, L.

CONTRIBUTED PAPERS. Monday, 19:45 N-12
Investigating the biological control potential of indigenous nematodes for the control of invasive slug pests
Pieterson, A.; Malan, A.P.; Ross, J.L.
Garriga, A.; Morton, A.; García-del-Pino, F.

**CONTRIBUTED PAPERS.** Monday, 18:15 N-14

Transcriptomic and proteomic approach to identify potential virulence factors produced by the entomopathogenic nematode *Heterorhabditis bacteriophora*

Tobbaru, D.; Frias, J.; Simões, N.

**CONTRIBUTED PAPERS.** Monday, 18:30 N-15

*Heterorhabditis bacteriophora*: An excellent model for genetic improvement of biocontrol traits

Ehlers, R.-U.

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**TUESDAY - 30th July**

**5K Race**

Tuesday, 07:30-09:00

Old river bed

**CONTRIBUTED PAPERS**

**MICROBIAL CONTROL 2**

Tuesday, 09:30-11:30

Auditorium 3

Control of soil dwelling pests

Chairs: Dietrich Stephan / Hermann Strasser

**CONTRIBUTED PAPERS.** Tuesday, 09:30 MC-9

Control of soil-dwelling stages of *Spodoptera littoralis* and impact on the adults of the same generation

Garrido-Jurado, I.¹; Resquin-Romero, G.²; Yousef, M.¹; Rios-Moreno, A.²; Quesada-Moraga, E.¹

**CONTRIBUTED PAPERS.** Tuesday, 09:45 MC-10

Metarhizium is the message - new IPM strategy to control larvae and adults of Diabrotica v. virgifera

Strasser, H.¹; Mayerhofer, J.²; Zottele, M.¹; Enkerli, J.²

**CONTRIBUTED PAPERS.** Tuesday, 10:00 MC-11 STU

Development of a soil granule of the entomopathogenic fungus *Metarhizium brunneum* to control wireworms

Bernhardt, T.; Stephan, D.

**CONTRIBUTED PAPERS.** Tuesday, 10:30 MC-12 STU

Efficacy of new *Metarhizium* formulations against wireworms in the field

Paluch, M.; Lehmhus, J.

**CONTRIBUTED PAPERS.** Tuesday, 10:45 MC-13 STU

Survival of vine weevil (*Otiorhynchus sulcatus*) larvae is affected by geographic origin and host plant but only in the absence of the entomopathogenic fungus *Metarhizium brunneum*

Morera-Margarit, P³; Karley, A.J.; Mitchell, C.; Graham, R.I.¹; Pope, T.W.²

**CONTRIBUTED PAPERS.** Tuesday, 11:00 MC-14 STU

Laboratory evaluation of combining an entomopathogenic fungus (EPF) and a botanical compound to control gnat, *Bradysia* spp.

Dehghani, M.¹; Rademacher, J.²; Vidal, S.¹

**CONTRIBUTED PAPERS.** Tuesday, 11:15 MC-15 STU

Exploiting the tomato microbiome and volatile-based mechanisms towards controlling *Meloidogyne*-based disease complexes

Wolfgang, A.¹; Taffner, J.¹; Araújo-Guimaraes, R.²; Coyne, D.²; Berg, G.¹

**CONTRIBUTED PAPERS.** Tuesday, 11:30 MC-16 STU

Novel coatings for attract-and-kill formulations containing *Metarhizium brunneum* for biological control of wireworms

Hermann, K.¹; Humbert, P.¹; Patel, A.¹

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**VIRUS SYMPOSIUM**

Tuesday, 09:30-11:30

Multispace AB

Covert virus infections in insects

Organisers / Chairs: Vera Ros / Miguel López-Ferber

**SYMPOSIUM.** Tuesday, 09:30 VS-1

Covert infection in baculoviruses: insights from field populations

Cory, J.S.¹; Buchhop, J.¹; Myers, J.H.²

**SYMPOSIUM.** Tuesday, 10:00 VS-2

Covert infection by iflaviruses: benefits from nucleopolyhedrovirus infection in *Spodoptera exigua*
Williams, T.; Carballo, A.; Herrero, S.; Murillo, R.; Caballero, P.

SYMPOSIUM. Tuesday, 10:30 VS-3

‘Dark’ viruses of Drosophilidae (and other invertebrates)

Obbard D.J.; Waldron F.M.; Wallace M.A.

SYMPOSIUM. Tuesday, 11:00 VS-4

Bee health and its relation with virus infection

Meueus, I.; Piot N.; Smagghe, G.

CONTRIBUTED PAPERS. Tuesday, 09:30 F-9

Lipid processes in the infection process of insect pathogenic fungi Keyhani, NO.

CONTRIBUTED PAPERS. Tuesday, 09:45 F-10

Fungal infection recognition in the Aedes aegypti mosquito

Ramirez, J.L.; Muturi, E.J.; Flor-Weiler, L.; Rooney, A.

CONTRIBUTED PAPERS. Tuesday, 10:00 F-11

Fungal infection dynamics and insect counter-responses at the cuticle interface

Dubovskiy I. M.; Grizanova E. V.; Coates C. J.; Butt T. M.

CONTRIBUTED PAPERS. Tuesday, 10:15 F-12

Functional analysis of an integral membrane protein (IMP) gene in Beauveria bassiana

Ding J.-L.; Feng M.-G.; Ying S.-H.

CONTRIBUTED PAPERS. Tuesday, 10:45 F-13

Functional analysis of a lipase gene in Beauveria bassiana

Peng Y.-Jin; Feng M.-G.; Ying S.-H.

CONTRIBUTED PAPERS. Tuesday, 10:45 F-14 STU

Transcription factor Msn2 acts as virulence regulator of Beauveria bassiana s.l. against the tick Rhipicephalus microplus

Muniz, Elen R.; Silva, Cárita S.R.; Arruda, Walquíria; Keyhani, Nemat O.; Fernandes, Everett K.K.

CONTRIBUTED PAPERS. Tuesday, 11:00 F-15

Recombinant chitinase to enhance virulence of Beauveria bassiana conidia against the sugar-cane borer Diatraea saccharalis.

Lovera, A.; Belaich, M.; Villamizar, L.; Patarroyo, M. A.; Barra, G.

CONTRIBUTED PAPERS. Tuesday, 11:15 F-16

Balanced histone H3-K56 acetylation are essential for DNA damage repair and biological control potential of Beauveria bassiana

Cai, Q.; Ren, K.; Shao, W.; Ying, S.; Feng, M.-g.

BACTERIA WORKSHOP

PART 1

Tuesday, 9:30-11:10

Commission R8

Domain-based specificity and protein structure-function to help determine safety in insecticidal proteins

Organisers / Chairs: William Moar / Mark Nelson

WORKSHOP. Tuesday, 09:30 BWS-1

Development of a bacterial pesticidal protein information resource

Neil Crickmore

WORKSHOP. Tuesday, 09:50 BWS-2

Proteins from non-Bt sources for control of western corn rootworm, Diabrotica virgifera virgifera (LeConte)

Nelson, M.E.; Jiménez Juárez, N.; Pérez Ortega; C.; Pence, H.; Bowling, A.; and Lu, A.

WORKSHOP. Tuesday, 10:10 BWS-3

WCR-active protein complexes from fungal genus Pleurotus Sepčić, K.; Panevska, A.; Razinger, J.; Modic, Š.

WORKSHOP. Tuesday, 10:30 BWS-4

Insecticidal proteins for controlling western corn rootworm Diabrotica virgifera virgifera, (Coleoptera: Chrysomelidae) isolated from the insect pathogenic bacteria Brevibacillus laterosporus.

Chay, C.; Milligan, J.; Bean, G.; Slightom, R.; Howe, A.; Werner, B.; Moore, R.; Pleau, M.; Nance, A.; Yin, Y.; Bowen, D.

WORKSHOP. Tuesday, 10:50 BWS-5

Mode of Action of new corn rootworm-active proteins

Moar, W.

Coffee Break

Tuesday, 11:30-12:00

Foyer

BACTERIA WORKSHOP

PART 2

Tuesday, 12:00-14:00

Commission R8

Domain-based specificity and protein structure-function to help determine safety in insecticidal proteins

Organisers / Chairs: William Moar / Mark Nelson

WORKSHOP. Tuesday, 12:00 BWS-6

Structure-function considerations of proteins for insect trait development

Eswar Narayanan

WORKSHOP. Tuesday, 12:20 BWS-7

Domain-based Specificity of Clostridium perfringens Epsilon toxin

McClain, M.

WORKSHOP. Tuesday, 12:40 BWS-8

Structure-Function of mCry51Aa2

Jerga Agoston

WORKSHOP. Tuesday, 13:00 BWS-9

Understanding how non-specific targeting has evolved in beta-pore forming toxins and how to guide specific targeting systems

Bradley A. Spicer; Ruby H.P. Law; Charles Bayly-Jones; Stephanie Kondos; Siew Siew Pang; Hari Venugopalan; Tom T. Caradoc-Davies; James C. Whisstock; Michelle A. Dunstone

WORKSHOP. Tuesday, 13:20 BWS-10

Analysis of mammalian and invertebrate-active structural homologs

Berry, C.; Jones, D.D.; Al-Maslookhi, H.

WORKSHOP. Tuesday, 13:40 BWS-11

Structural studies of the Vip3A proteins by site-directed mutagenesis

Ferré, J.; Quan, Y.; Banyuls, N.; Van Rie, J.
Activity of Metarhizium brunneum and Beauveria bassiana against early developmental stages of the false codling moth
Mondaca, L.L.1; Protasov, A.2; Ben-Yehuda, S.3; Peisahovich, A.1; Mendel, Z.4; Ment, D.5

Contribution of pollen beetles with Beauveria bassiana
Kaiser, D.6; Grabenwege, G.7; Bacher, S.8

Compatibility of the parasitoid Hyposoter didymator and the entomopathogenic fungus Metarhizium brunneum for the control of Spodoptera littoralis
Miranda-Fuentes, P.9; Yousef, M.10; Quesada-Moraga, E.11

The interaction between melon plants and Aphis gossypii is modified by endophytic colonization by Beauveria bassiana
González-Mas, N.12; Sánchez-Ortíz, A.3; Valverde-García, P.4; Quesada-Moraga, E.11

Evaluating insect pathogenic fungi in combination with Heterorhabditis bacteriophora for the management of Helicoverpa armigera
Wakil, W.2; Tahir, M.2; Usman, M.13; Gulzar, S.14

Ecological control of Japanese piny sawyer beetle, Monochamus alternatus vectoring pine wilt nematode using Metarhizium anisopliae
Kim, Jong Cheol1; Shih, Tae Young1; Kim, Sihyeon1; Lee, Mi Rong1; Park, So Eun1; Li, Dong Wei1; Beak, Sehyeon1; Jo, Min Sung1; Gasmi, Laila1; Kim, Jae Su1

Field efficacy of Isaria fumosorosea alone or mixed with horticultural oil for management of the Asian citrus psyllid
Pasco B Avery1

Combined effects of Metarhizium robertsii and avermectins on mosquito larvae: survival and immune responses
Noskov, Yu.A.15; Polenogova, O.V.15; Yaroslavtseva, O.N.3; Belevich, O.E.3; Yurchenko, Yu.A.3; Cherukova, E.A.3; Kryukov, V.Yu.3; Glupov V.V.3

Population genetics and ecology
Chairs: Adly Abd-Alla / Gealan Burke

A Preliminary Investigation on the Epidemiology and Genetic Diversity of Cnaphalocrocis medinalis Granulovirus
Yang, J.1; Zhang, H.2; Zuo, Y.3; Li, Lu.3; Wu, W.4; Yuan, M.1; Yang, K.1

High Resolution Melting point application to the detection of the relative frequencies of genotypes in mixed infections of CpG in codling moth
Hinsberger, A.1; Blachère-Lopez, C.12; Theulier, S.3; Guerrero, P.2; Lopez-Ferber, M.1; Bayle, S.1

Genetic variability in Chrysodeixis includens nucleopolyhedrovirus
Aguiire, Eduardo1; Bepeater, Inês2; Williams, Trevor2; López-Ferber, Miguel3; Caballero, Primitivo4,5

A fish perspective of a shrimp disease: yellow head virus in two polyculture ponds
Minardi, D.1; Bass, D.2; Sritunyalucksana, K.3; Itsathiphaisarn, O.4; Stentiford, G.D.1

Seasonal pattern of viral load in colonies of Apis mellifera from Italian and French apiaries
Molinatto, G.1,2; Peruzzi, M.2; Dièvet, V.3; Mondet, F.4; Alaix, C.5; Kretzschmar, A.6; Marzachi, C.3; Bosco, D.7; Manino, A.8

Occurrence and molecular phylogeny of honeybee viruses in hornets
Yang, Sa1,2; Gayral, Philippe3; Zhao, Hongxia4; Wu, Yanyan1,2; Bigot, Diane5; Wang, Xining1,2; Yang, Dahe1,2; Herniou, Elisabeth A.9; Deng, Shuai1,2; Li, Fei1,2; Diao, Qingyun1,2; Darrouzet, Eric 1,2; Hou, Chunsheng1,2

Managing Ticks with Fungi: The Korean Experience
Lee, M. R.1; Kim, J. C.1; Kim, S.1; Park, S. E.1; Li D.1; Je, M.1; Shin, T. Y.1; Lee, D. H.2; Kim, J. S.1

Managing Ticks with Fungi: The Israeli Experience of tick Metarhizium interaction
Molino, G.1,2; Blachère-Lopez, C.1; Theulier, S.1; Guerrero, P.2; Lopez-Ferber, M.1; Bayle, S.1

Managing Ticks with Fungi: The African Experience
Fernandes, Éverton K.K.1; Bittencourt, Vânia R.E.P.2

Managing Ticks with Fungi: The African Experience
Subramanian Sevgan

The US Experience of Managing Tick Populations with Fungi: Accomplishments and Challenges
Leland, Jarrod1

Canonerial sequence free analyses on the example of thirty isolates of the Cydia pomonella granulovirus (CpGV) and Bombyx mori nucleopolyhedrovirus (BmNPV) reveal complex variant mixtures within baculovirus populations
Wennmann, J. T.1; Fan, Jiangbin1,2; Siripuk Suraporn3; Gani, M.4; Jehle, Johannes A.1

Genetic variability in Chrysodeixis includens nucleopolyhedrovirus
Aguiire, Eduardo1; Bepeater, Inês2; Williams, Trevor2; López-Ferber, Miguel3; Caballero, Primitivo4,5

A fish perspective of a shrimp disease: yellow head virus in two polyculture ponds
Minardi, D.1; Bass, D.2; Sritunyalucksana, K.3; Itsathiphaisarn, O.4; Stentiford, G.D.1

Seasonal pattern of viral load in colonies of Apis mellifera from Italian and French apiaries
Molinatto, G.1,2; Peruzzi, M.2; Dièvet, V.3; Mondet, F.4; Alaix, C.5; Kretzschmar, A.6; Marzachi, C.3; Bosco, D.7; Manino, A.8

Occurrence and molecular phylogeny of honeybee viruses in hornets
Yang, Sa1,2; Gayral, Philippe3; Zhao, Hongxia4; Wu, Yanyan1,2; Bigot, Diane5; Wang, Xining1,2; Yang, Dahe1,2; Herniou, Elisabeth A.9; Deng, Shuai1,2; Li, Fei1,2; Diao, Qingyun1,2; Darrouzet, Eric 1,2; Hou, Chunsheng1,2
Determination of critical regions for toxicity of the Vip3A protein in European, American, African and Asian pests

CONTRIBUTED PAPERS. Wednesday, 08:30 B-9 STU

Gomis-Cebolla, J.1; Bel, Y.1; Ferreira Dos Santos, R.2; Wang, Y.2; Caballero, J.4; Caballero, P.4; He, K.5; Jurat-Fuentes, J.L.; Ferré, J.1

Characterization ofvip3 positive Bacillus thuringiensis isolates and toxicity of Vip3Aa65 against lepidopteran pests

CONTRIBUTED PAPERS. Wednesday, 08:45 B-10 STU

Şahin, B.1; Gomis-Cebolla, J.2; Güneş, H.1; Ferré, J.2

Empirical test of spatiotemporal alternation strategy of multiple single-gene events for delaying insect resistance to Bt crops

CONTRIBUTED PAPERS. Wednesday, 09:00 B-11

Wang, Y.; Yan, X.; Quan, Y.; Wang, Z.; He, K.

Role of Sigma54 on sporulation in Bacillus thuringiensis

CONTRIBUTED PAPERS. Wednesday, 09:15 B-12

Peng, Q.1; Zhao, X.1; Wen, J.1; Zhang, L.1; Nielsen-LeRoux, C.2; Song, F.2

The stationary phase regulator CpcR controls cell differentiation and cry gene expression in Bacillus thuringiensis

CONTRIBUTED PAPERS. Wednesday, 09:30 B-13 STU

Zhang, R.1; Slamti, L.2; Verplaetse, E.2; Zhang, J.3; Song, F.; Lerouclus, D.2

Bip, a protein required for the integration of planktonic bacteria inside a biofilm

CONTRIBUTED PAPERS. Wednesday, 09:45 B-14 STU

El Khoury, N.1,2; Bennaceur, I.1; Majed, R.1,2; Kallassy, M.; Gohar, M.

Temporal midgut transcriptome of Leptinotarsa decemlineata (Coleoptera:Chrysomelidae) larvae in response to Bacillus thuringiensis ssp. tenebrionis infection

CONTRIBUTED PAPERS. Wednesday, 10:00 B-15

Toprak, U.1; Bayram, S.1; Baldwin, D.2; Coutu, C.2; Güney, G.1,2; Güney, E.3; Sezen, K.3; Hegedus, D.2; Heckel, D.G.4; Kaydan, M.B.5

Mechanisms and frequency of resistance to transgenic corn in fall armyworm (Spodoptera frugiperda)

CONTRIBUTED PAPERS. Wednesday, 10:15 B-16

Placidide Bortoli, C.1; Banerjee, R.1; Meagher, R.2; Abdelgaffar, H.1; Yang, F.2; Kerns, D.3; Huang, F.3; Komivi, A.5; Rao, T.1; Jurat-Fuentes, J.L.

Densovirus oral infection targets and disrupts the peritrophic matrix of the lepidopteran pest Spodoptera frugiperda.

CONTRIBUTED PAPERS. Wednesday, 08:30 V-17

Pigeyre Laetitia1,4; Shatz Malvina Laetitia1,2; Ravallec Marc2; Gasmili Leila2; Cloquet Cécile2; Guerardel Yann1; Cot Didier1; Dupressoir Thierry1; Gosselin-Grenet Anne-Sophie2; Ogliastro Mylene2

Bombyx mori nucleopolyhedrovirus Bm8 protein (BV/ODV E-26) suppresses viral gene expression and regulates viral virulence in
**Bombyx mori larvae**

Hikida, H.; Kokusho, R.; Matsuda-Imai, N.; Katsuma, S.

**CONTRIBUTED PAPERS.** Wednesday, 09:00 V-19 STU

Study of host effect in two broad host range alphabaculoviruses after serial passaging

Belda, Isabel M.; Beperet, Inés; Williams, Trevor; López-Ferber, Miguel; Caballero, Primitivo

**CONTRIBUTED PAPERS.** Wednesday, 09:15 V-20

The Two Prevalent Genotypes of an Emerging Infectious Disease, Deformed Wing Virus, Cause Equally Low Pupal Mortality and Equally High Wing Deformities in Host Honey Bees

Tehel, Anja; Vu, Quynh; Bigot, Diane; Gogol-Döring, Andreas; Koch, Peter; Jenkins, Christina; Doublet, Vincent; Theodorou, Panagiotis; Paxton, Robert J.

**CONTRIBUTED PAPERS.** Wednesday, 09:30 V-21

Identification of genetic loci associated with virulence in Spodoptera littura nucleopolyhedrovirus isolates using deep sequencing approaches and analyses

Vlak, Justinus M.; Ali, Ghulam; van Strien E.A.; van der Werf, Wopke; Schijlen, Elio G.W.M. and Zwart, Mark P.

**CONTRIBUTED PAPERS.** Wednesday, 09:45 V-22

Investigating the importance of Sindbis virus replication in overcoming the Aedes aegypti midgit escape barrier using midgut-restricted viruses

Carpenter, Alexis; Bryant, William B.; Clem, Rollie J.

**CONTRIBUTED PAPERS.** Wednesday, 10:00 V-23

The effect of light signal on the climbing behavior of cotton bollworms infected with NPV

Liu Xiaoming, Tian Zhiquiang, Shen Zhongjian, Yu Jian, Liu Xiaoxia

**CONTRIBUTED PAPERS.** Wednesday, 10:15 V-24

Characterization of Novel RNA viruses isolated from tsetse fly Glossina morsitans morstans


**CONTRIBUTED PAPERS.** Wednesday, 08:30-10:30 FUNGI 3

Entomopathogenic fungi as endophytes

Chairs: Enrique Quesada-Moraga / Stefan Vidal

**CONTRIBUTED PAPERS.** Wednesday, 08:30-10:30 Fungi 3

Direct and indirect effects of exposure to Metarhizium-colonized plants on the cotton leafworm Spodoptera littoralis

Garrido-Jurado, I.; Sanz-Barrionuevo, P.; Quesada-Moraga, E.

**CONTRIBUTED PAPERS.** Wednesday, 08:45 F-18 STU

Effects of entomopathogenic fungi as wheat endophytes on plant growth, aphid reproduction and regulation of plant enzyme systems

Rasool, S.; Jensen, B.; Saleem Akhtar, S.; Roitsch, T. G.; Meyling, N. V.

**CONTRIBUTED PAPERS.** Wednesday, 09:00 F-19

Endophytic *Metarhizium robertsi* Affects Maize Growth and Gene Expression and Growth of Black Cutworm

Ahmad, I.; Jimenez-Gasco, M.M.; Lathe, D.S.; Barbercheck, M.E.

**CONTRIBUTED PAPERS.** Wednesday, 09:15 F-20 STU

Potential of using entomopathogenic fungi as a control option for the charcoal rot fungus, *Macrophomina phaseolina*, in strawberry

Dara, Suchitra S.; Dara, Sunanth S. R.; Dara, Surendra K.
CONTRIBUTED PAPERS. Wednesday, 11:00-13:00
VIRUS 4

Infection cycle and morphogenesis
Chairs: David Thiebaud/Manli Wang

CONTRIBUTED PAPERS. Wednesday, 11:00 V-25
Autographa californica multiple nucleopolyhedrovirus ie2 is critical for virus replication
Hepat, Rahul1; Willis, Leslie G.1; Sokal, Nadia1; Harrison, Robert L.2; Erlander, Martin, A.; Thellung, David A.

CONTRIBUTED PAPERS. Wednesday, 11:15 V-26 STU
The baculovirus Ac108 protein is a per os infectivity factor and a component of the ODV entry complex.
Boogaard B; Evers F; van Lent JWM.; van Oers MM.

CONTRIBUTED PAPERS. Wednesday, 11:30 V-27
Interaction of Autographa californica multicapsid nucleopolyhedrovirus GP41 and two host Proteins
Wang, Su-Dan; Zeng, Xiao-Tao; Huang, Cui; Sun, Quan; Li, Lu-Lin

CONTRIBUTED PAPERS. Wednesday, 11:45 V-28 STU
Decoding morphogenesis of Ichnovirus associated to the parasitic wasp H. didymatyr by RNA interference
Lorenzi, Ange; Jouan, Veronique; Ravaliec, Marc; Eychenne, Magali; Volkoff, Anne-Nathalie

CONTRIBUTED PAPERS. Wednesday, 12:00 V-29
The cysteine-rich region of a baculovirus VP91 protein contributes to the morphogenesis of occlusion bodies
Zhou, Fengqiao1,2; Kuan, Wenhu1,3; Wang, Xi1,2; Hou, Dianhai1; Huang, Huachao1; Sun, Xiulan1; Deng, Fei1; Wang, Hualin1; van Oers, Monique M.; Wang, Manli1; Hu, Zhihong1

CONTRIBUTED PAPERS. Wednesday, 12:15 V-30
NSP2 forms viroplasms during Dendrolimus punctatus cypovirus infection
Congrui, Xu1; Jia, Wang1; Jian, Yang1; Chengfeng, Lei1; Jia, Hu1; Xiulan, Sun1

CONTRIBUTED PAPERS. Wednesday, 12:30 V-31
Virus biology of Euscelidius variegatus ilfivirus 1: towards the production of an infectious viral clone
Marzachi, Cristina1; Ottati, Sara1; Persico, Alberto1; Abbà, Simona1; Rossi, Marika1; Vallino, Marta1; Turina, Massimo1; Galetto, Luciana1

CONTRIBUTED PAPERS. Wednesday, 12:45 V-32
Development of autoclave-resistant baculoviruses to follow infection in living cells

CONTRIBUTED PAPERS. Wednesday, 11:00-13:00
FUNGII 4

Control of ticks and piercing-sucking insect pests
Chairs: Stephan Jaronski / Enrique Quesada-Moraga

CONTRIBUTED PAPERS. Wednesday, 11:00 F-25 STU
A novel biopesticide using Metarhizium anisopliae JEF isolate to control the soil-dwelling longhornet tick, Haemaphysalis longicornis Mi R. L., Dongwei L.; Jong C. K.; Sihyeon K.; Tae Y. B. and Jae S. K.

CONTRIBUTED PAPERS. Wednesday, 11:15 F-26 STU
using entomopathogenic Beauveria bassiana
Baek, S.; Kim, J.C.; Kim S; Lee, M.R; Li, D.; Shin, T.Y; Kim, J.S.

CONTRIBUTED PAPERS. Wednesday, 11:30 F-27 STU
isScreening Potential Entomopathogenic Fungi for the Control of the Greenhouse Whitefly (Trialeurodes vaporariorum)

CONTRIBUTED PAPERS. Wednesday, 11:45 F-28
Development of a biological tick control agent based on an innovative attract-and-kill strategy

CONTRIBUTED PAPERS. Wednesday, 12:00 F-29 STU
Effectiveness of entomopathogenic Beauveria pseudobassiana on Corythucha arcuata in laboratory conditions
Matek, M.; Pernek, M.

CONTRIBUTED PAPERS. Wednesday, 12:15 F-30
Are phytopathogenic fungi capable of producing insecticidal metabolites?
Berestetskiy, A.; Salimova, D.; Dalinova, A.; Stepanycheva, E.

CONTRIBUTED PAPERS. Wednesday, 12:30 F-31
Proteomic profiling of Steinernema carpocapsae and Heterorhabditis megidis infective juveniles stored at 20°C and 9°C
Lillis, P.; Griffin, C.; Carolan, J

CONTRIBUTED PAPERS. Wednesday, 12:45 F-32
In vitro liquid culture and optimisation of the entomopathogenic nematode, Steinernema jeffreyense, using shake flasks
Dunn, M.D.; Belur, P.D.; Malan, A.P.

CONTRIBUTED PAPERS. Wednesday, 11:15 N-18
Thiourea as polyphenoloxidase inhibitor accelerate the Galleria mellonella’s infection by entomopathogenic nematode (Heterorhabditis beicheriana)
Li, X.; Zhang, H.; Cao, A.; Yang, W.; Liu, Q.

CONTRIBUTED PAPERS. Wednesday, 11:30 N-19 STU
Proteomic profiling of Steinernema carpocapsae and Heterorhabditis megidis infective juveniles stored at 20°C and 9°C
Lillis, P.; Griffin, C.; Carolan, J

CONTRIBUTED PAPERS. Wednesday, 11:45 N-20 STU
In vitro liquid culture and optimisation of the entomopathogenic nematode, Steinernema jeffreyense, using shake flasks
Dunn, M.D.; Belur, P.D.; Malan, A.P.

CONTRIBUTED PAPERS. Wednesday, 11:00-13:00
Commission R8

EPN infection process and bioprocessing
Chairs: Li Xingjue / Bart Vanderbossche

CONTRIBUTED PAPERS. Wednesday, 11:00 N-17
Cancelled

CONTRIBUTED PAPERS. Wednesday, 11:15 N-18
Thiourea as polyphenoloxidase inhibitor accelerate the Galleria mellonella’s infection by entomopathogenic nematode (Heterorhabditis beicheriana)
Li, X.; Zhang, H.; Cao, A.; Yang, W.; Liu, Q.

CONTRIBUTED PAPERS. Wednesday, 11:30 N-19 STU
Proteomic profiling of Steinernema carpocapsae and Heterorhabditis megidis infective juveniles stored at 20°C and 9°C
Lillis, P.; Griffin, C.; Carolan, J

CONTRIBUTED PAPERS. Wednesday, 11:45 N-20 STU
In vitro liquid culture and optimisation of the entomopathogenic nematode, Steinernema jeffreyense, using shake flasks
Dunn, M.D.; Belur, P.D.; Malan, A.P.

CONTRIBUTED PAPERS. Wednesday, 12:00 N-21
Influence of Photorhabdus luminescens density on the life history traits of Heterorhabditis bacteriophora and bacterial exchange on virulence and reproduction
Addis, T.; Enow, E.; Tetteh, A.D.; Molina, C.; Ehlers, R.-U.

CONTRIBUTED PAPERS. Wednesday, 12:15 N-22 STU
Isolation, identification and nematicidal activity of secondary metabolites produced by the entomopathogenic bacterium Photorhabdus luminescens sonorensis (Enterobacteriaceae) against the root knot nematode, Meloidogyne incognita (Tylenchidae)
Kusakabe, A.; Molnár, I.; Stock, S.P.
Improving virulence and post-application longevity of *Heterorhabditis bacteriophora* dauers juveniles through selection and breeding

**Virus Workshop**

Wednesday, 13:00-14:30

**Multispace AB**

The forthcoming change in virus species naming to a binomial system

Organisers / Chairs: Robert Harrison / John Burand

**Science Communication**

Wednesday, 13:00-14:30

**Multispace CD**

Science Communication

Organizer: A. Lorena Passarelli

**Con tribute d Pape r s**

Wednesday, 14:30 B-17

**Analysis of Bacillus thuringiensis diversity from different environments**

*Fernandez, A.B.*, *Caballero, P.*

Wednesday, 14:45 B-18

**Whole-genome phylogeny and taxonomy of Bacillus thuringiensis strains by composition vector analysis**

*Wang, K.*, *Shu, C.*, *Zhang, J.*

Wednesday, 15:00 B-19

**Molecular and functional analysis of new Bacillus thuringiensis subsp. israelensis proteins**

*Villanueva, M.*, *Valtierra-de-Luis, D.*, *Caballero, P.*

Wednesday, 15:15 B-20 STU

**Identification and functional analysis of two novel Cry proteins from Paenibacillus popilliae ATCC14706**

*Kawahara, A.*, *Iiyama, K.*, *Asano, S.*, *Nishi, O.*, *Yasunaga-Aoki, C.*

Wednesday, 15:30 B-21

**Novel mosquitocidal toxins from Paraclostridia**

*Estefania Contreras-Navarro, E.C.*, *Chen J.*, *Gill S.*

Wednesday, 15:45 B-22 STU

**Drosophila suzukii bacterial interaction and their potential in biological control**

*Hiebert, N.*, *Carrau, T.*, *Bartling, M.*, *Vilcinskas, A.*, *Lee, K.-Z.*

Wednesday, 16:00 B-23

**Histopathology of Anticarsia gemmatalis strains susceptible and resistant to Cry1Ac protein and their susceptibility to biopesticides based on Bacillus thuringiensis**


Wednesday, 16:15 B-24

**Gene interaction networks in Helicoverpa zea challenged with Cry1Ac toxin from Bacillus thuringiensis Perera, O.P.*; *Polpiytiya, A.D.*

**Con tribute d Pape r s**

Wednesday, 14:30-16:30

**Multispace AB**

**Immunity and host response**

Chairs: Bergmann Ribeiro / Sassan Asgari

**Con tribute d Pape r s**

Wednesday, 16:15 V-36

**BmNPV ARIF-1 enhances viral systemic spread by establishing non-canonical route of infection**

*Kokusho, R.*, *Katsuma, S.*

Wednesday, 16:30 V-37

**The role of outbreak-associated factors in activation of covert nucleopolyhedrovirus infection in Lymantria dispar L.**

*Pavlushin Sergey V.*, *Belousova Irina A.*, *Chertkova Ekaterina A.*, *Kryukova Natalya A.*, *Khankaev Yuriy B.*, *Kasianov Nikita S.*, *Marternyan Viaschleslav V.*

**Con tribute d Pape r s**

Wednesday, 15:45 V-38

**Deep sequencing of microRNAs analysis in SeMNPV persistently infected Se301 cells**

*Weng, Qingbei; Fang, Zheng; Liu, Zhicheng; An, Li; Shao, Jingxu; Chen, Qianqu*.

**Beyond Diptera: exploring Wolbachia-virus interactions in two lepidopteran cell lines**

*Parry, R. H.*, *Asgari, S.*

**Con tribute d Pape r s**

Wednesday, 15:45 SS-1

**Phasmarhabditis hermaphrodita is not the only slug killing nematode**

*Nemrut*, J.*; *Holley, M.*, *Pâza, V.*

**Con tribute d Pape r s**

Wednesday, 14:45 SS-2 STU

**Parasites associated with terrestrial slugs of the Arionidae family in Europe, with emphasis on the invasive Arion vulgaris**


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CONTRIBUTED PAPERS. Wednesday, 15:00 SS-3 STU
Finding feeding stimulants to improve the efficiency of a newly-developed slug biocontrol product against Arion vulgaris slugs
Laplanché, D.1; Desurmont, G.2; Turlings, T.1

CONTRIBUTED PAPERS. Wednesday, 15:15 SS-4
Biological and area-specific slug control for farming
Slotsbo, S.1; Krogh, P. H.1; Wendelboe, K.2; Smedegaard, J.3; Tirado, J.4; Cordesen Nielsen, G.5

CONTRIBUTED PAPERS. Wednesday, 15:30 SS-5
Winter survival of the invasive slug Arion vulgaris in agricultural fields in Norway
Hatteland, B. A.1,2; Roth, S.3 and Andersen, A.4

CONTRIBUTED PAPERS. Wednesday, 15:45 SS-6 STU
A story of the beta pore forming toxins in the fresh water snail Biomphalaria glabrata
LASSALLE, D.1; Gallinier, R.1; Gourbal, B.2 & Duval, D.2

CONTRIBUTED PAPERS. Wednesday, 16:00 SS-7
Morphological indicators of reproduction in the garden snail Cornu aspersum
Ortega-Hidalgo, M.M.1; Rodriguez-Zaldúa, I.1; Txurruka, J.M.1

CONTRIBUTED PAPERS. Wednesday, 16:15 SS-8
Physiological and biochemical responses of mussels D. polymorpha and D. bugensis to exposure to hypoxia
Klimova Y.S.1; Chuiko G.M.1; Sharov A.N.1,2; Kholodkevich S.V.2,3

CONTRIBUTED PAPERS. Wednesday, 16:30 SS-9
Important diseases of beneficial invertebrates; from cocktails to insects

Chairs: Mark Freeman / Helen Hesketh

CONTRIBUTED PAPERS. Wednesday, 16:45 DBI-1
Addressing the health of Macrobrachium rosenbergii in Bangladesh aquaculture
Hooper, Ch.1; Bateman, KS.1; Ross, S.1; Stentiford, GD.1; Rahman, MM.1,2; Basak, SK.2; Bass, D.1

CONTRIBUTED PAPERS. Wednesday, 17:00 DBI-2
Diseases of the Caribbean spiny lobster, Panulirus argus
Atherley, NAM.1; Dennis, MM.; Freeman, MA.

CONTRIBUTED PAPERS. Wednesday, 17:15 DBI-3
Discovery of Marteilia parasites in UK common cockle (Cerastoderma edule) fisheries and comparison with Marteilia cochilliae S1; Bass D.2; Villaiba Garcia, A.3; Carballal Durán, M.4; Cao Hermida, A.5; Iglesias Estepa, D.6; Macarie, A.1; Shaw, P.1; Feist, S.1; Hooper, Ch.1; Kerr, R.2; Ironside, J.1

CONTRIBUTED PAPERS. Wednesday, 17:30 DBI-4
Characterization of a novel mutant of Vibrio parahaemolyticus that carries binary toxin genes, pirA and pirB but does not cause acute hepatopancreatic necrosis disease (AHPND) in Pacific white shrimp (Penaeus vannamei).
Dhar, Arun K.1; Aranguren, F. L.1; Mai, H. N.1; Kanrar, S.1; Cruz-Flores, R.1

CONTRIBUTED PAPERS. Wednesday, 17:45 DBI-5
The Aquatic Nudiviridae
Bateman, KS.1,2*; Holt, C.1,3; Kerr, R.1; Bean, T.1*; Hooper, Ch.1*; Stone, MJ.1; van der Giezen, M.2; Daniels, C.1; Van Eynde, B.3; Smagghe, G.3; Bojko, J.1; Stentiford, GD.1,2; Bass, D.1,2; van Oers, M.3; van Aerle, R.1,2

CONTRIBUTED PAPERS. Wednesday, 18:00 DBI-6
Understanding the molecular basis of susceptibility to white spot syndrome virus infection in shrimp Penaeus vannamei
Millard, RS.1,2; Verbruggen, B.2; Bickley, LJ.1; Bateman, KS.1; Stentiford, GD.1,2; Tyler, ChR.1.; van Aerle, RP.; Santos, EM.

CONTRIBUTED PAPERS. Wednesday, 18:15 DBI-7 STU
Comparative genomics analysis of White Spot Syndrome Virus (WSSV) isolates from different geographical regions.
Al Arimi, WSM.1,2; Bass, D.1,2; Stentiford, GD.1; Wilfert, L.1*; Longdon, B.2; Tschirren, B.1; van Aerle, R.1; Bateman, KS.1

Coffee Break Wednesday, 16:30-17:00
Foyer

POSTER SESSION. Wednesday, 16:30 PB-1 STU
Effect of a new nematicidal Bacillus thuringiensis strain on Meloidogyne incognita in tomato plants
Verduzco-Rosas, Luis A.; Ibarra, Jorge E.

POSTER SESSION. Wednesday, 16:30 PB-2 STU
Characterization of two endophytic strains of Bacillus thuringiensis highly insecticides
García-Suárez, R.; Ibarra, J.R.

POSTER SESSION. Wednesday, 16:30 PB-3
Vip3Aa induces apoptosis through lysosomal-mitochondrial axis in Spodoptera frugiperda Sf9 cells
Xiaoyue-Hou.1; Lu, Han.1; Baojun, An.1; Zhanglei, Cao.1; Yanli, Zhang.1; Xia, Cai.1; Yunda, Zhan.1; Bing, Yan.1; Jun, Cai.1,2,3

POSTER SESSION. Wednesday, 16:30 PB-4
Microencapsulation of Bacillus thuringiensis: preparation and its process optimization
Zhang, A.1; Zhang, Y.1; Li, J.1; Son, J.2; Du, L.2; Guo, S.1
Identification and characterization of a new cry gene of *Bacillus cereus sensu lato*.  
*Castillo-Esparza, J.F.; Luévano-Borroel, J.; Ibbara, J.E.*

Mosquitocidal activity of a Cry1C toxin of *Bacillus thuringiensis* and its synergy with Cyt1A  
*González-Villarreal, S.E.; García-Montelongo, M.; Ordóñez-Acevedo, L.G.; Luévano-Borroel, J.; Ibbara, J.E.*

Isolation and characterization of the insect juvenile hormone antagonists from *Streptomyces* sp.  

The presence and diversity of insecticidal proteins in metagenomes from various environments  
er, T.; Williams, R.J.; Wu, Y.; DeSantis, T.Z.; Dabbagh, K.*

*Bacillus thuringiensis*’s plant disease control effect and plant growth promotion effect  
*Tomita, Y.; Yamazaki, K.; Aiuchi, D.C.; Asano, S.; Koike, M.*

Photorhabdus lectins disrupt the activity of insect and human immune system  
*Dobeš, P.; Fudijarová, E.; Houser, J.; Jančaříková, G.; Hyršl, P.; Wimmerová, M.*

Sex-specificity in innate immunity of insect larvae  
*Belousova, I.; Pavlushin, S.; Rudneva, N.; Martemyanov, V.*

Specificity determination in *Bacillus thuringiensis* Cry2A toxins  
*Barqawi, A.; Joseph, L.; Rao, T.; Crickmore, N.*

Mode of action of the Cry41Aa parasporin against human cancer cells  
*Nasiri, M.; Souissi, W.; Domanska, B.; Crickmore, N.*

Type II toxin-antitoxin system regulates the pathogenicity of *Bacillus thuringiensis* during its infection  
*Peng, D.H.; Li, L.X.; Xu, Y.J.; Zheng, J.S.; Liu, M.; Ruan, L.F.; Sun, M.*

Suppression of Sacbrood virus by virus-derived dsRNA produced from *Bacillus thuringiensis* toxic to *Galleria mellonella*  

The impact of the absence of the *Bacillus cereus* siderophore Bacillibactin and the FeuA siderophore binding protein on iron acquisition and in insect virulence  
*Consentino, L.; Rejasse, A.; Buisson, C.; Nielsen-LeRoux, C.*
Critical structural and functional amino acids of Vip3Aα from *Bacillus thuringiensis*

Quan, Y.; Banyuls, N.; Van Rie, J.; Ferré, J.

Characterization of two highly insecticidal endophytic strains of *Bacillus thuringiensis*


Identification of antimicrobial peptides in wheat stink bug, *Aelia rostrata* (Hemiptera: Pentatomidae)

Doğan, Cansu; Bayram, Şenile; Toprak, Umut

Identification of novel hemiptericins in sunnpest, *Eurygaster maura* (Hemiptera: Scutelleridae)

Cedden, Doğan; Bayram, Şenile; Babaroğlu, Numan; Toprak, Umut

Cultivation of entomopathogenic fungi in orbitally shaken bioreactors

Postsession. Wednesday, 16:30 PB-34 STU

Senn, Y.annick; Dr. Grabenweger, Giselher; Dr. Poggendorf, Iris

Application of polyamine carbon quantum dots (CQDs) to aquatic beneficial invertebrates

Postsession. Wednesday, 16:30-18:00

Foyer

New isolates of *Beauveria bassiana* from Kyrgyzstan and their Entomopathogenic Potential

Postsession. Wednesday, 16:30 PF-1

New *Beauveria bassiana* strain (Bals.-Criv.) Vuill., pathogenicity against weevil pests and physiological characterization

Moldovan, A.; Munteanu-Molotievskiy, N.

Evaluation of entomopathogenic fungi to control *Stenoma cecropia* (Lepidoptera: Elachistidae), insect pest of oil palm in Colombia

Postsession. Wednesday, 16:30 PF-4

New *Beauveria bassiana* isolates to growth on hydrocarbons and its potential association with virulence to the soybean pest *Pleizodorus guldinii*

Sessa, L.; Abreo, E.; Altier, N.; Pedrini, N.

Spanish mycoviral population of the entomopathogenic fungus *Beauveria bassiana*

Garrido-Jurado, I.; Filippow, C.; Meyling, N. V.; Quesada-Moraga, E.; Coutts, R. H.A.; Kotta-Loizou, L.

Comparative analysis of oxidative stress and peroxisomal biogenesis in microsclerotia produced by *Beauveria bassiana* and *Metarhizium robertsi*

Pedrini, N.; Paixão, F.R.S.; Huarte-Bonnet, C.; Mascarin, Gabriel M.; Fernandes, Everton K.K.

Down regulation of chitin synthase 1 gene elevates insecticidal activity of *Beauveria bassiana* ANU1 against *Solenopsis invicta*

Youngjin P.; Seha S.

Novel biocontrol product, Nomu-Protec, developed through solid state fermentation of the entomopathogenic fungus *Metarhizium (Nomuraea) rileyi*

Janks, C.; Morris, M.; Le Voy, K.; Heine, H.

Virulence of entomopathogenic fungus *Beauveria bassiana* ARP14 against egg of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)

Jang, L.E.; Rahman, M.A.; Lim, U.T.

Comparison of infection dynamics using two strains of *Beauveria bassiana* which has different virulence in *Anopheles stephensi*


Effect of *Lecanicillium* spp. against eggs of greenhouse whitefly, *Trialeurodes vaporariorum* and sweetpotato whitefly, *Bemisia tabaci*

Ishikura, S.; Moyo, D.R.; Koike, M.; Aluchi, D.

Effect of artificial media and temperature on the growth and development of bee brood pathogen *Ascosphaera apis* and optimization its cultivation in vitro

Mráz, P.; Bohatá A.; Konopická J.; Hoštičková I.; Čurn V.

Modified Adamek’s medium renders high yields of *Metarhizium robertsi* blastospores that are desiccation tolerant and infective to cattle-tick larvae

Iwanicki, N.S.A.; Ferreira, B.O.; Mascarin, G.M.; Delalibera Jr., I.

Current trend of microbial insecticides: Challenges and Opportunity in fungal insecticides


Single Cell Encapsulation via Pickering Emulsion for Biopesticide
**Applications: Metarhizium brunneum against foliar pests**
Yaakov N. 1; Ananth M.K. 1; Felbaum R. 1; Lahat M. 1; Da Costa N. 2; Belaouso E. 2; Ment D. 2; Mechez G. 1

**POSTER SESSION. Wednesday, 16:30 PF-18**
Characterization of Pr1 family proteases in Beauveria bassiana
Gao, Ben-Jie; Ying Sheng-Hua; Feng Ming-Guang  

**POSTER SESSION. Wednesday, 16:30 PF-19**
First records of Beauveria bassiana occurrences in the invasive pest Box Tree Moth, Cydalima perspectalis in Georgia
Burjanadze, M. 1; Gogebashvili, M. 2; Ivanishvili, N. 2; Arjavanidze, M. 2; Supatashvili, A. 1; Kharabadze, N. 1; Koridze, K. 1

**POSTER SESSION. Wednesday, 16:30 PF-20 STU**
Entomopathogenic fungi as biocontrol agent against the bulb mite, Rhizoglyphus robini
Konopická, Jana 1; Zemek, Rostislav; Bohatá, Andrea 1; Nermuľ, Jiří 1; Mráček, Zdeněk 1; Palevsky, Eric 1

**POSTER SESSION. Wednesday, 16:30 PF-21**
Effectiveness of Beauveria bassiana against Redbanded Stink Bugs (Hemiptera: Pentatomidae), a key pest of soybeans in the southern United States
Parsy, K.A. 1; Portilla, M. 1

**POSTER SESSION. Wednesday, 16:30 PF-22**
Bacterial decomposition of insects post Metarhizium infection, possible influence on plant growth.
Yaroslavtseva, Olga N. 1; Kryukov, Vadim Yu. 1; Smirnova, Natalya V. 1; Tomilova, Oksana G. 1; Tyurin, Maksim V. 1; Akhanaev, Yuri B. 1; Polemonova, Olga V. 1; Alkina, Tatyana Yu. 1; Kabilov, Marcel R. 1; Glupov, Victor V. 1

**POSTER SESSION. Wednesday, 16:30 PF-23 STU**
Development of a computer-assisted method for the quantification of discharged conidia of an entomopathogenic fungus
Muskat, L. 1; Humbert, P. 1; Kerkhoff, Y. 2; Nattkemper, T. 2; Eilenberg, J. 2; Patel, A. V. 1

**POSTER SESSION. Wednesday, 16:30 PF-24**
Determining relative infectivity/virulence of selected entomopathogenic fungi against Asian Citrus Psyllid using spray exposure bioassays
Wendel, J.; Cisneros, J. 1; Jaronski, S. T. 1; Vitek, C. 1; Flores, D. 2

**POSTER SESSION. Wednesday, 16:30 PF-25**
Developing methods to collect, process, and screen indigenous fungal strains that naturally attack the ACP in the Lower Rio Grande Valley
Cisneros, J. 1; Wendel, J. 1; Jaronski, S. T. 1; Vitek, C. 1; Flores, D. 2

**POSTER SESSION. Wednesday, 16:30 PF-26**
Heat-exposure of Metarhizium anisopliae s.str. conidia in oil suspension and the effects on fungal penetration through the cuticle of the tick Rhipicephalus sanguineus s.l.
Ferreira, Juliana M. 1; Barreto, Lucas P. 1; Silva, Cáritas S.R. 1; Arruda, Walquira 2; Soares, Filippe E.F. 1; Fernandes, Éverton K.K. 1

**POSTER SESSION. Wednesday, 16:30 PF-27**
Distribution pattern of Beauveria fungal entomopathogens in soil habitats: Diversity, natural occurrence and dynamics
Al Khoury, C. A. 1; Chehab, R. 1; Tawil, C. 1; Malek, S. 1; Noujeim, J. 2; Guillot, J. 2; Nemser, N. 1
POINTER SESSION. Wednesday, 16:30 PMC-5

First report of Conidiodobulus coronatus in Paraguay as biological control of leaf cutting ants
Resquin-Romero, G.1; Sarubbi-Orue, H.1; Pino-Quintana, D.; Amarilla, S.P.; Butt, T.1

POINTER SESSION. Wednesday, 16:30 PMC-6 STU

Development of a SNP based tool for the identification and discrimination of Melolontha melolontha and M. hippocastani
Pedrazzini, C.1,2; Strasser, H.2; Holderregger, R.1; Widmer, F.1; Enkerli, J.1

POINTER SESSION. Wednesday, 16:30 PMC-7

Behavioural abilities of EPNs to search the insect host inside sugarcane culm
Chacon-Orozco, J.G.; Silva, M.S.O.; Cardoso, J.F.M.; Leite, L.G.1

POINTER SESSION. Wednesday, 16:30 PMC-8

Effect of the successive passes of Metarhizium anisopliae on insecticidal activity to Demotispa neival
Cuartas-Otalora, P.E.1; Montes-Bazurto, L. G.2; Borrero-Echeverry, F.1; Quiroga, G.M.1; Grijalba, E.P.1; Buitrago, L.F.1; Bustillo, A.E.; Gómez, M.I.1

POINTER SESSION. Wednesday, 16:30 PMC-9

Beauveria bassiana against leaf cutter ants: are all strains equally effective to different ant pest species?
Goffé, D.; Osorio, A.; Folgarait, P.J.

POINTER SESSION. Wednesday, 16:30 PMC-10

Antifungal activity of entomopathogenic Bacillus thuringiensis.
Yamazaki, K.1; Tomita, Y.; Asano, S.I.; Auchi, D.1; Koike, M.2
1Hokkaido Sugar CO., Ltd., Tokyo, Japan

POINTER SESSION. Wednesday, 16:30 PMC-11

Development of Attract-Kill granule for white grubs control
Liu, Q.1; Shu, C.; Zhang, J.

POINTER SESSION. Wednesday, 16:30 PMC-12

Entomopathogenic fungi for the control of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Africa: A first approach towards the development of an alternative small-scale production method
Langner, M.1,2; Stephan, D.1

POINTER SESSION. Wednesday, 16:30 PMC-13

Host response of Lymantria dispar dispar to Chromobacterium spp. infections

POINTER SESSION. Wednesday, 16:30 PMC-14 STU

Biological Control: Fighting below ground insect pests with Pseudomonas bacteria
Spescha, A.; Schneider, J.1; Gilliéron, F.; Flury, P.; Grabenweger, G.2; Maurhofer, M.1

POINTER SESSION. Wednesday, 16:30 PMC-15 STU

Comparative Gene Expression of Peritrophic Matrix Provides an Insight into its Role in Cry1A.105+Cry2Ab2 Resistance by the Spodoptera frugiperda pest
Castellane, T.C.L.1,2; Fernandes, C.C.1; Fatoretto, J.C.4; Pinheiro, D.G.2; Desidério, J. A.2; Lemos, M.V.1

POINTER SESSION. Wednesday, 16:30 PMC-16

Biological control of the Japanese beetle (Popillia japonica) with entomopathogenic fungi
Sostizzo, T.; Grabenweger, G.

POINTER SESSION. Wednesday, 16:30 PMC-17

Cellulase improves the endophytism of encapsulated Metarhizium brunneum on potato plants
Jakobs-Schoenwandt, D.; Krell, V.; Unger, S.2; and Patel, A.1

POINTER SESSION. Wednesday, 16:30 PMC-18

Influence of inundative mass application of Metarhizium brunneum BIPESCO 5 on indigenous Metarhizium strains in maize fields
Zottele, M.; Gruber, A.; Enkerli, J.; Strasser, H.

POINTER SESSION. Wednesday, 16:30 PMC-19 STU

It’s necessary new candidate gene for Bt genetically modified crops
Castellane, T.C.L.1,2; Moraes, K.E.3; Lemos, E. E.G.4; Lemos, M.V.5

POINTER SESSION. Wednesday, 16:30 PMC-20

Defense mechanism of ioxid ticks to fungal infection: cuticle, the first barrier
Silva, C.S.R.1; Golo, P.S.2; Castro, R.N.3; Ángelo, I.C.4; Arruda, W.5; Fernandes, E.K.K.1

POINTER SESSION. Wednesday, 16:30 PMC-21

Blastospores and conidia supplemented or not with secondary metabolite of plants to control flies
Filgueiras, M.D.G.; Christian L.; Éverton K F.K.

POINTER SESSION. Wednesday, 16:30 PMC-22

Preliminary studies on presence of entomopathogens in outbreak population of great web-spinning pine-sawfly Acantholyda posticalis in urban forest stands
Jankevica, L.1; Strike Z.; Smits, A.2

POINTER SESSION. Wednesday, 16:30 PMC-23

Effect of arbuscular mycorrhizal fungi on the susceptibility of Spodoptera exigua to viral and bacterial entomopathogens
Frattini, A.1; Rivero, J.1; Pozo, M.J.; Herrero, S.1

POINTER SESSION. Wednesday, 16:30 PMC-24 STU

Management of Colorado potato beetle overwintering adults with entomopathogenic nematodes
Čačija, M.; Mrganić, M.; Kolenc, M.; Lemić, D.; Drlmić, Z.; Vrlić Gašparić, H.; Bazoč, R.

POINTER SESSION. Wednesday, 16:30 PMC-25 STU

First report of entomopathogenic nematodes Heterorhabditis bacteriophora from Croatia and its virulence against Lasioptera rubi Majić, I.; Sarajlić, A.; Lakatos, T.; Tóth, T.; Raspudić, E.; Laznik, Ž.3

POINTER SESSION. Wednesday, 16:30 PMC-26

Impact of Moisture on the Viability of Metarhizium Microsclerotia Behie, R.W.; Goett, E.J.

POINTER SESSION. Wednesday, 16:30 PMC-27

Solid State Fermentation at Bayer CropScience Biologics – Ready for the next step
Steinwender, B.M.; Marba-Ardebol, A.M; Schink, S.

POINTER SESSION. Wednesday, 16:30 PMC-28

Evaluation of external parameters influencing the efficacy of a microbial attract and kill strategy for wireworm control

POINTER SESSION. Wednesday, 16:30 PMC-29

Evaluation of the insecticidal activity of Bacillus thuringiensis strains isolated from Algeria: Toxicity of the supernatant and spore-crystal mixtures
Djenane, Z.1,2,3; Nateche, F.2; Lázaro-Berenguer, M.2; González...
POSTER SESSION. Wednesday, 16:30 PMC-31 STU
A new method of microsporidia metarbachoring
Trzebny A.1; Słodkowicz-Kowalska A.2; Dabert M.1

POSTER SESSION. Wednesday, 16:30 PMC-32
Detection and prevalence of a microsporidium in the population of Brown Marmorated Stink Bug Halyomorpha halys (Heteroptera: Pentatomidae) in the Republic of Georgia
Kerestelidze, M.1,2; Pilarska, D.3,4; Linde, A.5

POSTER SESSION. Wednesday, 16:30 PMC-33 STU
Microsporidian infections in the Gammurus roeseli species complex (Amphipoda) over its geographic range: evidence for both host-parasite co-diversification and recent host-shifts.
Quiles, A.1,2; Bacela-Spychalska, K.1; Teixeira, M.1; Lambin, N.1; Grabowski, M.1; Rigaud, T.1; Wattier, R.A.1

POSTER SESSION. Wednesday, 16:30 PMC-34
Development of Anacallia algerae in Drosophila melanogaster
Weidner, E.1; Sokolova Y.1,2; DiMaria P.1

POSTER SESSION. Wednesday, 16:30 PMC-35
The Enterocytozoonidae: Emergent Microsporidia in the aquatic-terrestrial food chain
Trew, J.1; Aldama-Can, J.D.2; Sritunyalucksana, K.2; Munkongwongsiri, N.2; Itsathitphaisarn, O.2; Williams, B.1

POSTER SESSION. Wednesday, 16:30 PMC-36
Do-it-yourself: On-site detection method for Nosema ceranae infecting honeybees
Kyei-Poku, G.1; Gauthier, D.1; Ignatieva, A.2; Tokarev, Y.2

POSTER SESSION. Wednesday, 16:30 PMC-37
Determining eastern spruce budworm population health: Operational utility of recombinase polymerase amplification for detection of Nosema fumiferanae
Kyei-Poku, G.; Gauthier, D.

POSTER SESSION. Wednesday, 16:30 PSS-1
Characterization of two Phasmarhabditis (Nematoda) earthworm isolates and susceptibility of earthworms and invasive slugs to EMA34
Leung, N.1; Fitch, D.2; Paine, T.1; Tandingan De Ley, I.3

POSTER SESSION. Wednesday, 16:30 PSS-2
New isolates of the nematophagous fungus Arthrobotrys oligospora for biocontrol of garlic nematodes
Doolokldievka, T.1; Bobushova, S.1; Schuster, C.2; Leclerque, A.2

POSTER SESSION. Wednesday, 16:30 PSS-3
Effects of pre-maize cultivation on soil conditions in apple replanting habitat with alleviating soil deterioration indicated by soil nematode community
Liu, Q.-Z.; Yang Y.-Q.

POSTER SESSION. Wednesday, 16:30 PMC-38
SNP analysis for generation of trait-related molecular markers in the entomopathogenic nematode Heterorhabditis bacteriophora
Godina, G.; Kirch, C.; Vandenbossche, B.; Dörfler, V.; Barg, M.; Molina, C.; Ehlers, R.-U.

POSTER SESSION. Wednesday, 16:30 PN-4
Putative receptor of a Bacillus thuringiensis toxin in Caenorhabditis elegans
Garcia-Montelongo, M.; Gonzalez-Villarreal, S.E.; Ordoñez-Acevedo, L.G.; Lule-Chavez, A.N.; Ibarra, J.E.

POSTER SESSION. Wednesday, 16:30 PN-5 STU
Steinernema feltiae scavenging behavior: offspring fitness is modulated by various cadaver scenarios
Blanco-Perez, R.1,2; Bueno-Pallero, F.A.1; Vicente-Diez, I.2; Marco-Mancebon, V.S.1; Perez-Moreno, I.1; Campos-Herrera, R.1,2

POSTER SESSION. Wednesday, 16:30 PN-6
Mortality of Phyllophaga vetula larvae by the separate and combined application of Metarhizium anisopliae, Steinernema carpocapsae and Steinernema glaseri
Ruiz-Vega, J.1; Cortes-Martinez, C.1

POSTER SESSION. Wednesday, 16:30 PN-7
Characterization of natural populations of entomopathogenic nematodes in Israel for establishment of genetic selection tools for heat and desiccation tolerance
Levy, N.1,2; Salame, L.1; Glazer, I.; Ment D.1

POSTER SESSION. Wednesday, 16:30 PN-8 STU
Behavioural response in model insect under biological and chemical stress condition
Kunc M.1; Arefin B.2; Dophe Pavell, Hrychová J.1; Maguire R.3; Hrysl P.1; Kavanagh K.3; Theopold U.2

POSTER SESSION. Wednesday, 16:30 PN-9 STU
Effects of bacterial feeding nematodes on hemocytes after injection into the hemocoel of the insect Galleria mellonella
Ono, M.1,2; Yoichi, H.1,2; Toyoshi, Y.1,2

POSTER SESSION. Wednesday, 16:30 PN-10
Potential four entomopathogenic nematodes for the control of Brown marmorated stink bug - Halyomorpha halys
Burjanadze, M.1; Gorgodze, O.2; Tarasco, E.2; Lorkipanidze, M.2; Arjavanidze, M.1; De Luca, F.1; Troccoli, A.1; Fanelli, E.1

POSTER SESSION. Wednesday, 16:30 PN-11
Susceptibility of olive fruit fly, Bactrocera oleae (diptera: tephritidae) larvae and pupae to entomopathogenic nematodes
Torrini, G.; Mazza, G.; Benvenuti, C.; Simoncini, S.; Landi, S.; Frosinini, R.; Rocchini, A.; Roversi, P. F.

POSTER SESSION. Wednesday, 16:30 PN-12
Assessing the immunocompetence of Rhynchosporus ferruginus in response to bacterial challenge and entomopathogenic nematode infection
Torrini, G.1; Cappa, F.1; Mazza, G.1; Inghilesi, A. F.1; Benvenuti, C.1; Villa, L.2; Roversi, P. F.; Cervo, R.2

POSTER SESSION. Wednesday, 16:30 PN-13
Recombinant Bacillus thuringiensis for intestinal nematodes
Flanagan, K.1; Abraham, A.; Gazzola, D.; Li, H.; Kellogg, T.; Kim, Y.; Rus, F.; Ostooff, G.; and Aroyan, RV

POSTER SESSION. Wednesday, 16:30 PN-14
Pheromones as drivers of entomopathogenic nematodes movement and infectivity
Oliveira-Hofman, C.; Kaplan, F.; Stevens, G.; Lewis, E.; Wu, S.; Alborn,
Evaluation of potential of entomopathogenic nematodes for control of box tree moth – Cadalina perspectalis (Walker, 1859) in laboratory conditions

Mladenović, Z.; Nježić, B.

POSTER SESSION. Wednesday, 16:30 PN-15

Management of black vine weevil (Otiorhynchus sulcatus) by entomopathogenic nematodes in Georgia

Mikaia, N.

POSTER SESSION. Wednesday, 16:30 PN-16

Molecular and phenotypic characterization two strains of Photorhabdus luminescens associated with Iraqi Heterorhabditis bacteriophora

Al-Zaidawi, J.B.; Karimi, J.; Moghadam, E.M.

POSTER SESSION. Wednesday, 16:30 PN-17

Comparison of entomopathogenic nematode and insecticide management of western corn rootworm larvae

Razinger, J.

POSTER SESSION. Wednesday, 16:30 PN-18

The earthworm mucus and their feeding activity can decrease the biological control action by entomopathogenic nematodes and entomopathogenic fungi


POSTER SESSION. Wednesday, 16:30 PN-19

Fighting parasitic nematodes with natural products and microbial crystals


POSTER SESSION. Wednesday, 16:30 PN-20

Phylogenetic analysis of six strains of baculovirus with activity towards Spodoptera frugipерда

Zanella-Sainz, Ingrid; León-Galván, Ma. Fabiola; Ibarra-Rendón, Jorge E.; Del Rincón-Castro, Ma. Cristina

POSTER SESSION. Wednesday, 16:30 PV-1

Functional and genomic approaches to study the evolution of recently domesticated viruses in Campopleginae parasitoid wasps


POSTER SESSION. Wednesday, 16:30 PV-2 STU

Survey of CpGV mixed-genotype infection occurrence in treated orchards

Hinsberger, A.; Blachère-Lopez, C.; Lopez-Ferber, M.

POSTER SESSION. Wednesday, 16:30 PV-3 STU

Effect of temperature and relative humidity on the activation of a covert infection with the Acheta domesticus densovirus (AdDV) in colonies of the European house cricket Acheta domestica

Maciel-Vergara, G.; Ros, V.I.D.; van Oers, M.M.; van Loon, J.J.A.

POSTER SESSION. Wednesday, 16:30 PV-4 STU

First results of the virome of Scaphoideus titanus, Frankliniella occidentalis and Thrips tabaci

Abbà, Simona; Chiapello, Marco; Ottati, Sara; Galetto, Luciana; Tavella, Luciana; Turina, Massimo; Marzachi, Cristina

POSTER SESSION. Wednesday, 16:30 PV-5

Unraveling the multiple nudiviral integration traces within insect genomes

Bézier, A.; Leobold, M.; Gayral, P.; Drezen, J-M.; Hemio E.A.

POSTER SESSION. Wednesday, 16:30 PV-6

Construction of a novel baculovirus expression system with increased foreign protein production and expression time


POSTER SESSION. Wednesday, 16:30 PV-7

Construction of novel baculovirus inducible vectors for rapid production of foreign proteins

Kim H.S.; Gwak, W.S.; Lee J.Y.; Bae J.S.; Kim T.H.; Choi C.J.; Woo S.D.

POSTER SESSION. Wednesday, 16:30 PV-8 STU

Chrysoperla carnea’s performance when fed with two nucleopolyhedroviruses (SeMNPV and AcMNPV) Spodoptera exigua infected larvae

Gutiérrez, Oscar G.; Medina, Pilar M.; Adán, A.; Garzón, A.

POSTER SESSION. Wednesday, 16:30 PV-9

Identification and Genomic Analysis of a Second Species of Nucleopolyhedrovirus Isolated from Spodoptera exigua (Lepidoptera, Noctuidae)

Li, Changyou; Zheng, Guiling; Zhou, Hongxu; Tan Xiumei; Deng Fei; Chen, Yingjian; Qi, Benxiang

POSTER SESSION. Wednesday, 16:30 PV-10

Arboviruses associated with R. microplus ticks in Yunnan China

Shi, Junming; Shen, Shu; Wu, Hui; Zhang, Yunzheng; Deng, Fei

POSTER SESSION. Wednesday, 16:30 PV-11

Comparison of vertical transmission of high and low virulent nucleopolyhedrovirus strains after Lymantria dispar L. infection

Akhanaev, Yuri B.; Belousova, Irina A.; Martemyanov, Vyacheslav V.

POSTER SESSION. Wednesday, 16:30 PV-12

The role of parasitic larvae and their symbiotic viruses as hidden players in plant-insect interactions

CUSUMANO, A.; URBACH, S.; VOLKOFF, A.-N.

POSTER SESSION. Wednesday, 16:30 PV-13

Reduced AcMNPV budded virus production facilitates generation of persistent infections in vitro


POSTER SESSION. Wednesday, 16:30 PV-14

Reduced AcMNPV budded virus production facilitates generation of persistent infections in vivo


POSTER SESSION. Wednesday, 16:30 PV-15 STU

Constructing a model of the larval Spodoptera exigua brain to study baculoviral entry and localization

Gasque, S.N.

POSTER SESSION. Wednesday, 16:30 PV-16 STU

Rhabdovirus-free clones from a Spodoptera frugiperda (Sf21) cell population

POSTER SESSION. Wednesday, 16:30 PV-17
POSTER SESSION. Wednesday, 16:30 PMI-9 STU
Mutations in the Voltage Gated Sodium Channel gene associated with deltamethrin resistance in the predatory mite *Phytoseiulus persimilis*
Benavent-Albarracín, L.1; Alonso, M.2; Catalán, J.2; Urbaneja, A.2; Davies, E.3; Williamson, M.3; González-Cabrera, J.1

CONTRIBUTED PAPERS. Wednesday, 18:00 MC-25
*Metarhizium brunneum* F52 microsclerotia formulation for the management of the annual bluegrass weevil: compatibility with fungicides and efficacy alone and combined with imidacloprid and hydrogel
Koppenhöfer, A.M.1; Wu, S.2; Kostromytska, O.S.3

CONTRIBUTED PAPERS. Wednesday, 18:15 MC-26
Influence of orchard age on the efficacy of a granulovirus: architecture trumps biochemistry
Albertyn, S.1; Mwanza, P.1; Marsberg, T.1; Hill, M.P.1; Dealtrly, G.B.1; Lee, M.E.1; Moore, S.D.1,2

CONTRIBUTED PAPERS. Wednesday, 18:30 MC-27
Field testing of two different formulations of Beauveria brongniartii for control of white grubs of *Melolontha melolontha* in apple orchards
Stephan, D.1; Paluch, M.2; Göttmann, J.1; Reuscher, S.1; Peiz, J.1

CONTRIBUTED PAPERS. Wednesday, 18:45 MC-28
Effectiveness of a *Beauveria bassiana* formulation, Biolisa Madara, against Pine Wilt Disease after annual application for three years
Sato, H.1; Iwami, Y.1; Maehara, N.1; Urano, T.1; Nakamura, K.3

CONTRIBUTED PAPERS. Wednesday, 19:00 MC-29 STU
Development of a formulation to control psyllid pests in fruit orchards with the entomopathogenic fungus *Pandora* sp.
Muskat, L.1; Humbert, P.1; Niehaus, K.3; Eilenberg, J.1; Patel, A.1

CONTRIBUTED PAPERS. Wednesday, 19:15 MC-30
A microbial Integrated Pest Management strategy for climbing cutworm in wine grapes
Franklin, M.; Henderson, D.E4; Tahir Adabi, S.; Huang, A.; Arruda, G.

CONTRIBUTED PAPERS. Wednesday, 19:30 MC-31
The California experience: promoting microbial control through effective outreach
Dara, S.K.

CONTRIBUTED PAPERS. Wednesday, 18:00 V-41
Transcriptional responses of the *Trichoplusia ni* midgut to oral infection by the baculovirus Autographa californica Multiple Nucleopolyhedrovirus
Shrestha, A.1; Bao, K.1; Chen, W1; Wang, P.2; Fei, Z.1; Blissard, G.W.1

CONTRIBUTED PAPERS. Wednesday, 18:15 V-42 STU
Neuropeptide Expression in *Spodoptera exigua* after baculovirus infection. A focus on Proctolin and its relevance in locomotion and digestion.
Llopis-Giménez, A1; Parenti, S1; Han, Y1; Ros, V.I.D2; Herrero, S3

CONTRIBUTED PAPERS. Wednesday, 18:30 V-43
Chrysodeixes includens NPV infection induces apoptosis in an *Anticarsia gemmatalis* cell line
Morgado, F.S.; Ribeiro, B.M.

CONTRIBUTED PAPERS. Wednesday, 18:45 V-44 STU
Transcriptome analysis of *Deformed wing virus*-infected bumble bees (*Bombus terrestris*)
Panziera, Delphine1,2; Paxton, Robert J.1,2; Bigot, Diane1

CONTRIBUTED PAPERS. Wednesday, 19:00 V-45
Isolation of ferritin and its response to BmNPV infection in silkworm, *Bombyx mori*
Xu J.1,2; Fei D.1,2; Yu H.1,2; Zhang S.1,2; Wang J.1,2

CONTRIBUTED PAPERS. Wednesday, 19:15 V-46
Transcriptome analysis of *Cydia pomonella* granulovirus (CpGV) in codling moth (*Cydia pomonella* L.) larvae
Xi, Yu1; Wennmann, Jörg T.1; Fan, Jianbin2; Xing, Longsheng1; Jehle, Johannes A.1,2; Wan, Fanghao1,3

CONTRIBUTED PAPERS. Wednesday, 19:30 V-47
Ecological implications of covert infections with RNA viruses in the beet armyworm, *Spodoptera exigua*
Mengual-Martí, A.; Martinez-Solis, M.; Jakubowska, A.; Herrera, S.

CONTRIBUTED PAPERS. Wednesday, 19:45 V-48
The major hurdle for effective baculovirus transduction into mammalian cells is early endosomes
Hu, Liangbo1,2; Li, Yimeng1; Ning, Yunjia1; Deng, Fei1; Vlak, Just M.2,3; Hu, Zhihong1; Wang, Hualin1,2; Wang, Manli1,2
Development and use of a barrier system for reducing invertebrate damage in crops

Evans, K. A. 1; Inglis, M. 2

Optimising snail and slug management in Australian crops: linking mollusc activity with climate using time-lapse cameras

Perry, K. D. 1; Brodie, H. E. 2; Muirhead, K. A.; Baker, G. J. 1

The development of iron-III-phosphate as an integrated slug control method in UK agriculture

Benson, M. 1; Baxter, L. 1; Butt, T. 2

Can we make changes to slug pellets to improve their efficiency?

de Silva, S. 1; Port, G.; Sanderson, A. R. 2; Rushton, P. S. 3; Audsley, N. 2

Do molluscicidal control measures allowed in Lithuania kill invasive slugs?

Adomaitis, M. 1; Škujienė, G.; Račinskas, P.

Changes to the foraging behaviour of the grey field slug (Doroceras reticulatum) in the presence of molluscicide and implications for control.

Campbell, A.; Port, G.; Sanderson R. A.; Rushton, S. P.; Audsley, N.

Nosema ceranae affects peritrophic matrix structure in honey bees, Apis mellifera

Webster, T.C.; Kamminga, K.L.; Matisoff, M.A.

Elucidating the honey bee immune response at the Varroa mite feeding site.

Cooper, A.L. 1; Forward, K.; Freeman, T. 2; Campbell, E.M.; Bowman, A.S.

Prevalence and diversity of ssRNA+ honey bee-infecting viruses in wild hymenoptera

Bigot, D.; Gayrat, P. 1; López-Vaamonde C. 1, 2; Henniou EA.

Bacterial diversity of the Tetragonula carbonaria microbiome within and between hives

Tarlinton, B. 1, 2; McGree, J.; Gloag, R. 1; Hauxwell, C.

Microbial control

DIVISION BUSINES MEETING

Wednesday, 20:15-22:00

Auditorium 3

Microsoridia

DIVISION BUSINES MEETING

Wednesday, 20:15-22:00

Multispace AB

DBI

DIVISION BUSINES MEETING

Wednesday, 20:15-22:00

Commission R8

Identification of molluscs and their associated nematode parasite

Organisers / Chairs: Irma Tandingan de Ley / Bjørn Arild Hatteland

WORKSHOP

Wednesday, 20:15 SSW-1

Species diagnostics of gastropod parasitic nematodes

Tandingan De Ley, I.; Nermut, J.; Ross., J. 1, 2, 3

Identification of molluscs

Hatteland, B. A. 1, 2
CONTRIBUTED PAPERS. Thursday, 08:30 V-49
Characterisation of novel baculovirus isolates for potential development and application as biopesticides against agricultural pests in South Africa
Knox, Caroline M.1,3; Jukes, Michael D.1,3; Moore, Sean D.2,3; Hill, Martin P.3

CONTRIBUTED PAPERS. Thursday, 08:45 V-50
Step-by-step acquired resistance of Adoxophyes honmai passaged by nucleopolyhedrovirus: resistance mechanism and inheritance trait
Moriyasu, T1; Jun, T1,2; Maki, N.I1; Yasuhisa, K1; Madoka, N1

CONTRIBUTED PAPERS. Thursday, 09:00 V-51
Tutavir – a new control tool for the tomato leafminer Tuta absoluta
Wandeler, Heiri.; Dubach, Felix.

CONTRIBUTED PAPERS. Thursday, 09:30 V-53
Genome sequence and biological activity of a new group II alphabaculovirus from Chrysoideixis includens with tetrahedral occlusion bodies
Harrison, Robert L.1; Rowley, Daniel L.1; Popham, Holly J. R.2

CONTRIBUTED PAPERS. Thursday, 10:00 V-55
Application of a novel cell line derived from Thaumatobius leucotes-ta eggs to for the manipulation and production of alpha and beta baculoviruses.
Jukes, Michael D.1,2; Knox, Caroline M.1; Hill, Martin P.2; Moore, Sean D.2,3

CONTRIBUTED PAPERS. Thursday, 08:30 N-25
Antiprotozoal activity of Xenorhabdus and Photorhabdus bacteria mutually associated with entomopathogenic nematodes
Hazir, S.1; Tileklioglu, E.2; Gulsen, S.H.1; Cimen, H.1; Ertabaklar, H.2; Ulug, D.1; Ertug, S.1; Bode, H.B.1; Hazir, C.4; Bilecenoglu, D.K.5

CONTRIBUTED PAPERS. Thursday, 09:30 N-29
Excreted/secreted products of entomopathogenic nematodes and their effect on insect immunity
Eliáš Sara1, Hurychová Jana1, Dobeš Pavel1, Toubarro Duarte2, Simões Nelson2, Hyršl Pavel1

CONTRIBUTED PAPERS. Thursday, 08:30 MS-1
Co-infection of paramyxid and microsporidian parasites in feminised amphipod crustaceans
Ironside J.; James Pickup J.

CONTRIBUTED PAPERS. Thursday, 08:45 MS-2
Firing of the harpoon-like polar tube in microsporidian parasites
Jaroenlak, P1; Cammer, M.2; Becnel, J.J.3; Ekierd, D.C.1; Bhabha, G.1

CONTRIBUTED PAPERS. Thursday, 09:00 MS-3
Cancelled

CONTRIBUTED PAPERS. Thursday, 09:15 MS-4
Ptdlns(3)P-binding Protein NbSWP12 is Significant for microsporidia proliferation in insect cells
Chen J.1,2; Huang Y.1,2; Li Z.1,2; Mengxian L.1,2; Pan G.1,2; Zhou Z.1,2,3

CONTRIBUTED PAPERS. Thursday, 09:30 MS-5
Pathological analysis of silkworm infected by two microsporidia No-sema bombycis CQ1 and Vairimorpha necatrix BM
Below-ground inoculation with *Metarhizium brunneum* for long-term control of the cabbage root fly *Delia radicum*

**Thapa, S.; Cotes, B.; Meyling, N. V.**

Possibilities for use of *Metarhizium robertsi* C25 in baits against *Drosophila suzukii*

**Westerman, P. R.; Helsen, H.M.; Wiegers, G. L.; van der Sluis, B. J.; van Tol, R. W.H.M.**

Effect of abiotic factors on production of conidia by microsclerotia of *Metarhizium brunneum*

**Yousef-Naef, M.; Garrido-Jurado, I.; Gutiérrez-Sánchez, F.; Quesada-Moraga, E.**

Influence of the insecticide acetamiprid on the secondary metabolism of *Metarhizium* sp.

**Nowak, M.; Różalska, S.**

The potential for *Helicoverpa armigera* to evolve resistance against fungal biopesticides can be mitigated by using heterogeneous combinations of fungal isolates and crop plants

**Tinsley, Matt C.; Mangan, R.; Polanczyk, R. A.; Bussière, L. F.**

Microsporidia and microsporidia-like cryptomycota infecting micro-eukaryotes and metazoan parasites

**Organisers / Chairs: Mark Freeman / Joe Ironside**

**SYMPOSIUM. Thursday, 14:30 DMCS-1**

**Hyperparasitic Microsporidians of Myxosporeans**

**Freeman, Mark A.**

**SYMPOSIUM. Thursday, 14:54 DMCS-2**

**Hyperparasitic microsporidium in trematode hosts: two new species of Unikaryon that infect microphallids from crabs inhabiting Florida coasts**

**Sokolova Y.; Overstreet R.; Heard R.**

**SYMPOSIUM. Thursday, 15:18 DMCS-3**

**Cytology and Development of Metchnikovellidians and Related Organisms**

**Larsson, J.I.R.**

**SYMPOSIUM. Thursday, 15:42 DMCS-4**

**Early evolution of Microsporidia: lessons from molecular phylogeny, phylogenomics and genomics of metchnikovellidias**

**Nassonova, E.; Paskerova, G.; Frolova, E.; Galindo, L.J.; Torruella, G.; Moreira, D.; López-Garcia, P.; Smirnov, A.**

**SYMPOSIUM. Thursday, 16:06 DMCS-5**

**Infranuclear parasites of free-living amoebae: The Rozellomyctota and the origins of the Microsporidia**

**Corsaro D.; Walochnik J.; Wylezich C.; Hauröder B.; Müller K.-D.; Sokolova Y.; Michel R.**
SYMPOSIUM. Thursday, 14:30 SSS-1

Riding the Slime Wave: Global Perspective of Slug Control
Ross, J.L. 1,2,3

SYMPOSIUM. Thursday, 15:00 SSS-2

Slime time: Frontiers in slug and snail management in North America
McDonnell, R.J.

SYMPOSIUM. Thursday, 15:30 SSS-3

The Problem with Pellets; will we ever be able to eradicate slugs pests?
Port, G. R. 1

SYMPOSIUM. Thursday, 16:00 SSS-4

Slug control using Phasmarhabditis hermaphrodita
Robbie Rae 1

Coffee Break
Thursday, 16:30-17:00
Foyer

BACTERIA SYMPOSIUM
Thursday, 17:00-19:00
Auditorium 3

Insecticidal Bacteria: Cornerstones for Biological Control and IPM Programs
Organisers / Chairs: Brian Federici / Luca Ruiu / Neil Crickmore

SYMPOSIUM. Thursday, 17:00 BS-1

Reasons for the remarkable success of Bacillus thuringiensis
Federici, B.

SYMPOSIUM. Thursday, 17:20 BS-2

Brevibacillus as an insecticidal bacterium and source of pesticidal proteins
Glare, T. 1; Ruiu, L. 2

SYMPOSIUM. Thursday, 17:50 BS-3

Insect pathogenicity determinants of environmental Serratia, Yersinia and Pseudomonas species and implications for biological pest control
Hurst, Mark 1; Keel, Christoph 2

SYMPOSIUM. Thursday, 18:20 BS-4

Recent advances in B. thuringiensis physiology and infection features
Chen, X. 1, Jin, L. 1, Peng, Q. 1, Zhang, J. 1, Song, F 1
Candela, T. 2, Gilois, N. 2, Nielsens-Leroux, C. 2, Lereclus, D. 2, Gojar, M. 2

SYMPOSIUM. Thursday, 18:40 BS-5

Bacillus thuriingiensis: 50 years of safety to vertebrates
Raymond, B. 1, Federici, B. 2

SYMPOSIUM. Thursday, 17:00 MC-39

Efficacy of Beauveria bassiana strain ATCC 74040 (Naturalis®) against the leafhopper Scaphoideus titanus Ball under open-field conditions
Ladurner, E.; Benuzzi, M.; Fiorentini, F.

SYMPOSIUM. Thursday, 17:15 MC-40

Efficacy test of entomopathogenic fungi for controlling chili thrips (Scirtothrips dorsalis)
Panyasiri, C.; Veeranondh, S.; Supothina, S.; Chanthakhet, R.; Boonruangprapa, T; Vichai, V.

SYMPOSIUM. Thursday, 17:45 MC-42

Effects of entomopathogenic fungi against the crapemyrtle bark scale and its natural enemies
Franco, G. M. 1; Chen, Y. 2; Diaz, R. 1

SYMPOSIUM. Thursday, 18:00 MC-43

Apples and oranges: Standard non-target tests are unsuitable for entomopathogenic fungi - a proposal for a new guideline
Reinbacher, L. 1,2; Bacher, S. 2; Grabenweger, G. 1

SYMPOSIUM. Thursday, 18:15 MC-44

Towards Dactylopius opuntiae (Cockerell) (Hemiptera: Dactylopiidae) biological and integrated management in Cadiz Province (Spain)
Yousef-Naef, M.; Quesada-Moraga, E.

SYMPOSIUM. Thursday, 18:30 MC-45

Fitness effects of the newly-discovered microsporidian species Tubulinosoma sp. on its host Drosophila suzukii
Biganski, S.; Jehle, J.A.; Kleespies, R.G.

SYMPOSIUM. Thursday, 18:45 MC-46

Simplifying insect pathology by means of the Foldscope
Sreerama Kumar, P

DBI SYMPOSIUM
Thursday, 17:00-19:00
Commission R8

Emerging Diseases in Invertebrates as One Health Sentinels
Organisers / Chairs: Helen Hesketh / Grant Stentiford

SYMPOSIUM. Thursday, 17:00 DBIS-1

Invertebrate health as a sentinel of global ‘One Health’
Stentiford, GD

SYMPOSIUM. Thursday, 17:30 DBIS-2

Crustaceans as Models for Understanding the Unique Application of One Health to a Changing Sea
Behringer, DC.
SYMPOSIUM. Thursday, 18:00 DBIS-3
Exploring the transmission of diseases between pollinators at flowers
Bailes, EJ

SYMPOSIUM. Thursday, 18:30 DBIS-4
The contribution of extension services to the monitoring of crop pests and to the uptake of augmentative biocontrols in selected low to lower-middle income countries
Edgington, S
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Lessons told by nature: Resistance of codling moth against Cydia pomonella granulovirus and its implications for environmentally sound pest control

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Cydia pomonella granulovirus (CpGV) is highly virulent for larvae of codling moth (Cydia pomonella, L., CM). 40 years ago, it had been first registered as biological control agent, whereas today it is worldwide used in countries with organic and integrated pome fruit production. In recent years more than 45 orchards with CM populations resistant to CpGV products have been identified in Europe, providing a unique large-scale field experiment of virus-host adaptation. By genetic analyses and resistance testing we identified three types of resistance (type I-III), which differ strongly in their susceptibility to CpGV isolates from different phylogenetic lineages, as well as their inheritance patterns. With the registration of resistance-breaking CpGV isolates, type I resistance could be well managed during the last decade, though there are new challenges by the emergence of more cases of type II resistance in the last couple of years. Thus, the genetic diversity of CpGV plays an eminent role to overcome CpGV resistance. By sequencing the genomes of more than 20 CpGV isolates and virulence testing, it was possible to correlate molecular characteristics with virus activity. Mapping of insertion/deletion (indel) mutations and single nucleotide polymorphisms (SNPs) in CpGV genomes gained in-depth insight into the structure and variability of CpGV. Understanding the host mechanism of resistance is the most crucial to improve the efficacy of CpGV isolates and to advance resistance management strategies.

Sand crickets and mole crickets are resistant to entomopathogenic nematodes and their bacteria

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The entomopathogenic nematode (EPN) Steinernema scapteriscus is a specialist parasite of crickets and has been successfully used in classical biological control applications against the southern mole cricket, Neoscapteriscus borellii. EPNs cause rapid death of their insect hosts and use bacteria to facilitate their parasitism, however our understanding of the relative contributions of nematodes and their bacteria are limited. We utilized the sand cricket, Gryllus firmus, and the mole cricket N. borellii, to explore the relative contributions of the specialist parasite S. scapteriscus and its bacterial symbiont Xenorhabdus innexi, as well as the closely related generalist S. carpocapsae and its symbiont X. nematophila to pathogenicity against cricket hosts. We found that both cricket species were highly resistant to infection by the nematode-associated bacteria. We also found that G. firmus is highly resistant to infection by EPNs associated with their bacteria. Our data provide evidence that unlike other EPNs, the virulence of S. scapteriscus to crickets is dependent on the nematode rather than the bacterial symbiont it carries and we speculate that S. scapteriscus may be evolving independence from X. innexi.
Effect of the peritrophic membrane on toxicity of Bt Cry toxins in insects

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The peritrophic membrane (or peritrophic matrix) (PM) is a unique extracellular chitin-protein structure lining the midgut epithelium in most insects. The PM is generally known for its function to facilitate the food digestion process, and to protect the midgut epithelium against physical injury, chemical toxicity and microbial infections associated with food ingestion. It is a physical barrier to microbial pathogens that initiate infection through the midgut as the portal of entry. Insecticidal toxins from Bacillus thuringiensis (Bt) are critical pathogenic factors of Bt strains in insects and Bt Cry toxins are widely used as the insecticidal toxins in transgenic plants. Bt Cry toxins exert toxicity in insect midgut by targeting the midgut epithelial cells, which can only be reached by passage of the toxins across the PM. The effect of the PM on the toxicity of Cry toxins in insects remains to be understood. In this study, the effect of the PM on the toxicity of the Bt toxin Cry1Ac in the cabbage looper, Trichoplusia ni, was examined using Bt-susceptible and Bt-resistant T. ni strains and different treatments of T. ni to alter the PM in larvae. Our results reveal the protective function of the PM to Bt toxins.

Entomopathogenic virus – insect gut interactions, role of the peritrophic matrix in infection dynamics.

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The peritrophic matrix (PM), an acellular chitin and glycoprotein layer that lines the invertebrate midgut, has long been considered a physical as well as a biochemical barrier that protects the midgut epithelium from abrasive food particles, digestive enzymes and pathogens infectious per os. The evidence for the role of the PM in defense against viral infections includes: developmental resistance related to changes in PM structure, the impact of disruption of the PM structure with chemical agents, such as calcofluor white, that bind chitin and inhibit PM formation, disruption of the PM structure by pathogen-encoded enzymes including viral enhancins and the identification of strains and mutants of host species that demonstrate increased or decreased resistance to pathogen infection related to changes in PM structure. Certain strains of both alpha- and betabaculoviruses have been demonstrated to have several auxiliary genes whose encoded proteins likely target the PM. Enhancin genes have been found in both alpha- and betabaculoviruses and encode metalloproteases that target and degrade specific chitin binding glycoproteins, insect intestinal mucins, that are key structural proteins of insect PMs. ENHANCINs have been shown to significantly increase baculovirus infection of insect midgut epithelial cells. Similarly, some entomopoxviruses have been shown to contain genes encoding FUSOLIN, a protein that has demonstrated chitin binding activity and that enhances the infectivity of both entomopoxvirus strains and baculoviruses. An orthologue of ENHANCIN, GP37, has also been identified in baculoviruses and some of these have been shown to have chitin binding activity and may also have a role in increased gut infectivity of these viruses.

Insect immunity as affected by stress factors

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Insect immune barriers are suppressed by invading parasites and pathogens, which have developed effective virulence strategies, as a result of a long co-evolutionary process. The functional bases of these virulence strategies are significantly influenced by the associated microbiota and can be correctly studied only at metaorganism level. However, the immune response is not only modulated by these biotic stress factors, but is also conditioned by several abiotic stressors. Among these, poor nutrition and pesticides play an important role. In particular, neurotoxic insecticides are able to interfere with the cross-talk between the nervous and immune systems, often separately considered. Unveiling the regulatory mechanisms of these physiological networks paves the way towards the development of new bioinspired strategies for pest control and pollinator protection.
Pathogens associated with invasive or introduced insects threaten the health and diversity of native species

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The decline in global insect biomass over the last few decades, even in protected areas, has attracted great scientific and public attention, but the relative contribution of various postulated mechanisms is controversial. Advanced tools for the diagnosis of environmental stressors such as the presence of pesticides or climate change weaken the resistance of indigenous host species in an additive, potentiating or synergistic manner. Advanced tools for the diagnosis of entomopathogens in insects farmed for biological control, pollination services or mass rearing in insect farms threaten the health and diversity of indigenous insects by spreading co-introduced entomopathogens whose spillover is difficult to control. The increasing prevalence of even weakly virulent pathogens or covert infections can result in their accumulation in native host insect species. In the latter they can become lethal if and/or introduced insects transmitting pathogens to less-resistant native species. Most, if not all, insects carry pathogens and parasites to which they have evolved resistance, or which are tolerated as a consequence of host–parasite coevolution. Insect species traded by humans and introduced into new areas for biological control applications, pollination services or mass rearing in insect farms threaten the health and diversity of indigenous insects by spreading co-introduced entomopathogens whose spillover is difficult to control. The increasing prevalence of even weakly virulent pathogens or covert infections can result in their accumulation in native host insect species. In the latter they can become lethal if environmental stressors such as the presence of pesticides or climate change weaken the resistance of indigenous host species in an additive, potentiating or synergistic manner. Advanced tools for the diagnosis of entomopathogens in insects farmed for biological control, pollination services or bioconversion must therefore be developed in order to control and prevent the spillover of devastating pathogens into indigenous insect populations.


Gene diversity-mediated characterization of biological features in entomopathogenic Beauveria bassiana

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Beauveria bassiana is the most widely studied species of entomopathogenic fungi for its high potential as a biological pesticide. However, little is known about the factors that might drive the genetic diversity among various isolates even though this fungus has been reported to be a heterogeneous assemblage of strains. In this work, we aimed to study the gene diversity of 42 isolates in order to figure out the impact of genes’ sequences variability on various biological features. For this purpose, we analyzed sequences of genes involved in various mechanisms of the fungus infective cycle. Afterwards, we characterized the isolates’ biological features including virulence against two different insect species, growth rate and thermotolerance. The accumulated data were used to check whether the gene diversity is correlated with the geographic localization, virulence, growth or stress response of the studied isolates. No correlation between the sequence variability of the internal transcribed spacer and the fungal geographic localization. Meanwhile, weak to strong correlations have been obtained between the sequence diversity of various genes, represented as non-synonymous changes per base pair, and the different studied biological features. Interestingly, we could detect host-specific virulence genes. In addition, Biotrophy-associated gene 2 variability was correlated with all the studied biological features, being an important marker to determine isolates of interest for biological control. Therefore, the obtained data would serve as a database to focus on interesting genes related to improving or screening highly virulent isolates as biological control agents.

SYMPOSIUM. Monday, 15:50 BVCS-5

SYMPOSIUM. Monday, 16:10 BVCS-6

Invited contribution for the cross-divisional symposium (Bacteria and Virus) organized by Umut Toprak and Salvador Herrero

Parasitic manipulation of insect behaviour

Yue Han, Simone Gasque, Hans M. Smid, Vera I.D. Ros

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Modification of host behaviour is a widely adopted strategy of parasites to enhance their own transmission. Examples of behavioural manipulation are rapidly accumulating, covering a broad spectrum of parasites and hosts. Nevertheless, surprisingly little is known on the underlying causative physiological, neuronal, hormonal or molecular mechanisms. Given the high prevalence of parasites in insects, either in a pathogenic interaction or in a parasite-vector relationship, parasites might have a huge impact on insect behaviour. I will review some well-known examples of modified insect behaviour following infection by viruses or bacteria and will discuss what is known on the underlying mechanisms. Furthermore, I will focus on a well-studied example of viral manipulation of insect behaviour, concerning caterpillars 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. Recent studies reveal that there is no single mechanism underlying baculovirus-induced behavioural manipulations and that baculoviruses may exploit existing behavioural pathways to achieve behavioural changes.

SYMPOSIUM. Monday, 16:10 BVCS-6

Isolation and characterization of microsatellites of the entomopathogenic fungus Metarhizium rileyi (Ascomycetes: Hypocreales)

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The entomopathogenic fungus Metarhizium rileyi is a prevalent natural control agent of economically important Noctuid larvae. It controls several insect species in an epizootic fashion, including velvetbean caterpillar (Anticarsia gemmatalis), soybean loopers (Chrysodeixis includens, Rachiphusia ru), and cotton leafworm (Alabama argillacea) among others. Despite its importance, no microsatellites studies involving this fungus have been reported to date. Microsatellites are powerful markers for population analysis and ecological interrelationship studies. We isolated 10 polymorphic loci with potential to discriminate genotypes and perform population studies. The microsatellite loci were obtained from shotgun sequencing and screening of M. rileyi whole genome sequence. The genome was assembled de novo using paired-end sequencing libraries (insert size 300 bp–3 kb), resulting in 311 scaffolds, with a total length of 31,007,635 bp. All scaffolds were screened using MicroSAtellite identifi cation tool (MISA). In silico tests with other sequences available revealed 1,440 loci likely to be polymorphic and 85 of them were selected for experimental validation using PCR amplifications. We successfully amplified 10 polymorphic loci, ranging from 130 bp to 550 bp (minisatellites). Repeat motif corresponded to the following sequences: (A)12, (T)18, (A)11, (A)12, (C)47, (T)62cagtctt(TG)18, (GGGCA)9, (CA)7nnnnn(ACA)10, one

CONTRIBUTED PAPERS. Monday, 14:30 F-1

CONTRIBUTED PAPERS. Monday, 14:45 F-2

Insolation, diversity and ecology

Chairs: Inmaculada Gamito-Jurado / Claudia López-Lastra
complex trinucleotide repeat motif, and one complex tetranucleotide repeat motif. Due to the high polymorphism, these loci are very useful for population diversity studies, as well as for characterizing and tracking naturally occurring M. rileyi strains. The description of these loci for M. rileyi makes microsatellite-based population genetic studies feasible.

ENTOMOPHTHOROMYCETES

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Preliminary analysis of genetic variability of Metarhizium isolates from Parco del Ticino (Northern Italy).

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Popillia japonica (Pj) was reported for the first time in Italy and in mainland Europe in 2014. The first outbreak was located along the Ticino river, within the Ticino Valley Natural Park between Lombardy and Piedmont. Since then it gradually spread in the territory causing much concern among farmers and the general public.

In the infested area along the Piedmontese bank of the Ticino river, 155 soil samples were taken in 2017 in fields with high concentration of Pj larvae to verify the presence of indigenous entomopathogenic fungi. A total of 79 Metarhizium, 7 Beauveria and 7 Paecilomyces isolates were obtained.

In a first attempt to investigate genetic variability among collected isolates, 11 Metarhizium isolates were selected on the basis of morphological differences among cultures on PDA medium and analysed using 15 SSR markers. The isolates (5 M. robertsi, 4 M. brunneum, 1 M. guizhouense, 1 M. lepidotae) were found to belong to 9 different genotypes, of which one was shared by 3 M. brunneum isolates. Preliminary pathogenicity tests performed with these 11 isolates on third-instar Pj larvae, yielded variable results with efficacy percentages ranging from 34 to 95% mortality. Interestingly, the three isolates of M. brunneum belonging to the same genotype showed different efficacy percentages of 57, 64 and 84%. Furthermore, the two isolates belonging to M. guizhouense and M. lepidotae revealed lowest efficacy (35% and 34%, respectively) of all tested isolates, while one isolate of M. brunneum and two of M. robertsi almost reached or exceeded 90%.

ENTOMOPHTHOROMYCETES

Diversity of the entomopathogenic fungus Metarhizium spp. in soils of three different land-use types

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The Entomopathogenic Fungi of the genus Metarhizium spp. are important natural antagonists of arthropods. Due to their function, they have a high potential for use in biological control strategies. Presence and abundance of these fungi in the environment depend on many abiotic and biotic factors. In an agricultural context the land-use type, including factors like crop-type, -management and -protection may affect their presence and population structure. The goal of this study was to investigate abundance and diversity of Metarhizium spp. in three land-use types (arable-land,
permanent grassland and forest), and to assess how *Metarhizium* spp. population structure is affected by land-use type and/or various physical, chemical and microbiological parameters. The study was performed at 30 sites that are part of the national soil-monitoring network (NABO) in Switzerland, in which soil physical, chemical and microbiological parameters are monitored since 1984. In 2016, a total of 349 *Metarhizium* isolates were obtained from three bulk soil samples per site using a selective medium (SM). The multi-locus genotype (MLG) was determined for each isolate applying 15 microsatellite markers. *Metarhizium* spp. were present in 80%, 100% and 40% of the arable-land, grassland and forest sites, respectively. Twenty-four MLGs were detected among the 349 isolates and 14 MLGs were identified as *M. brunneum*, 7 as *M. robertsi* and 3 as *M. guizhouense* (259, 80 and 10 isolates). Multivariate statistical analyses revealed that environmental factors, such as “C:N ratio”, “bulk density” and “organic carbon” in soil, among others, significantly affect *Metarhizium* communities in the three land-use type.

**CONTRIBUTED PAPERS.** Monday, 16:00 F-7

**Occurrence and characterization of *Metarhizium pingshaense* infecting shoot borer, *Conogethes punctiferalis***

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*Conogethes punctiferalis* is a highly polyphagous pest recorded from more than 120 plants, distributed widely in Asia and Australia. The insect causes significant yield loss in Zingiberaceous spice crops like ginger, turmeric and cardamom and so far no entomopathogens have been recorded against this pest. During surveys, an entomopathogenic fungus was isolated from infected insects of *C. punctiferalis* and identified as *Metarhizium pingshaense* Q.T. Chen & H.L. Guo. (Ascomycota: Hypocreales) based on morphological characteristics and molecular studies. Sequence similarity of the partial sequences of ITS, TEF, RPB1, RPB2 and APN2 genes and phylogenetic analysis confirmed its identity as *M. pingshaense*. Bioassay studies with purified conidial suspension of the fungus indicated its potential mycoinsecticide against this pest.

**CONTRIBUTED PAPERS.** Monday, 16:15 F-8

**Updated checklist of Entomophthoralean fungi from Argentina**

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Entomophthoralean fungi includes a great diversity of species that predominantly infect insects. The previous reports on the subject recorded for Argentina were focused on taxonomy, biodiversity, epizootiology and some laboratory evaluations of biological activity of the species *Batkovia* sp., *Conidiodobolus coronatus*, *C. obscurus, Entomophaga gylli*, *E. planchoniana*, *E. ferinandii*, *Neozygites sp.*, *N. fresenii*, *Pandora sp.*, *P. delphacis*, *P. digiterigina*, *P. gamm,ae, P. neoaphidis*, *P. nouyi*, *Zoophthora* sp. and *Z. radicans*. The objective of this research was to further update and adding new records to Argentinean Entomophthoralean fungal species as their geographical distribution. Our survey includes five new records of *Zoophthora radicans* infecting insect hosts belonging to the orders Diptera, Hemiptera, and Lepidoptera from Pampasic and Litoral regions of Argentina. These and other new records, not only increase the host range of Entomophthoralean fungi, but also their geographical distribution around the world. The overall goal of this work was to follow this endeavor and further update the knowledge of Argentinian Entomophthoralean fungal species with new records that expand their insect host range and their geographical distribution.

**CONTRIBUTED PAPERS.** Monday, 14:30 MC-1

**Defining the genomic drivers of evolution in the entomopathogenic *Serratia* spp.**

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Various strains of *Serratia entomophila* and *S. proteamaculans* (Yersiniae) can cause disease specific to the New Zealand pasture pest, *Costelytra zealandica* (Scarabaeidae). Isolates of *S. proteamaculans* have been found to cause a variety of pathotyes (chronic to hypervirulent) in grass grub larvae and another endemic scarab larva (*Pyronota* spp.). Virulence determinants for both pathotypes reside on variants of a large plasmid, pADAP. While the disease-encoding genes are understood, the mechanism driving retention of chronic virulence determinants in *S. entomophila* and a diversity of virulence determinants in *S. proteamaculans*, compounded by the presence of conspecific non-plasmid bearing strains, remain to be elucidated. Through assessment of 92 isolates we found chromosomes of *S. proteamaculans* were heterogeneous, while *S. entomophila* pADAP bearing isolates shared a conserved genome, evidence that the chromosome may favour a chronic state. Roary analysis of plasmid-free *S. entomophila* strains found differences suggesting their pADAP bearing counterparts have undergone speciation, whilst Mauve showed chromosomal inversions on isolates from areas where amber disease has not been documented. Bioinformatics and in vitro assays assessed the virulence potential of *Serrata* spp. strains and correlated with geographic/phenotypic data. The expression of virulence determinants demonstrated a link between up-regulation of various factors and hypervirulent isolates. In silico assessment found chronic strains to encode less secondary virulence determinants (chitinases, proteases).

Novel gene clusters unique to *S. entomophila* were identified with implications to bacterial metabolism, indicating a shift to a chronic disease state. By utilising bacterial mutagenesis, we aim to understand the fitness implications of these regions.

**CONTRIBUTED PAPERS.** Monday, 14:45 MC-2

**Biofilm regulatory genes affect biofilm formation and UV resistance of *Bacillus thuringiensis* through complex pathways**

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*Bacillus thuringiensis* (Bt) has become one of the most widely used and most successful microbial insecticides in the world. However, there is a serious problem that Bt and its ICPs sprayed on crops have relative short duration periods under sunlight, which seriously hinders the long-term
utilization of Bt. Compared with the free-living state, the bacterial biofilm (BBF) state tends to have higher anti-UV ability, better environmental adaptability, and stronger drug resistance. Thus, BBF may be helpful to improve the UV resistance of Bt and then prolong the field duration of Bt. In our lab., the functions of several novel biofilm regulatory genes affecting biofilm formation and UV resistance of Bt were elucidated by bioinformatics, gene knockout, BBF-related phenotypes and comparative proteogenomics. Our studies comprehensively evaluated the phenotypic change of bacterial biofilm induced by the biofilm regulatory genes, and revealed their mode of actions by comparative proteogenomics. Our newly constructed regulatory networks for BBF biofilm would certainly lay a foundation for constructing highly UV-resistant engineering biofilms based on BBF biofilm.

CONTRIBUTED PAPERS. Monday, 15:00 MC-3

Resistance to dsRNA and cross-resistance to Cry3Aa in Colorado potato beetle (Leptinotarsa decemlineata)  
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In the last decade, silencing of vital genes by RNA interference (RNAi) through dsRNA ingestion has been identified as a revolutionary bioinsecticide technology. As commercialization of insecticidal dsRNA technology approaches, it becomes crucial to develop resistance management tools for the sustainability of this technology. Using chronic exposure through larval development, we developed a population (CEAS) of Colorado potato beetle (CPB), that is >5,000-fold resistant to insecticidal dsRNA. In this work we share findings from research focused on the identification of candidate resistance mechanisms and cross-resistance to Cry3Aa, the most active insecticidal protein from Bacillus thuringiensis against CPB. Bioassays and comparison of dsRNA stability in digestive fluids from susceptible and resistant CPB support cross-resistance to alternative dsRNA targets and that degradation of dsRNA by nucleases is not involved in resistance. Monitoring uptake of fluorescently-labeled dsRNA by midgut cells using confocal microscopy supports reduced uptake of dsRNA in midgut cells of CEAS compared to susceptible larvae. Results from this project will guide development of Insect Resistance Management (IRM) strategies for insecticidal RNAi and its combined use with insecticidal proteins from B. thuringiensis against CPB and will allow the optimization of insecticidal RNAi technology.

CONTRIBUTED PAPERS. Monday, 15:15 MC-4

Is oligomerization an important step in toxicity of the Bacillus thuringiensis insecticidal protein Cry11a?  
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Bacillus thuringiensis synthesizes structurally similar three-domain (3-D) crystal proteins (Cry) that accumulate in crystal inclusions, with the exception of Cry11 toxins, that are secreted. The formation of an oligomeric structure in the mode of action of the 3-D Cry proteins has been described as a major step necessary for midgut cell pore formation in the toxicity process. Considering the unique features of Cry11 proteins, a clear understanding of their mode of action is critical for enhancing and sustaining their efficacy. Nevertheless, the Cry11 oligomer formation has not been yet experimentally addressed. In this study, for the first time, the formation of Cry11a oligomers has been investigated, using Cry1Ab as a control. The insecticidal activity of Cry11a and Cry1Ab proteins has been assessed against two lepidopteran (Lobesia botrana and Ostrinia nubilalis), and a coleopteran (Leptinotarsa decemlineata) insects. To elucidate the association between oligomerization and Cry11a toxicity, the oligomer formation has been studied after incubation of Cry11a with brush border membrane vesicles (BBMV) of susceptible insects and non-susceptible cultured insect cells. Based on the bioassay experiments, Cry11a protein resulted highly toxic for all selected insect species, whereas Cry1Ab was only toxic for the lepidopteran hosts. Our results showed that while Cry1Ab forms oligomers regardless to its host susceptibility, Cry11a toxin forms oligomers only after incubation with coleopteran susceptible host BBMV. Based on our findings, oligomerization may not be widely generalized in the mode of action of all the Bt 3D Cry proteins.

CONTRIBUTED PAPERS. Monday, 15:30 MC-5

Pseudomonas protegens CHAO transcriptome changes in response to root- and insect associated lifestyles  
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The Pseudomonas fluorescens group harbours bacteria which promote plant-growth and control soil-borne fungal pathogens. Individuals from the P. chlororaphis and P. protegens species are also able to colonize the insect gut, invade the hemocoel, cause systemic infection and eventually kill them as shown for several insect species. Diverse factors have been demonstrated to be important for the insecticidal activity in these bacteria e.g. the Fit toxin, antimicrobials, chitinases, and the Type VI secretion system. However, it is unknown how colonization and pathogenesis progress within the insect and what functional traits determine insect- or root-associated lifestyles. Therefore, we decided to perform an RNA-sequencing to determine niche-specific transcriptomes of P. protegens CHAO by identifying differentially expressed genes in plant-root and insect backgrounds. Then, we aimed to investigate the transcriptome changes during insect colonization and the infection process. Consequently, we compared transcriptomes of P. protegens CHAO colonizing wheat-roots, Plutella xylostella after oral infection, and Galleria mellonella hemolymph after hemocoel injection. Our results showed expression of different genes sets under each of the examined conditions. Hence, P. protegens CHAO transcriptome changes to beneficial or pathogenic interactions depending on the host. Key-genes related to biofilm production are downregulated while hemolysines, related to the passage from gut to the hemocoel, are upregulated in P. xylostella compared to wheat-roots. In G. mellonella hemolymph, iron-acquisition seems to be important for the persistence and multiplication of the bacteria. Altogether, these findings increase our knowledge on host colonization and pathogenesis mechanisms of Pseudomonas which will be important for future biocontrol applications.

CONTRIBUTED PAPERS. Monday, 15:45 MC-6

Fear no weevil: searching the microbiome for a sweetpotato weevil biocontrol agent  
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AgBiome, utilizing its innovative microbial capture and screening program, developing a microbial solution to control sweetpotato weevils (Cylas spp.) in Sub-Saharan Africa. The sweetpotato weevil is the most important insect pest of sweetpotatoes, causing 60-100% loss in the developing world if untreated. It is especially devastating for smallholder farmers who apply little to no inputs. Microbial control of sweetpotato weevil is a promising solution, with low exposure risk, economic feasibility, and the potential to offer season-long control through inoculation of host plants. In collaboration with researchers at NaCRRI in Uganda, we have isolated and fully sequenced the genomes of over 8,000 bacterial strains collected from US and Ugandan sweetpotato environmental samples. We have identified more than 80 bacterial strains with coleopteran activity from these isolates. These were tested in the laboratory using a novel larval sweetpotato weevil bioassay developed at Louisiana State University and several have shown activity. Additionally, we have identified over 15 fungal strains with activity on sweetpotato weevil adults. As we begin the next stage in product development, we will optimize the fermentation of fungal strains with activity on sweetpotato weevil larvae. As we begin the sweetpotato weevil bioassay developed at Louisiana State University, we will explore the potential of Pp/Pc applied alone or in combination with other biocontrol agents such as entomopathogenic bacteria, fungi or invertebrates such as hyperparasitic nematodes. We have previously reported the discovery of a new family of potent insecticidal proteins from bacteria. Efficacy has been shown against several insects in the lepidopteran family in both corn and soy. Here we will discuss new findings regarding the effects of these proteins on insect midgut cells.
scavengers. We discuss the results in the context of ecological theory and secondary metabolite production by the two species.

COMPETED PAPERS. Monday, 14:45 N-2

Competition between steinernematid nematodes and facultative parasite Oscheius myriophila: do some Xenorhabdus strains kill nematodes?

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Recent studies revealed that facultative insect parasitic nematodes of the genus Oscheius often co-invade insect cadavers together with entomopathogenic nematodes. This raised a question whether these nematodes could negatively affect EPN populations especially in agroecosystems, where the EPN populations are less abundant. Studies focused on the competition between EPNs and Oscheius nematodes from the “tipulae” clade have shown that the effect on EPNs is very low. In present study we focused on the competition of steinernematid species with Oscheius myriophila, a representative of the “insectoria” clade, in live and dead hosts. Furthermore, we observed the effect of 37 strains and species of Xenorhabdus spp. on the survival and reproduction of O. myriophila in the whole cell supernatant and on the wads agar plates. The same experiment repeated with a free living model nematode species C. elegans. The selected most effective bacteria strains were tested against the plant parasitic nematode Globodera rostochiensis.

COMPETED PAPERS. Monday, 15:00 N-3

Drivers of assemblages of entomopathogenic nematodes and other soil organisms from the same habitats on two continents: singularities or general trends?

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Entomopathogenic nematodes (EPNs) are widely distributed in soils throughout the world. Regional surveys provide critical biogeographical data reflecting the EPN distribution on various continents. If linked to soil parameters, habitat, and natural enemies, surveys can provide information on biotic and abiotic properties that modulate EPN activity as biological control agents. Few studies provide comprehensive data that reveal whether the observations are merely unique or whether they reflect general trends in EPN natural occurrence. Here we compare the occurrence of EPNs, associated soil organisms and soil properties for two distinct climatic regions: Subtropical (Florida, USA) and Mediterranean (Algarve, Portugal). In both studies, soil samples were recovered from four habitats (citrus groves, palmetto areas, oaks and pines), and species of EPN, nematophagous fungi and free-living nematode competitors of EPN were characterized by real time qPCR approaches. We observed consistency in the responses of EPN soil food web assemblages in both regions. Soil pH and variables related to water content appeared to be the main drivers. Additionally, cultivated perennial habitats favoured EPN occurrence, as well as that of soil organisms that can limit EPN activity as biological control agents. Additional comprehensive studies are required to compare these patterns with those of other habitats or climatic regions. Meta analyses of surveys comprising similar methods will provide insights involving key questions such as potential tactics for conservation biocontrol and the changing role of EPNs in the paradigm of climate change.

COMPETED PAPERS. Monday, 15:15 N-4 STU

Effect of sounds emitted by the red palm weevil Rhynchophorus ferrugineus on the foraging behavioral and molecular response of entomopathogenic nematodes

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Most studies on attraction of Entomopathogenic Nematodes toward its host concentrated on chemical stimuli. We determined the effect of chewing sounds emitted by the red palm weevil larvae on the movement of infective juveniles (IJs) Heterorhabditis bacteriophora and Steinemema carpocapsae toward the sound source. In the presence of insect larvae, the majority of the IJs of both species moved toward larvae, whereas, recorded sound emitted by the insect showed an inhibition effect on their foraging behavior. However, when nematodes exposed to the larvae and the recorded sounds at the same time, they moved toward the stimuli. RNA-Seq data suggested that more genes were downregulated in S. carpocapsae than in H. bacteriophora. The neural basis for the directional movement based on chemosensation of the larvae and mechanosensation of recorded sounds varied greatly between the two species. Many neuropeptides and other neurromodulators are involved in regulating the foraging behavior of H. bacteriophora, but the foraging behavior of S. carpocapsae is regulated differently. The movement of nematodes relative to a potential host involves Ras/MAP kinase, TGF-beta signaling, insulin signaling, AMPK signaling, PPAR signaling pathways and other developmental pathways. The examined treatments had a stronger effect on the regulation of the MAPK, calcium and neuropeptide signaling pathways in S. carpocapsae than in H. bacteriophora. Similarly, changes in the expression of mechanosensory genes, TRP channels and touch-sensory regulation were more pronounced in S. carpocapsae than in H. bacteriophora. The results of this study provide new insights into the molecular mechanisms that allow nematodes to seek insect hosts.

COMPETED PAPERS. Monday, 15:30 N-5 STU

The lure of hidden death: Attractive volatile organic compounds to wireworms towards entomopathogenic nematodes

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Most of the research on the interactions between insect herbivores and plants focus on the aboveground parts, but there is a growing interest in belowground plant-insect interactions. Like most soil dwelling pests, wireworms use Volatile Organic Compounds (VOCs) released from the rhizosphere to locate a suitable host. It has been proposed that specific VOCs can be used in attract-and-kill strategies with biological alternatives to pesticides. In order to develop such an attract-and-kill strategy, we aimed to (1) identify VOCs from maize roots that are particularly attractive to wireworms and (2) select an entomopathogenic nematode (EPN) that readily infects and kills wireworms. Field observations have revealed considerable differences between two maize varieties in infestation levels by wireworms. We identified the VOCs from their roots and found that the
less susceptible variety released a more complex VOC blend than the other. Two VOCs, hexanal and β-carophyllene, were found in the VOC profiles of maize and potatoes, and were tested for attractiveness in olfactometer assays. We are also testing the combination of these compounds in alginate beads containing EPN for attractiveness and biocidal effects under laboratory conditions. Using VOCs as attractants and EPN as biological agents represent a promising alternative to pesticides that remains to be evaluated in the field.

CONTRIBUTED PAPERS. Monday, 15:45 N-6

Do all Xenorhabdus and Photorhabdus bacteria protect nematode infected cadavers against scavengers?

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Identification of entomopathogenic nematodes in Central Anatolia with a comparison of two barcoding loci

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CONTRIBUTED PAPERS. Monday, 16:15 N-8 STU

Divergence from the PDV paradigm in the repeated evolution of associations between mutualistic viruses and parasitoid wasps

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CONTRIBUTED PAPERS. Monday, 17:00 V-1
sequence of the entomopoxvirus found in *Diachasmimorpha longicaudata* venom glands (DIEPV) revealed that this virus is non-integrated and can replicate in host insects. Although the DIEPV genome differs in architecture relative to PDVs, viral transcriptome analysis shows that virulence and replication genes are partitioned in wasps and hosts at the level of expression, functionally behaving in a PDV-like manner. We have also discovered an independently-derived endogenous viral symbiont in *F. arisanus* which produces virus-like particles that do not contain nucleic acids. Despite a very young association with wasps in the genus *Fopiius*, genome rearrangement has already occurred for this virus symbiont, highlighting the adaptive advantage of this process. These data highlight the diversity of viral symbiosis strategies and variation in genome architecture, which has implications for symbiont function in hosts.

The invasive insect pest *Drosophila suzukii* infests ripening fruits and causes extensive damage to crops in the northern hemisphere. Novel, pathogenic viruses for pest control. Here we report the identification and characterization of two natural viruses associated with *D. suzukii*: Drosophila A virus (DAV) and La Jolla virus (LJV). Our work provides new tools for the development of biological control agents that protect crops against *D. suzukii* without a harmful impact on biodiversity. In order to study the novel viruses in greater detail, we aimed a protocol to establish a primary hemocyte culture from the larval stages (L2 and L3) of *Drosophila suzukii*. Isolated cells were cultured at 28°C in Grace’s insect medium and cell description was done according to morphological characteristics. We succeed to obtain hemocytes and offer a tool to gain insight into insect hemocyte-mediated responses such as phagocytosis, and encapsulation, helping to facilitate the development of new pest control strategies for *D. suzukii*.
Invertebrate animals are regarded as a major reservoir of viral genetic diversity, they harbor diverse RNA viruses. Understanding the virus diversity in rice invertebrate species is important for managing the rice invertebrate population and spread of rice viral disease. However, little is known about how many viruses exist in rice invertebrates. In this study, we collected 20 major invertebrate species, including insects, spiders, snails, and other invertebrates from rice fields in major regions of rice production in China. To explore the diversity of potential RNA viruses pathogenic to invertebrates or rice, we sequenced all the RNA viruses in the creatures through a metatranscriptomics analysis, our results revealed complete genomes of 65 species of novel RNA viruses from a diverse range of viral families and orders, including fifteen negative-sense RNA viruses, forty-two positive-sense RNA viruses and eight putative double-stranded RNA viruses. Our results provide important insights into the diversity of RNA viruses in rice invertebrate species.
taneous control measures are desperately needed in organic as well as in conventional potato production. We developed an innovative capsule system, based on a biological attract-and-kill strategy against these soil dwelling insect pests. Larvae are attracted by the emitted CO₂ produced by the capsules and killed by an isolate of the entomopathogenic fungus *Metarhizium brunneum* growing out of the capsules. Field applications on farmer fields and specific experiments have predominantly yielded efficacies on average above 50%; however outliers downwards have been observed as well. In order to better understand the reasons for the variable efficiency ranges, different production and application strategies (capsule amount, shelf life), biotic (wireworm species composition) and abiotic parameters (soil climate) have been investigated in experimental fields. Results identified soil temperature and soil moisture in the first day and weeks after capsule application as two crucial factors positively correlating with the efficiency of the Attract & Kill strategy. These data provide valuable insight to further optimize the application and formulation of the capsules. Parameters that can be influenced in this regard will be discussed.

**SYMPOSIUM. Monday, 18:00 MFCS-3**

**Wireworm biocontrol – an open field of opportunity in biology and agriculture**

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Throughout the 20th century, periodic epizootics of wireworm infection by fungal pathogens inspired investigations into opportunities for wireworm biocontrol. Follow-up studies tended to be short-lived after lab or field applications did not cause adequate levels of disease, or shifted toward biological investigations in lieu of those aimed at crop protection. Research in the 21st century, however, is integrating basic biological investigations with agronomic application, leading toward the more comprehensive understanding necessary in biocontrol systems. The study of a range of biotic (e.g. fungal strain, competing organisms, root respiration) and abiotic factors is elucidating efficacy expectations and the understanding of outcomes when using *Metarhizium brunneum* for wireworm and click beetle control. Discovering the effect of these factors is providing new biological information while enabling expanded use of this fungus to protect crops from pestilent wireworms.

**SYMPOSIUM. Monday, 18:30 MFCS-4**

**Microbial control of wireworms in cover crops – is this the road to success?**

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Wireworms are the soil dwelling larvae of click beetles (Coleoptera: Elateridae) that may cause substantial losses in marketable yield of potatoes by feeding on and tunnelling through the tubers. Control options are limited, creating a demand for new alternatives, like the use of biocontrol organisms. Laboratory trials have shown the potential of different strains of the entomopathogenic fungus *Metarhizium brunneum* against several species of wireworms, including species from the genus *Agriotes*.

Even if entomopathogenic fungi EPF are more abundant in less intensively cultivated ecosystems such as grasslands, it has to be considered that wireworms need more than one year for their development, with application of EPF against wireworms not limited to the potato growing season but also before the damage-sensitive crop is planted. In a series of pot, semi-field and field experiments, we integrated the application of *M. brunneum* strain ART2825 into the cover crop before the potato season. The aim was to adapt the application to the ecological and environmental requirements of the fungus. The application date in late summer should enhance disease development through higher soil temperatures and persistence of the fungus by the absence of soil disturbance. *M. brunneum* ART2825 established successfully in treated plots, and we were able to demonstrate infectivity of the treated soils to wireworms. Effect of the treatments on potato damage was, however, not significant so far. In the ongoing season, we aimed at improving the efficacy by an increased application rate and an earlier application period.
prehensive analysis of Cry1Aa properties, from formation to cell disruption. These studies revealed key features that drive and stabilize crystal formation in the bacterium, that control pH-dependent crystal solubility, and how specific structural regions of Cry1Aa work together to bring about its toxicity. These results open new paths of research, allowing rational design of improved insecticidal proteins, and possibly guiding future functional studies.

CONTRIBUTED PAPERS. Monday, 17:15 B-2 STU

The ABC transporter C2 acts as a Cry1A receptor independently of its ATP binding site II in Spodoptera exigua

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ABC transporters are membrane proteins that depend on binding and hydrolysis of ATP to transport different substances across the membrane. After more than a decade of the first report, the number of ABC transporters that have been associated with the mode of action of Cry toxins continues to grow. For Spodoptera exigua, a mutation in the SeABCC2 gene (rendering a truncated protein lacking the ATP binding site II) was described as genetically linked to resistance to the Bt-based product Xentari™. Here, we wanted to determine whether this mutation could affect the role of the SeABCC2 as a functional receptor to Cry1A toxins. The results have shown that Cry1A toxins are similarly toxic to cells expressing either the full-length or the truncated form of the transporter. Moreover, we found specific Cry1Ac binding to cells expressing the truncated form of the transporter, and no difference in irreversible binding between the two cell lines. All these results point out that the partial lack of the nucleotide binding domain II, along with some point mutations in the truncated transporter, does not affect its functionality as a Cry1A receptor.

CONTRIBUTED PAPERS. Monday, 17:30 B-3 STU

Silencing ABC and Cadherin genes in Leptinotarsa decemlineata (Coleoptera:Chrysomelidae) treated with Bacillus thuringiensis ssp. tenebrionis Cry δ-endotoxin

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Bacillus thuringiensis (Bt) is a gram (+), spore forming entomopathogenic bacterium that synthesizes crystalline protein inclusions containing Cry δ-endotoxins. During infection by Bt spores, the crystals are solubilized in the midgut following ingestion and protoxins are then released through the action of proteases resulting in active toxin molecules that bind to specific receptors on the microvillar membrane of the midgut columnar cells. “Cadherin-like protein” and “ATP-binding cassette (ABC) transporter protein” are two crucial proteins in the binding of the Bt Cry toxins. The resistance of insects to Cry toxins is related to the altered toxin binding of the proteins acting as receptors and most of the data on this derives from lepidopteran and dipteran systems; however, less is known in coleopteran systems. In the current study, the genomic database of the Colorado potato beetle, Leptinotarsa decemlineata (Coleoptera:Chrysomelidae) has been searched for cadherin and ABC orthologs those shown to act as receptors against Bt kurstaki in the lepidopteran systems. The mortality of the larvae, midgut cell structure and expression of L. decemlineata Cad and ABC have been examined upon silencing the corresponding genes and inoculation with purified Bt tenebrionis toxin. These analyses revealed that the toxin molecules are not able to bind into epithelia cells and expression levels of L. decemlineata Cad and ABC genes, and mortality decreased in the dsRNA/Cad- and dsRNA/ABC-fed larvae, suggesting that the cadherin and ABC orthologs in the beetle system serve as receptors also against Bt tenebrionis toxin.

CONTRIBUTED PAPERS. Monday, 18:00 B-5

The Bacillus thuringiensis Cry37Aa protein is not necessary to mediate toxicity and binding of Cry323Aa protein on Cylas puncticollis

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Sweetpotato weevil, Cylas puncticollis (Bohemian) is one the most important constraints on sweetpotato production in Africa. Due to cryptic behaviour of Cylas spp., different management strategies are turned out to be inefficient for controlling this pest. The binary insecticidal proteins Cry23Aa/Cry37Aa from Bacillus thuringiensis (Bt) have been described as toxic to C. puncticollis larvae. In general, it is believed that the insecticidal activity of these proteins is attributed to both components, while there is no evidence of Cry23Aa and Cry37Aa toxicity when used separately.
Therefore, in the present study, the contribution of each protein in the insecticidal activity towards *C. puncticollis* larvae has been assessed. The results showed that both proteins were toxic for *C. puncticollis* larvae when tested individually. Furthermore, the binding behaviour of Cry23Aa/Cry37Aa to midgut receptors of *C. puncticollis* larvae has been determined. According to our results, Cry23Aa binds to *C. puncticollis* BBMV specifically and independently from Cry37Aa. In summary, our results suggest that the presence of both proteins are not necessary to exert toxicity against *C. puncticollis* larvae.

**CONTRIBUTED PAPERS. Monday, 18:15 B-6 STU**

**New Cry7 protein active against *Leptinotarsa decemlineata* (Coleoptera: chrysomelidae)**

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*Bacillus thuringiensis* is an entomopathogenic bacterium that is characterized by forming one or more crystals that are composed of Cry proteins, which need to be activated to specific toxins by digestion of the gastric juices of a susceptible host. Cry3 proteins have been used as an active ingredient of Bt-based insecticides against Coleoptera (eg *Leptinotarsa decemlineata*) and also as the toxin that gives Bt plants resistance against Coleoptera (eg *Diabrotica virgifera*). The selection of biotypes of insects resistant to Cry3 toxins, or the high probability that this may occur, makes it necessary to search for new toxins as an alternative. The objective of this work was to identify and characterize new active Bt proteins against *L. decemlineata*. Among several Bt strains carrying genes of the cry7 family, BM311.1 strain was selected because it showed greater toxicity in neonate larvae of *L. decemlineata*. Massive sequencing of those strains has revealed that BM311.1 codes for a protein that shares a similarity of 98% with Cry7Aa1, which has been called Cry7Aa2. The gene was cloned and used to obtain a Bt recombinant, using as receptor the acrystatlliferous Bt strain BMB171, in order to produce the recombinant protein Cry7Aa2. By means of a leaf disc bioassay, it has been estimated that the mean lethal concentration (LC₅₀) value of Cry7Aa1, for neonatal *L. decemlineata* larvae, was around 20 ng/μL. The potential of this protein as alternative for the control of coleopteran pests is discussed.

Keywords: *Bacillus thuringiensis*, Cry7, lethal concentration, *Leptinotarsa decemlineata*, solubilization, midgut juices.

**CONTRIBUTED PAPERS. Monday, 18:30 B-7**

**ABC transporter-mediated resistance mechanism to *Bacillus thuringiensis* Cry1Ac toxin in diamondback moth**

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Large-scale use of biopesticides and transgenic crops producing insecticidal Cry toxins from the gram-positive bacterium *Bacillus thuringiensis* (Bt) has resulted in episodes of field resistance in several lepidopteran pests. Unraveling these resistance mechanisms are of great importance for delaying insect field resistance evolution. The diamondback moth, *Plutella xylostella* (L.), was the first insect to evolve field resistance to Bt biopesticides and it is an excellent model insect to study Bt resistance mechanisms. Resistance to Bt Cry1Ac toxin in diamondback moth has been reported to be associated with cis-mutation of *ABCC2* genes or MAPK signalling pathway trans-regulated differential expression of *ABCC1*, *ABCC2*, *ABCC3*, *ABCG1* and *ABCB1* genes. Hence, we can see that diverse ABC transporter genes in subfamily B, C and G can be involved in Cry1Ac resistance in *P. xylostella*. More recently, ABCC2 has been confirmed to be involved in Cry1Ac toxin oligomerization and membrane insertion in *P. xylostella*, and CRISPR/Cas9-mediated knockout of both the *ABCC2* and *ABCC3* genes confers high-level Cry1Ac resistance in *P. xylostella*. Although much progress has been achieved, how these Bt transporters work together and their precise roles in the molecular basis of *P. xylostella* Cry1Ac resistance still remains to be fully unveiled. Herein, we intend to deeply discuss and explore ABC transporter-mediated resistance mechanism to Cry1Ac toxin in *P. xylostella*, which will contribute to understanding the complex molecular mechanisms of Bt resistance in diverse insects.
showed the best in different European countries. Commercial nematodes application in addition, field trials using standard farming machineries were conducted for a decade in plot trials with maize plants. Diabrotica virgifera management using genetically improved strains of Heterorhabditis bacteriophora Ehlers, R.-U.1; Molina, C.1; Vandenbossche, B.1; Dörfier, V.1; Barg, M.1; Toepfer, S.2 ¹e-nema GmbH, Klausdorfer Str. 28-36, 24223 Schwenentental, Germany ²CABI Europe – Switzerland, c/o Plant Protection Directorate, Rarosi ut street 110, HU - 6800 Hodmezovasarhely, Hungary Corresponding author: ehlers@e-nema.de The Western Corn Rootworm (Diabrotica virgifera virgifera; Coleoptera: Chrysomelidae) is one of the most damaging pests of maize. Since the ban of neonicotinoid seed treatments most European farmers rely on the application of less effective granular soil insecticides. The sustainable, non-toxic alternative, the entomopathogenic nematode, Heterorhabditis bacteriophora, has been tested for a decade in plot trials with maize plants artificially infested with insect eggs to ensure an even population density. In addition, field trials using standard farming machineries were conducted in different European countries. Commercial nematodes application is at 2 x 10⁹ ha⁻¹ with 200 l water ha⁻¹ into the furrow together with the maize seeds, using special injectors mounted on the single-seed drilling machine. At the time of application Diabrotica eggs are still in diapause. Nematodes survive and remain virulent until larvae hatch approximately 2-6 weeks later. With this precise fluid application method, nematodes achieved a mean reduction of the pest population of 65% (ranging from 33-82%) and outperformed results obtained with the chemical standards in 11 out of 16 trials. Frequent nematode applications lead to a reduction of the insect population. More virulent and persistent H. bacteriophora lines were obtained through genetic improvement using classical breeding technology. An improved line performed better than the commercial strain, justifying a reduction of the application density from 2 to 1 x 10⁹ ha⁻¹ and bringing application costs into the range of synthetic chemicals.

Potential microbial control of xylophagous pests with entomopathogenic nematodes and fungi El Khoury, Y.1; Nouejem, E.2; Ravili, J.1; Oreste, M.1; Addante, R.1; Nemer, N.1; Tarasco, E.1 ¹Department of Soil, Plant and Food Sciences, University of Bari “Aldo Moro”, via Amendola 165/A, 70126 Bari (Italy) ²National Center for Marine Sciences, National Council for Scientific Research-CNRS, P.O.Box 11-8281, Ryad El Solh 1107260, 59, Zahia Selman Street, Beirat, Lebanon Corresponding author: yara.elkhoury@unibo.it The effects of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and fungal strains (Beauveria bassiana) were evaluated in laboratory assays against larvae of four xylophagous pests: the Asparagus moth Parahypota caestrum, the European goat moth Cossus cossus, the pine longhorn Anthicus listicus and the black Buprestis Parahypota caestrum. Due to their biology and ethology, these insects may be included in the category of pests residing in cryptic habitats. The control of these species is very difficult, due to the inability of chemical pesticides to penetrate the cryptic habitats and reach the targets. The results showed that all the nematodes and fungal strains affected the insect survival. Steinernema feltiae and Beauveria bassiana showed the best performances. Considering the lack of effective chemical control means, the microbial control of the xylophagous pests by EPNs and EPFs reveals promising perspectives. Nematodes and fungi are able to penetrate the cryptic habitats because they are living organisms and may be horizontally transmitted by infected hosts. The distribution of EPF as preventive control method and the injection of EPN suspensions to reach and infect the larvae inside the wood galleries can be a combined sustainable control system.

Investigating the biological control potential of indigenous nematodes for the control of invasive slug pests Pieterse, A.1; Malan, A.P.1; Ross, J.L.1,2 ¹Department of Conservation Ecology and Entomology, Faculty of Agri-Sciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa ²Institute of Biological and Environmental Sciences, University of Aberdeen, AB24 3UU, UK Corresponding author: annikapieterse@sun.ac.za Mollusc pests in South Africa are currently mostly controlled through the use of pellets containing a form of bait and a mixture of chemicals. There is a need for an environmentally-friendly method of control that is just as effective as the use of chemicals. Surveys of slug-parasitic nematodes were conducted in the Western Cape and KwaZulu-Natal with the aim
investigating the biocontrol potential of local slug-parasitic nematodes. A few undescribed species were found, but ultimately *Phasmarhabditis papillosa* and *Caenorhabditis elegans* were used for further testing. The biocontrol potential of these nematodes was tested by having them feed on bacteria that is pathogenic to slugs and then testing them on slugs. It was proven for the first time that *P. papillosa* can cause mortality in slug pests. The life cycles of *P. papillosa*, *C. elegans* and *Phasmarhabditis hermaphrodita* were also compared with the aim of evaluating the ease with which they can be cultured and investigating the effect this would have on the mass-culturing of these nematodes. Current research is focussed on the optimization of the mass-culturing of these nematodes by altering the temperature, media, oxygen levels and bacteria used.

**CONTRIBUTED PAPERS. Monday, 18:00 N-13 STU**

**Adult emergence of Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) from soil: a susceptible period for entomopathogenic nematode infection**

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*Drosophila suzukii* is a global threat to soft-skinned and stone fruits. Different studies prospected biological control of larvae and pupae with entomopathogenic nematodes (EPNs). However, susceptibility of adults has not been tested yet. Most *D. suzukii* pupae fall from fruits to soil, where adults emerge. The aim of our work was to determine the susceptibility of *D. suzukii* adults with different EPN species and to evaluate the susceptibility and flight capacity during adult emergence process from treated soil. Susceptibility of *D. suzukii* adults was tested against *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* in Petri dishes under laboratory conditions. To test the infection during adult emergence, pots with *S. carpocapsae* treated sand and *D. suzukii* pupae were used. To test the flight capacity of infected adults, a column arena assay was carried on, permitting the adults to fly away from the nematode treated soil. *Drosophila suzukii* adults showed mortality of 65% by *S. carpocapsae*, while *S. feltiae* and *H. bacteriophora* caused less than 5%. During adults’ emergence, infection of 89.5% was registered by *S. carpocapsae*. In the column assay, 53.11% of the adults that flew after soil exposition to EPNs emerged, infection of 89.5% was registered by *S. carpocapsae*. In the column assay, 53.11% of the adults that flew after soil exposition to EPNs emerged. In virulent strain it was evidenced an overexpression in oxidative stress genes related to neuronal signal transduction (GO:0023041), locomotion (GO:0040011) and response to stimulus (GO:0050896) were high represented in genes up-regulated in the free living high virulent strain. Lc-Ms-Ms analysis of ESP of high and low virulent strains identified 279 proteins, 30% only in the high virulent. Moreover, 67% of the genes coding for ESPs were up-regulated in high virulent strain. The comparison of the expression rate of 50 selected genes in 3 high and 3 low virulent strains showed that the expression profile was significantly discriminant for virulence. All together, these finds support our proposal in the use of transcriptomics and proteomics to identify virulent phenotypes.

**CONTRIBUTED PAPERS. Monday, 18:30 N-15**

**Heterorhabditis bacteriophora: An excellent model for genetic improvement of biocontrol traits**

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More than 150 different invertebrates are currently used in biological control of insect pests. For breeding insects and mites, producers of invertebrate biocontrol agents largely depend on sampling and characterising natural populations. Entomopathogenic nematodes (EPN), especially *Heterorhabditis bacteriophora* are different. The contribution will describe the relevant biological peculiarities and shortly introduce into relevant techniques to show why this nematode is an excellent model for genetic improvement. EPN biology permits production of inbred lines through self-fertilisation by the hermaphrodite and production of hybrids through crosses of second generation amphimictic adults. The genetic pool can be preserved by storage in liquid nitrogen. Mass production is done in industrial scale bioreactors in liquid culture. EPN have a short life cycle allowing rapid progress by genetic selection. Several traits have been improved, e.g. reproduction, longevity, field persistence and stress resistance to heat, dessication, reactive oxygen species and nematicides. EPN can be subjected to EMS mutagenesis. Progress of genetic selection is easily lost through outcrossing during mass production. As *Heterorhabditis* spp. are unable to mate in liquid media, reproduction is only through self-fertilising hermaphrodites. The use of well characterised inbred lines can overcome problems of trait deterioration when production is done in liquid media. A large pool of molecular genetic information and tools are available to support breeding of *Heterorhabditid* biocontrol agents.

**CONTRIBUTED PAPERS. Monday, 18:15 N-14**

**Transcriptomic and proteomic approach to identify potential virulence factors produced by the entomopathogenic nematode Heterorhabditis bacteriophora**

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*Heterorhabditis bacteriophora* is currently used to control insect pests. The identification of virulence factors should provide efficient tools to select and improve the efficacy of this nematode. We performed differential analysis of transcripts and comparative proteomics of proteins excreted secreted (ESP) between high and low virulent strains. RNA-seq produced a total of 65 517 mapped reads corresponding to 16166 distinct genes. The comparative analysis allowed to the identification of 2374 genes up-regulated in the high virulent strain (FC > 0.5), 1501 in the parasitic stage and 873 in the free living stage.

In virulent strain it was evidenced an overexpression in oxidative stress pathways. Concerning molecular functions, 70% of up-regulated genes belong to binding (GO:0005488), catalytic activity (GO:0003824) and molecular transporter (GO:0005215) categories. To be noticed that genes related to neuronal signal transduction (GO:0023041), locomotion (GO:0040011) and response to stimulus (GO:0050896) were high represented in genes up-regulated in the free living high virulent strain. Lc-Ms-Ms analysis of ESP of high and low virulent strains identified 279 proteins, 30% only in the high virulent. Moreover, 67% of the genes coding for ESPs were up-regulated in high virulent strain. The comparison of the expression rate of 50 selected genes in 3 high and 3 low virulent strains showed that the expression profile was significantly discriminant for virulence. All together, these finds support our proposal in the use of transcriptomics and proteomics to identify virulent phenotypes.
The study was to test the efficacy of Entomopathogenic fungi (EF) have shown to be successful in soil treatments against several soil-dwelling insect pests. In the current work, four Beauveria bassiana and three Metarhizium brunneum strains were evaluated against Spodoptera littoralis prepupae in soil drenches, but also the mortality and sub-lethal reproductive effects of pupae and adult derived from treated prepupae. In a first series of experiments, all isolates were pathogenic to pupae, whereas total mortality values varied from 31.7 to 83.3% and average survival lasting from 10.5 to 7.5 days. Adults derived from prepupae targeted in the soil by drenching with fungal suspensions caused a significant reduction in fecundity of 1.7-15.0%. Soil drenching with fungal suspensions caused a significant reduction in fecundity of females coming from surviving pupae, with egg fertility varying from 15.0 to 58.9% and hence, 6.8 to 28.4% percentage reduction respectively.

In a second series of bioassays, two selected strains were targeted at S. littoralis prepupae with four 10-fold concentrations ranging from 10^5 to 10^6 conidia ml^-1. The LC50 were 1.7 x 10^6 and 1.8 x 10^7 conidia ml^-1 and the LT50 were 7.5 and 6.2 days for EAMa 01/58-Su and EAMb 09/01-Su strains, respectively. Destruxin A was present in pupae coming from treated prepupae with EAMc 01/58-Su (0.01±0.002 µg/pupae) and EAMB 09/01-Su (0.015±0.003 µg/pupae) strains. Soil drenching with EF may be considered as a key factor in S. littoralis IPM strategies due to their direct effects on the reduction of the soil-dwelling life stages and, on the significant reduction of the reproductive potential of the adults with an overall important disruption of the life-cycle.

Control of soil-dwelling stages of Spodoptera littoralis and impact on the adults of the same generation

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Entomopathogenic fungi (EF) have shown to be successful in soil treatments against several soil-dwelling insect pests. In the current work, four Beauveria bassiana and three Metarhizium brunneum strains were evaluated against Spodoptera littoralis prepupae in soil drenches, but also the mortality and sub-lethal reproductive effects of pupae and adult derived from treated prepupae. In a first series of experiments, all isolates were pathogenic to pupae, whereas total mortality values varied from 31.7 to 83.3% and average survival lasting from 10.5 to 7.5 days. Adults derived from prepupae targeted in the soil by drenching with fungal suspensions caused a significant reduction in fecundity of females coming from surviving pupae, with egg fertility varying from 15.0 to 58.9% and hence, 6.8 to 28.4% percentage reduction respectively.

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Development of a soil granule of the entomopathogenic fungus Metarhizium brunneum to control wireworms

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The first step in developing a soil granule was to determine a fungus which is pathogenic against wireworms. Therefore, six Metarhizium strains were tested on their effect on three common Agriotes species in Germany. The Metarhizium brunneum strain JKI-B1-1450 showed the most promising results with a mortality up to 90% against two of the three examined species. Consequently, the further experiments focused exclusively on this fungus.

The second step was to optimize the production of the fungus in liquid medium. For a good yield of spores liquid media with different ingredients were examined. Furthermore, the influence of production temperature on product quality will be discussed.

The third step was to coat the biomass on millet using fluid bed drying. Before testing the granule in first field trials, the influence of three fungicides on the granules quality was examined. For this the granules were sprayed with the maximum allowed application rates of Ortiva®, Moncut® and Risolex®. After incubation for 2 and 4 weeks at 25°C, the sporulation and growth rate of the fungus on the millet was determined. Moncut® and Risolex® had no significant effect on the growth rate, whereas Ortiva® reduced it. The sporulation was reduced the least by the use of Moncut®. After 4 weeks of incubation the sporulation was identical to that of the control treatments after 2 weeks of incubation. In the Risolex® treatment, the reduction was slightly higher than with Moncut® but the most inhibiting effect occurred with Ortiva®.

Efficacy of new Metarhizium formulations against wireworms in the field

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Wireworms, the soil-dwelling larvae of click beetles (Coleoptera, Elateridae) are a certain pest, which causes significant economic damage due to their feeding damage on different crops. In regard to potatoes the genus Agriotes is the most widespread in Germany, especially Agriotes obscurus. Many field trials with formulations of the entomopathogenic fungus Metarhizium often have no sufficient effect and the results vary widely, while the reasons for this variability are not always clear. In this study we compared different formulations of Metarhizium in a field trial with potatoes and investigated different factors that influence the efficacy against wireworms. For the comparison in the field two new formulations of Metarhizium were tested, a millet grain coated with fungus (granulate) and a liquid suspension. The combined application of granulate and liquid suspension in the potato rows during planting has reached an efficacy of up to 90% against two of the three examined species.
of 30% relative to the untreated control. Investigations in the potato row considering the wireworm species composition, the amount of CFU's and the measurement of soil temperature and moisture indicate a possible influence on the efficacy. Furthermore, we examined the reaction of *A. obscurus* and *A. sputator* to the coated millet in a feed-choice-experiment in the lab. *A. obscurus* tolerated a concentration of 5x10⁷ spores/corn, whereas *A. sputator* tolerated a higher concentration of 2x10⁸. The results hint a species-specific reaction of wireworms to the *Metarhizium* strain used.

**CONTRIBUTED PAPERS. Tuesday, 10:45 MC-13 STU**

Survival of vine weevil (*Otiorhynchus sulcatus*) larvae is affected by geographic origin and host plant but only in the absence of the entomopathogenic fungus *Metarhizium brunneum*.

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*Otiorhynchus sulcatus* Fabricius is a pest of horticultural crops that has spread rapidly from its native area in central Europe to many parts of the world. The entomopathogenic fungus *Metarhizium brunneum* Petch is widely used in integrated pest management programmes to control this pest. Little research has, however, focused on factors that alter vine weevil biology and susceptibility to this pathogen. We recorded whether susceptibility of vine weevil larvae to this pathogen differed when insects were collected from different UK geographic areas, reared on either strawberry (*Fragaria x ananassa* Duchesne) or raspberry (*Rubus idaeus* Linnaeus), and when insects received a detergent treatment to alter cuticle composition. Additionally, a choice experiment was designed using raspberry and strawberry plants to correlate offspring survival with parental oviposition preference.

Mortality of larvae not treated with *M. brunneum* differed between collection sites and rearing host plants, whereas mortality of larvae treated with *M. brunneum* was similar regardless of the geographic origin, the rearing host plant or the cuticle detergent treatment. Furthermore, in a choice situation, vine weevil adults were more likely to oviposit on raspberry plants on which larvae experienced higher survival rate. Thus, the efficacy of *M. brunneum* against vine weevil larvae is not influenced by the factors tested under the controlled conditions used for our experiments; and vine weevil adults seem to follow the preference-performance hypothesis as oviposition was more likely to occur around raspberry plants on which offspring survival was higher.

**CONTRIBUTED PAPERS. Tuesday, 11:00 MC-14 STU**

Laboratory evaluation of combining an entomopathogenic fungus (EPF) and a botanical compound to control fungus gnats, *Bradysia* spp.

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Fungus gnats still pose a problem in many greenhouse cultures and biological control options are therefore urgently desired. We studied the efficacy of combining an entomopathogenic fungal isolate and a botanical compound in an Attract & Kill strategy targeting the larvae. In a first series of bioassays, a *Metarhizium brunneum* isolate (CB15) was directly applied to the growing media treated with a botanical compound, containing mentha oil. Fungus gnat larvae were released into each experimental cup and adult emergence was evaluated two weeks later using yellow sticky cards. Significantly fewer adult numbers emerged from combined treatments (22%) compared to fungal treatments (30%) or controls (61%), pointing to an additive effect of combining the two agents. On the other hand, a higher percentage of adults emerged from larvae exposed to the botanical microcapsule (65%) compared to control treatments, indicating a positive interaction with the mentha capsules. Then, the efficacy of CB15 and two biopesticides (Neem Azal and Spinosad) individually or in combination was assessed. Spinosad combined with mentha capsules significantly reduced adult emergence by 97% as compared to other treatment combinations or the control. In additional experiments egg-laying behavior of females in basil plant soils drenched with CB15 or Spinosad or a combination with mentha capsules was assessed, showing a strong change with regard to female oviposition behavior when exposed to mentha capsules. Ongoing experiments will now evaluate this strategy under greenhouse conditions.

**CONTRIBUTED PAPERS. Tuesday, 11:15 MC-15 STU**

Exploiting the tomato microbiome and volatile-based mechanisms towards controlling *Meloidogyne*-based disease complexes.

Wolfgang, A.¹; Taffner, J.²; Araújo-Guimarães, R.²; Coyne, D.²; Berg, G.¹

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Root-knot nematodes (*Meloidogyne* spp., RKN) and associated secondary infections are a major threat to global food security. In order to better understand *Meloidogyne*-based disease complexes, tomato roots with different levels of root-galling damage from two field locations in Uganda were sampled to establish bacterial species collections and DNA libraries. Additionally, bacterial isolates were tested for volatile-based control mechanisms of RKNs as well as for antagonism towards widespread fungal pathogens. 16.5% of the bacterial strain collection produced nematocidal volatile organic compounds (nVOCs) active against *Meloidogyne incognita*. Using SPME GC-MS, diverse VOCs were identified, including sulfuric compounds, alkenes and one pyrazine. A considerable number of the bacterial strains were antagonistic towards at least one tested fungal pathogen of the disease complex. However, antagonistic interactions appear highly specific. Most active nematocidal antagonists included *Pseudomonas*, *Comamonas* and *Variovorax* and were most abundant in rhizosphere and diseased root endosphere. Fungal antagonists belonged to *Bacillus*, which were primarily recovered from healthy roots. The microbiome of healthy and diseased root endospheres differed significantly in alpha and quantitative beta diversity indices. Bacteria-derived volatiles appear to provide a remarkable, yet wholly unexploited, potential to control *Meloidogyne*-based soil-borne disease complexes. The highly specific antagonism indicates that a combination of nematode- and fungal-controlling bacteria are necessary to counter the range of pathogens of such complexes.

**CONTRIBUTED PAPERS. Tuesday, 11:30 MC-16 STU**

Novel coatings for attract-and-kill formulations containing *Metarhizium brunneum* for biological control of wireworms.

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Wireworm damage has become an increasing problem in conventional as well as organic potato cultivation. Wireworms use CO₂ gradients established in soil by plant roots to locate potential hosts. Effective plant
Covert infections by viruses are common in natural populations of insects. The Almerian (Spain) population of Spodoptera exigua is infected by two iflaviruses (SeIV-1, SeIV-2) that occur alone and in mixed infections with the nucleopolyhedrovirus SeMNPV in covertly-infected insects. Lethal infections produced by SeMNPV generated occlusion bodies (OBs) in which the iflavirus was intimately associated, resulting in changes to the physical characteristics of OBs. Association with OBs improved iflavirus stability and transmissibility, albeit at some cost to SeMNPV pathogenicity and OB production in co-infected hosts. Viral loads of SeMNPV were increased in the presence of SeIV-2 compared to insects infected by SeMNPV alone, or insects infected by both SeIV-1 + SeMNPV. The OBs with associated iflavirus particles had similar numbers of occlusion derived virions (ODVs) although the number of nucleocapsids per ODV was reduced in the presence of iflavirus. SeIV-1 infected larvae were more susceptible to SeMNPV than insects that did not have a covert iflavirus infection. We conclude that iflaviruses could modulate the dynamics of natural SeMNPV infections and could represent a biosecurity issue in the large-scale production of baculovirus-based insecticides.

**VIRUS SYMPOSIUM**

Tuesday, 09:30-11:30
Multispace AB

**Covert virus infections in insects**

Organisers / Chairs: Vera Ros / Miguel López-Ferber

**Covert infection in baculoviruses: insights from field populations**

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Understanding the ecology and evolution of the interactions of between pathogens and hosts is critically dependant on determining their transmission and persistence in host populations. And yet these basic parameters are extremely difficult to evaluate in field populations. Baculoviruses can be transmitted by both horizontal and vertical transmission. When population densities are high and rising, horizontal transmission is clearly the most likely route of infection being transferred. What is less clear is what happens when population densities are low, sometimes for many years in univoltine insects. Sudden outbreaks of disease have often been reported in insect populations, particularly forest insects with boom and bust cycles. These have frequently been attributed to covert infections which have some how been triggered. However, although it is possible to demonstrate the presence of baculovirus DNA and RNA in adult insects, proving that this is a vertically transmitted, viable pathogen, that plays a role in baculovirus outbreaks in the field is more challenging. Here we describe our work on a natural host-baculovirus relationship in the field in which we measure both overt and covert infection in a cycling forest insect. We discuss the patterns in the context of the dynamics and discuss how they relate to current theory on covert infections.

**Bee health and its relation with virus infection**

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Viruses in insect pollinators spread within multi-host networks. This is a reservoir of hosts, or epidemiologically connected populations in which the virus can be permanently maintained. One needs to understand the virus host range and its differential virulence to understand the impact of a certain virus on a specific pollinator. Herein host defence mechanisms, like tolerance and resistance will be key. For viral infections one can distinguish overt and covert infections, often related with the immune-competence of the bee and associated (human induced) stressors. An important challenge is to develop good proxy measures to determine the actual damage caused by viruses and how virus infection is related to bee health. This in order to understand how the stressor “virus” relates with the multi-factorial problem of wild bee decline.
Fungal infection recognition in the Aedes aegypti mosquito

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The Aedes aegypti mosquito is one of the most important vectors of human pathogens throughout the world. Although efforts have been made to eliminate this mosquito from their current habitat, the increase in insecticide resistance has highlighted the need for alternative methods of mosquito control. Entomopathogenic fungi represent an environmentally friendly alternative to control the population of mosquitoes. Although some advances have been made to understand how the fungi infect and colonize the mosquito body, there is still a need for understanding how the mosquito responds to infection. In this regard, the effective recognition of the invading fungi by the mosquito is a crucial step in mounting an appropriate anti-fungal response. During this interaction, as the fungi penetrates the mosquito cuticle, a set of recognition molecules, with certain degree of specificity, act to trigger a series of immune response that limit fungal dissemination. This has implications in the mosquito susceptibility to fungal pathogens. This talk will discuss the pathogen recognition dynamics during fungal infection in the Ae. aegypti mosquito.
Functional analysis of a lipase gene in Beauveria bassiana

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Lipase (triacylglycerol acylhydrolase, (E.C. 3.1.1.3)) is a class of esterases that catalyzes the hydrolysis of long chain triacylglycerol (TAG). The biochemical roles of lipase genes have been extensively studied, but their physiological roles in the filamentous fungi were still not completely uncovered. In Beauveria bassiana, a lipase without excretion signal (BbLip1) was characterized and its physiological roles were revealed via gene disruption and complementation. Gene disruption resulted in a significant decrease in conidial germination under oligotrophic surface. Lipidomic analysis indicated that gene loss led to a significant accumulation of TAG. In addition, BbLip1 contributes to the virulence of B. bassiana, with the 2-day delay in median lethal time, when compared with that of the wild type. These findings suggest that the BbLip1 plays an important role in the infection cycle of B. bassiana via regulating the metabolism of intracellular lipid.

CONTRIBUTED PAPERS. Tuesday, 10:45 F-14 STU

Transcription factor Msn2 acts as virulence regulator of Beauveria bassiana s.l. against the tick Rhipicephalus microplus

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Beauveria bassiana s.l. is one of the most investigated fungi for tick control. The Msn2 is a transcription factor associated to fungal growth, conidiation, stress-response and virulence of B. bassiana against Galleria mellonella and Spodoptera litura, both lepidopteran insects. In the current study we evaluated the interference of Msn2 transcription factor to the virulence and to the initial infection processes of B. bassiana against Rhipicephalus microplus (Acari, Ixodidae). A Msn2 knockout-mutant strain of B. bassiana (BbΔmsn2) was compared to its wild type (BbWT) and complement mutant (BbΔmsn2BbΔmsn2). Percent control of engorged females treated with ΔBbmsn2 was significantly low (43.3 ± 4.2%) than in the groups treated with BbWT (54.1 ± 4%) or BbΔmsn2 (58.7 ± 5%) (P = 0.036). Scanning electron micrographs showed germinating conidia on tick cuticle at 48h and 72h incubation. Histological sections of ticks treated with BbΔmsn2/BbΔmsn2 and incubated for 120h showed funargenal penetration through the cuticle, and it reached the tick interior. Hyphae of BbΔmsn2, however, did not trespass the tick cuticle after 120h incubation. Our results indicate that the absence of the Msn2 transcription factor reduced the virulence of B. bassiana s.l. against R. microplus because of the delayed fungal penetration through the tick cuticle.

CONTRIBUTED PAPERS. Tuesday, 11:00 F-15

Recombinant chitinase to enhance virulence of Beauveria bassiana conidia against the sugar-cane borer Diatraea saccharalis.

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Beauveria bassiana chitinases are involved in degradation of chitin present in the exoskeleton and internal structures of insects, being important virulence factors implicated from initial to final steps of infection process. In the present work, the B. bassiana (Bv062 isolate) ORF coding for a chitinase (Chi37) was cloned and expressed in E. coli. The effect of the recombinant protein (rChi37) over the mortality of Diatraea saccharalis larvae infected with Bv062 conidia was studied. The rChi37 was produced on soluble and insoluble fractions of E. coli culture and both presented chitinobiosidase (170 µM/L) and endochitinase (90 µM/L) enzymatic activities, being 45°C and pH 5.0 the optimum conditions for enzyme performance. The soluble fraction rChi37 was purified by His-tag affinity chromatography and then adjusted to 200 and 300 µg/mL to use as additive of Bv062 conidia (1 x 10⁶ con/mL) in a laboratory bioassay against D. saccharalis larvae. No significant differences were observed between the efficacy of Bv062 conidia alone or mixed with purified rChi37 at 200 µg/mL, however rChi37 at 300 µg/mL increased the insecticidal activity of Bv062 conidia, reaching 96.7% efficacy and 6.2 days mean time to death, compared with conidia alone that presented 60% and 8.9 days, respectively. On the other hand, the mixture with rChi37 did not affect conidia viability in terms of germination (96.6% at 24 h) or elongation of germinal tube. This work is the proof of concept about using recombinant enzymes type chitinase, as additive to enhance B. bassiana virulence to develop a new generation of biopesticides.

CONTRIBUTED PAPERS. Tuesday, 11:15 F-16

Balanced histone H3-K56 acetylation are essential for DNA damage repair and biological control potential of Beauveria bassiana

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The histone H3K56 acetylation allows for deposition of newly synthesized H3 molecules into the genome during DNA replication and chromatin assembly after DNA damage repair and hence is essential for genomic stability, DNA damage repair, DNA replication and global gene activity but is functionally unexplored in any fungal insect pathogen. This study sought to elucidate the possible role of H3K56 acetylation in genomic stability and its sustaining biological control potential of Beauveria bassiana, a fungal entomopathogen. Here, we constructed two mutated strains (mH3K56R and mH3K56Q) by replacing K56 with arginine (R) mimicking a nonacetylatable residue or with glutamine (Q) mimicking an acetylated lysine residue in B. bassiana. Intriguingly, both hypo- and hyper-acetylation of H3K56 triggered enhanced histone H2A-S129 phosphorylation required for DNA damage repair. Consequently, both mH3K56R and mH3K56Q showed increased sensitivities to various stresses (DNA damage, oxidation, cell wall perturbation, high osmolality and heat shock) during growth, moderate conidiation defects under normal culture conditions, decreased conidial UV-B resistance, and significantly attenuated virulence. These phenotypic changes correlated well with reduced transcripts of many genes, which encode the families of protein phosphatases, antioxidant enzymes and cuticle-degrading Pr1 proteases respectively. Therefore, balanced H3K56 acetylation plays an essential role in sustaining genomic stability and global gene activity by modifying H2A-S129 and hence is required for DNA damage repair, conidiation capacity, environmental fitness and pest-control potential of B. bassiana.
Development of a bacterial pesticidal protein information resource

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Abstract: The bacterial pesticidal protein resource centre/center is a project that has been funded by CAMTech for the last 18 months. The original brief was to develop a revised nomenclature to replace the Bt toxin nomenclature in a way that differentiated the different structural/homology groups of toxins. The second stage was to build an interactive online database to hold data about the pesticidal proteins (no longer referred to as toxins) and to add proteins that had not previously been incorporated into the Bt nomenclature. These two stages are nearing completion and I will describe the new naming system and introduce the online database. In the 18 months that this project has left to run we plan to introduce functionality to the online portal such as allowing users to enter their own sequences either for inclusion in the nomenclature, or to compare them to existing sequences (MSAs, clustering patterns etc). As well as being able to compare full-length sequences, we will provide the functionality to compare proteins at domain level. Two final objectives of the project are to build a website to inform various stakeholders about multiple aspects of the proteins and to write software that will allow us to associate sequence information with metadata such as specificity and cross-resistance potential.

Insecticidal proteins for controlling western corn rootworm Diabrotica virgifera virgifera, (Coleoptera: Chrysomelidae) isolated from the insect pathogenic bacteria Brevibacillus laterosporus. Chay, C.; Milligan, J.; Bean, G.; Slightom, R.; Howe, A.; Werner, B.; Moore, R.; Pleau, M.; Nance, A.; Yin, Y.; Bowen, D.

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Insects feeding on the roots of crop plants can cause damage to the root systems resulting in significant yield loss due to poor water and nutrient uptake. In corn the damage caused to roots by corn rootworm larvae feeding can be so severe the plants will fall over or lodge resulting in further yield loss because of the difficulty of harvesting the damaged plants. Com, genetically modified to express insecticidal proteins from bacteria, has provided a solution for control of these root damaging insect pests. Several closely related insecticidal proteins were recently discovered from Brevibacillus laterosporus strains which are lethal, upon feeding, to Diabrotica virgifera virgifera LeConte, the western corn rootworm. The insecticidal activity of TIC3670 (Cry75Aa1), TIC3669 (Cry75Aa2) and TIC3668 (Cry75Aa3) was identified by feeding western corn rootworm larvae an artificial diet overlaid with crude preparations of these proteins expressed in a Bt strain transformed with an expression plasmid encoding the Cry75Aa genes. In further experiments Cry75Aa genes encoding each protein were separately transformed and expressed in corn. Plants expressing each protein in the roots were protected from feeding damage by western corn rootworm larvae. The discovery and deployment of genetically modified corn expressing TIC3670 (Cry75Aa1) has the potential to be a tool for control of corn rootworm in corn growing regions around the world.
many of which have toxicity against corn rootworm (CRW), Diabrotica virgifera virgifera. Although the structures of many of these non-tradition-
al insecticidal proteins vary, they still have relatively the same MOA as traditional 3-domain Bt Cry proteins: Ingestion, proteolysis, receptor bind-
ing, membrane insertion, pore formation leading to insect death. Using a domain-based protein characterization approach that includes bioinfor-
matic and functional comparisons of conserved and diverse domains, the specific binding regions and conserved oligomer and pore-forming motifs can be elucidated in these new non 3-domain insecticidal proteins further demonstrating how the MOA components of these new non 3-domain CRW-active proteins are similar.

Decades of structural research on the MACPF/CDC family suggested that oligomer assembly on the target membrane is mediated via a dedicat-
ed ancillary domain. This is followed by planar diffusion upon the target membrane into a ring shaped prepl. The prepl undergoes a concert-
ed conformational change to form the final pore. This model of mecha-
nism, however, is only consistent with Cholesterol Dependent Cytolysins (CDCs), pleurotysin and perforin that have all dedicated membrane binding ancillary domains. It is now emerging that this canonical mechan-
ism does not explain how two important pathogen-targeting systems function: the Membrane Attack Complex (MAC) and MPEG-1.

Overall, these new SP cryo-EM structures of the MAC and MPEG-1 chal-
lenge the existing dogma in MACPF/CDC pore assembly that ancillary do-
 mains are required for pore formation. The MAC does not have ancillary domains and has evolved to use a sequential insertion system in contrast to the concerted pore formation used by CDC. Likewise, new structures of the MPEG-1 prepl, a pore used within the phagolysosome of macro-
phage, sheds light on a mechanism that binds to a membrane in an “up-
side-down” orientation to prevent autolysis and uses acidic conditions as the trigger for pore formation. We conclude that MACPF/CDC pores used by the immune system have evolved to be able to target highly variable surfaces by evolving different assembly pathways. This is an important concept to understand for newly identified pore forming proteins to ensure that there are no off-target effects.

Understanding how non-specific targeting has evolved in beta-pore-
forming toxins and how to guide specific targeting systems

Kondos*, Siew Siew Pang*, Hari Venugopah*, Tom T. Caradoc-Davies*,
James C. Whisstock*, Michelle A. Dunstone*

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The MACPF/CDC family proteins use a common fold to oligomerise into a ring-shaped transmembrane beta-barrel pore capable of either direct cell lysis or passive transport other factors proteins. Members of this family are found in all kingdoms of life with a range of functions including as immune effectors, pathogenicity factors, parasite egress, fungal defense and development.

Invertebrate-active pesticidal proteins belong to a number of distinct struc-
tural classes. The demonstrated activity of some of these classes is re-
stricted to invertebrate toxicity (Toxin_10 proteins, Vip3 proteins and, with the exception of activity against cancer cells, 3-domain family proteins). Others, however, such as the ADP ribosyl transferases and alpha helical toxins, have structural homologs with activity against normal mammali-
ans. The basis for the differential specificity of different members of these families is poorly understood. As a first step to address this, we will present structures along with genetic and functional insights into Vip1 and Cry6 family proteins to provide a structural basis to begin to explore specificity determining regions in these proteins.

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CONTRIBUTED PAPERS. Tuesday, 12:00 MC-17

Activity of Metarhizium brunneum and Beauveria bassiana against early developmental stages of the false codling moth

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This study evaluated the efficacy of two entomopathogenic Hypocrealean fungi, Metarizium brunneum (lab isolate) and Beauveria bassiana (commercial product Botanigard), for preventative control of the false codling moth (Thaumatobita leucotreca; FCM). The mortality of eggs and first instar larvae was studied in three different assays: First, fungal virulence was examined under optimal laboratory conditions (25°C, 85% RH) by placing FCM eggs on conidia-impregnated filter paper. One-day-old eggs and 1st instar larvae were susceptible to both fungi. In contrast, 5-day-old eggs were susceptible only to M. brunneum. The activity of both fungi against eggs was assessed under two humidity regimes: an optimal for fungal germination - 85% RH, ambient humidity in the lab - 60% RH. The second assay examines whether treated paper will reduce the number hatching eggs? For this purpose pieces of parchment paper that served for laying eggs were treated with each of the fungi and introduced to gravid females at different points in time after they had been inoculated (0, 2, 7 and 14 days). Both fungal species reduced the hatching rate under both humidity regimes compared to control treatments. The third assay evaluated the level of contamination of fruit by FCM that treated with either type of fungi versus control. All citrus fruits were treated with each of the fungi. Eggs were placed on the fruit peels and the fruits were maintained under room conditions (25°C, 60% RH), each of the fungal treatments reduction in fruit colonization by FCM larvae.

CONTRIBUTED PAPERS. Tuesday, 12:15 MC-18 STU

Biological control of pollen beetles with Beauveria bassiana

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Pollin bees (Brassiccogethes spp.) are a main insect pest in colza cultivation across Europe and intensively treated with insecticides. Entomopathogenic fungi, Beauveria bassiana and Metarhizium anisopliae, showed promise in laboratory but only limited impact in field trials when sprayed directly. To improve the efficacy of entomopathogenic fungi in open field applications, we explored co-formulations of B. bassiana spores with natural substances for synergistic interactions or UV-protection. Laboratory results showed a synergistic interaction between blastospores of a Swiss B. bassiana strain and an emulsified colza oil resulting in a pollen beetle mortality of up to 70.8 ± 5.0 (mean ± SE). The synergistic effect accounted for an increase in efficacy of 28%. A stone dust application caused a high pollen beetle mortality of up to 70% ± 13.4 (mean ± SE). However, there was no synergistic interaction when combined with the fungal spores.

Humic acid showed up to 100% UV-protection of UV-B exposed B. bassiana conidia in the laboratory and a 7.8 times higher conidia survival on colza buds in a field trial. Ongoing field evaluations will show if both, synergistic effects and UV protection, help to increase the efficacy of B. bassiana treatments against pollen beetles under real practice conditions.

CONTRIBUTED PAPERS. Tuesday, 12:45 MC-20 STU

The interaction between melon plants and Aphis gossypii is modified by endophytic colonization by Beauveria bassiana

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Entomopathogenic fungi are effectively used for aphid control in spray
treatments, with several mycoinsecticides commercially available in horticulture under greenhouse conditions. In addition, the use of these fungi as a new systemic biological control method by delivering conidia onto the plant surfaces to achieve an artificial endophytic colonization could influence aphid life-table parameters and behavior while reducing plant damage. In this study, two strains of Beauveria bassiana and one of Metarhizium brunneum were assayed against the cotton aphid Aphis gossypii Glover in surface sprayed and endophytically colonized leaves to investigate lethal and sublethal effects. Moreover, dual-choice behavior assays were also performed to assess aphid preference to non-colonized or colonized melon plants. Moreover, volatile profile from entomopathogenic fungi colonized plants were qualitatively and quantitatively analyzed and compared with the one obtained from non-colonized plants. Aphid mortality rates ranged between 48.15 and 56.92 % in leaves epiphytically and endophytically colonized (leaves sprayed with the fungal suspension), and between 37.71 and 49.99 % in endophytically colonized leaves (leaves not sprayed with the fungal suspension). Besides that, there was a significant effect of endophytic colonization on aphid fecundity patterns but did not result in a total increase on the aphid population. Endophytic colonization of the leaves by entomopathogenic fungi did not influence host plant selection by aphids, but they were observed differences in qualitative and quantitative terms between the blend of volatiles released by EF colonized and control plants. Despite these differences did not involve changes in the herbivore behavior, they could provide other benefits to the plant, such as indirect defense, pathogen resistance or abiotic stress tolerance.

CONTRIBUTED PAPERS. Tuesday, 13:00 MC-21

Evaluating insect pathogenic fungi in combination with Heterorhabditis bacteriophora for the management of Helicoverpa armigera

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The current study revealed the impact of Beauveria bassiana and Metarhizium anisopliae alone and in their respective combination with Heterorhabditis bacteriophora on the mortality of second instar larvae of Helicoverpa armigera at 3, 5 and 7 days of post-treatment. In case of alone treatment, the maximum observed mortality was 28.57% against H. bacteriophora followed by B. bassiana (22.44%) and M. anisopliae (17.34%) at 7 days of post-treatment. In case of combined treatments when both applied simultaneously, all intervals showed additive interaction except B. bassiana vs. H. bacteriophora that showed synergism causing mortality (61.85%) only at 7 days of post-application. At 24 hour interval, M. anisopliae vs. H. bacteriophora showed synergistic interaction at 7 days of post-exposure. While the B. bassiana x H. bacteriophora showed additive synergistic interaction at 5 and 7 days of post-application. At 48 hours interval, the combination M. anisopliae vs. H. bacteriophora showed synergism at 5 and 7 days while B. bassiana vs. H. bacteriophora exhibits synergism among all the exposure intervals. The treatment B. bassiana vs. H. bacteriophora yield none at 48 hour interval, while M. anisopliae vs. H. bacteriophora gave 11.67, 9.55 and 7.72% pupae, adult and viable eggs, respectively. Increase trend in incubation, larval, pre-pupal, pupal, total immature and pre-oviposition period was observed among all the treatments, however, reduction was noticed in adult longevity of both sexes, oviposition duration and fecundity among all the tested treatments. Overall the combined application of B. bassiana and H. bacteriophora outperformed if H. bacteriophora applied after 48 hour interval of B. bassiana.

Key words: B. bassiana, M. anisopliae, H. bacteriophora, mortality, sub-lethal impact on development

CONTRIBUTED PAPERS. Tuesday, 13:30 MC-23

Field efficacy of Isaria fumosorosea alone or mixed with horticultural oil for management of the Asian citrus psyllid Pasco B Avery

CONTRIBUTED PAPERS. Tuesday, 13:45 MC-24

Combined effects of Metarhizium robertsi and avermectins on mosquito larvae: survival and immune responses

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Chemical insecticides are still the most important element in the mosquito vector control programs, despite the development of resistance and direct/indirect toxic effects on non-target organisms, including human. Using various microorganisms combined with other biological insecticides may be a promising tool for mosquito vector control. Here we report the susceptibility of A. aegypti larvae and its physiological and immunological parameters to combined treatments of avermectins and entomopathogenic fungus M. robertsi. We also studied the influence of the insecticides on mosquito’s intestinal microbiota. Significant differences in the dynamics of larval mortality between the treatments were observed. Avermectins and M. robertsi conidia lead to 57 and 55% mortality, respectively, and combined treatment – to 99% mortality at the 6th day post treatment (pt). LT50 under combined treatment occurred twice as fast as with avermectin or with fungus treatments. Treatment with fungus decreased phenoloxidase activity, although avermectin increased the enzyme activity. Fungus also decreased dopamine concentration. At initial stage of toxicoses (12 h post treatment) we observed inhibition of glutathione-S-transferase activity under combined treatment. Fungus and avermectins increased protease activity and greatest increase was observed under combined treatment.
Lysozyme activity decreased 12 h pt under combined treatment, but significantly increased 48 h pt. Both protease and lysozyme activity increase may indicate the cells destruction process. Fungus and avermectins affected the microbiome community structure. The greatest changes of the intestinal microbiota were observed under fungal infection. Possible reasons of the synergy are discussed.

A Preliminary Investigation on the Epidemiology and Genetic Diversity of Cnaphalocrocis medinalis Granulovirus

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Body text Full justified, calibri 10 roman/italics, exactly 11 point line spacing 16 points full space after this paragraph. Keep the length of the abstract body text a maximum of 250 word Cnaphalocrocis medinalis is one of the three major pests of rice in China, and the larva roll rice leaf blades longitudinally into tubular chamber by fastening the edges, feed on the epidermis and mesophyll, resulting in restricted growth and even death of rice. The Cnaphalocrocis medinalis granulovirus (CnmeGV) Empty strain was first isolated from a C. medinalis larva in the rice field of Enping city, Guangdong Province, P.R. China in 1979 and genome-sequenced in 2015. CnmeGV can trigger the prevalence of granulosis disease in the population of C. medinalis in the field. Here, we report a recent survey on the CnmeGV epidemiology in southern China and the genetic variation among several CnmeGV isolates.

High Resolution Melting point application to the detection of the relative frequencies of genotypes in mixed infections of CpGV in codling moth

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The High resolution melting (HRM) was adapted to discriminate between two CpGV genotypes that can be present together in treated apple orchards: CpGV-M and CpGV-R5. CpGV-M is the prototype of the Cydia pomonella granulovirus. When a larva carrying the type I resistance ingest CpGV-M alone, the virus replication is blocked. CpGV-R5 is a virus isolate which replication is not affected by the presence of the type I resistance; it replicates similarly in insects carrying or not the type I resistance gene. When both genotypes are present in the same larva, both replicate, and it replicates similarly in insects carrying or not the type I resistance gene. When both genotypes are present in the same larva, both replicate, and it replicates similarly in insects carrying or not the type I resistance gene.

Samples containing 90% of one genotype and 10% of the second are reliably quantified.

Canonical sequence free analyses on the example of thirty isolates of the Cydia pomonella granulovirus (CpGV) and Bombbyx mori nucleopolyhedrovirus (BmNPV) reveal complex variant mixtures within baculovirus populations

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Baculovirus (Baculoviridae, dsDNA) populations, such as field isolates collected from single larvae or limited geographical locations, show fewer intraspecific variations than viruses with high spontaneous mutations rates, such as RNA viruses, where the concept of quasispecies has been applied. The relative stable pass of the replication cycle of baculovirus even within their arthropod host larvae is reflected by the close genetic relationship of geographical far distinct baculoviruses isolates, as it was demonstrated for various Cydia pomonella granulovirus (CpGV) isolates sampled from Mexico, Canada, England, Iran and China. Although CpGV isolates share high colinear genomes, they bear unique genomic features, such as insertion/deletion mutations and most suspiciously single nucleotide polymorphisms (SNPs) that occur randomly all over the genome. In the present study, the genome NGS data of 30 isolates of CpGV and BmNPV were submitted to a consensus sequence free analysis and the entirety of hundreds of variable SNP positions for each baculovirus species was determined. Frequencies of alternative occurring variants at each SNP positions were used for isolate and isolate mixtures detection revealing a high genetic diversity inside CpGV and BmNPV isolates, ranging from highly pure to complex mixtures of natural isolates. A newly developed SNP detection tool circumvented the extraction of consensus sequences for each sequenced isolate; instead a hierarchal clustering method based on principal component analysis was used on an all isolates comprising matrix including all detected SNP positions and frequencies allowing the adequate reflection of taxonomic groups and considering variant mixtures even at very low frequency levels. With the established pipelines for the rapid identification of baculovirus mixtures an important contribution for the understanding of baculovirus population dynamics, such as the co-evolution of variants within natural and commercial isolates, is made that will not only influence quality control measures in commercial baculovirus production systems but will also have impact on the registration process of baculoviruses as biocontrol agents.

Genetic variability in Chrysoideixis includens nucleopolyhedrovirus

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Genetic variability in Chrysoideixis includens nucleopolyhedrovirus inoculated in codling moth larvae in Navarra- Spain.

The research was conducted on Chrysoideixis includens codling moth larvae reared in the laboratory of the University of Navarra. The larvae were infected with CpGV isolate I4-11 that was isolated from a larva collected in the region of Navarra, Spain. The larvae were reared and infected under controlled conditions as described in a previous study (Aguirre et al., 2016). The efficacy of the virus was assessed by counting the number of healthy and infected larvae at different time points after infection.

The results showed that the virus infects the larvae and induces a granulosis disease. The majority of the larvae died within 5 days post-infection, while a few larvae survived and developed into adult moths. The adult moths were reared and infected with a second dose of CpGV to assess the intra-host variability of the virus. The results showed that the virus infects the adult moths and induces a granulosis disease. The majority of the adult moths died within 5 days post-infection, while a few adult moths survived and developed into egg-laying females. The egg-laying females were reared and infected with a third dose of CpGV to assess the inter-host variability of the virus. The results showed that the virus infects the egg-laying females and induces a granulosis disease. The majority of the egg-laying females died within 5 days post-infection, while a few egg-laying females survived and developed into adults. The adults were reared and infected with a fourth dose of CpGV to assess the inter-generational variability of the virus. The results showed that the virus infects the adults and induces a granulosis disease. The majority of the adults died within 5 days post-infection, while a few adults survived and developed into egg-laying females. The egg-laying females were reared and infected with a fifth dose of CpGV to assess the inter-sequential variability of the virus. The results showed that the virus infects the egg-laying females and induces a granulosis disease. The majority of the egg-laying females died within 5 days post-infection, while a few egg-laying females survived and developed into adults.

In conclusion, the results showed that the CpGV isolate I4-11 is effective in controlling the codling moth larvae in Navarra- Spain. The virus infects the larvae and induces a granulosis disease. The majority of the larvae died within 5 days post-infection, while a few larvae survived and developed into adult moths. The adult moths were reared and infected with a second dose of CpGV to assess the intra-host variability of the virus. The results showed that the virus infects the adult moths and induces a granulosis disease. The majority of the adult moths died within 5 days post-infection, while a few adult moths survived and developed into egg-laying females. The egg-laying females were reared and infected with a third dose of CpGV to assess the inter-host variability of the virus. The results showed that the virus infects the egg-laying females and induces a granulosis disease. The majority of the egg-laying females died within 5 days post-infection, while a few egg-laying females survived and developed into adults. The adults were reared and infected with a fourth dose of CpGV to assess the inter-generational variability of the virus. The results showed that the virus infects the adults and induces a granulosis disease. The majority of the adults died within 5 days post-infection, while a few adults survived and developed into egg-laying females. The egg-laying females were reared and infected with a fifth dose of CpGV to assess the inter-sequential variability of the virus. The results showed that the virus infects the egg-laying females and induces a granulosis disease. The majority of the egg-laying females died within 5 days post-infection, while a few egg-laying females survived and developed into adults.
Genetic variability within baculoviruses is becoming increasingly recognized, although the mechanisms that maintain this diversity and its consequences at the genotype and population levels are only just attracting the attention of virologists and ecologists. We examined the genetic diversity of Chrysoideixis includens nucleopolyhedrovirus (ChinNPV) in infected field insects and the genotypic structure of natural isolates. Of a total of eleven baculoviruses isolated from larvae killed by viral infection, collected in soybean fields, nine had genomic DNA restriction profiles distinguishable from each other. An equimolar mixture of these isolates, named Mex-1, was produced to represent the diversity present in the natural ChinNPV population in this region. The insecticidal characteristics of Mex-1 were determined and indicate that this virus population could form the basis for a biocontrol agent. From this mixture, 23 genotypes were isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay. A single-dose mortality assay with each of the genotypes isolated identified “ChinNPV-R” as the most lethal for the insect isolated by plaque-assay.
Managing Ticks with Fungi: The Israeli Experience of tick-Metarhizium interaction
Ment, D.
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In Israel, tick control relies solely on chemical acaricides. Although entomopathogenic fungi (EPF) are natural enemies of veterinary pests as well as tick there is no commercial application of EPF based products against veterinary pests including ticks. As our long-term aim is to promote EPF based products in Israeli agriculture we evaluate EPF application from lab to field through comprehensive studies that evaluate both the intimate host-pathogen interaction during disease progression and EPF efficacy under field conditions. The ixodid ticks, Rhipicephalus annulatus, R. sanguineus and Hyaolomma excavatum were found to be susceptible to Metarhizium and Beauveria. Summarizing our current knowledge regarding tick-Metarhizium interaction, we observed: 1) a great deal of variation in the susceptibility of different tick species to fungal infection, 2) substantial differences in virulence among different Metarhizium species and strains, 3) different fungal species and strains possess unique tolerance to heat stress, 4) formulation effect on fungal efficacy in field trials. By live imaging, we monitored fungal development on ticks with varied susceptibility to fungal infection. Based on these observations, the dynamic of disease progression was characterized and the critical stages leading to successful mycosis were determined. Live imaging to study the dynamic of disease progression is used as our state of the art tool to evaluate the effects of abiotic and biotic factors on disease progression in the research and development of EPF as commercial products.

SYMPOSIUM. Tuesday, 12:48 FS-3

Advances and challenges on the use of entomopathogenic fungi for tick control in Brazil
Fernandes, Everton K.K.; Bittencourt, Vânia R.E.R.
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Tick resistance to chemical acaricides is an incentive to develop fungal products, although currently no product is registered in Brazil. One fungal product registered for biological control of spittlebug in sugarcane is commercially accessible in Brazil and has claimed to control ticks (Acari:
Ixodidae). Entomopathogenic fungi against ticks have been intensively investigated in the last decades; however, their effective use for tick control remains puzzling, and few research groups have dedicated to this purpose worldwide. The development of an appropriate fungal formulation for tick control is necessary to enhance the potential of propagules to tolerate natural abiotic factors and keep them viable and infective. Biological control of ticks faces two major challenges: 1) application of fungi on infested animals, and 2) application on infested environments. Applying fungi on the host is not less or more important than applying fungi in the field; combined, they may be more beneficial to designing effective programs for tick control. Strategies for improving the efficacy of fungi against ticks are the focus of many studies, and conidial formulation in mineral or vegetable oil or oil-in-water emulsion has provided encouraging results and relevant advances. Additionally, fungal propagules other than conidia, e.g., blastospores and microsclerotia, were effective against *R*. microplus in recent laboratory tests. Resistance of ticks to chemical acaricides is a hard and chronic reality in Brazil, particularly for the cattle tick. A well-designed integrated program with use of multiple-effective control strategies seems to be the best approach to minimize the tick burden in livestock.

**SYMPOSIUM. Tuesday, 13:12 FS-4**
Managing Ticks with Fungi: The African Experience  
*Subramanian Sevgan*

**SYMPOSIUM. Tuesday, 13:36 FS-5**
The US Experience of Managing Tick Populations with Fungi: Accomplishments and Challenges  
*Leland, Jarrod*  
1Novozymes BioAg

**Lunch Boxes**  
Tuesday, 14:00-15:00  
Multispace 2

**Excursions and tours**  
Tuesday, 15:00-18:30  
Main entrance

**BBQ**  
Tuesday, 20:00-23:00  
Jardines de la Hacienda

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**SYMPOSIUM. Tuesday, 13:36 FS-5**
The US Experience of Managing Tick Populations with Fungi: Accomplishments and Challenges  
*Leland, Jarrod*  
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**SYMPOSIUM. Tuesday, 13:36 FS-5**
The US Experience of Managing Tick Populations with Fungi: Accomplishments and Challenges  
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Tuesday, 15:00-18:30  
Main entrance

**BBQ**  
Tuesday, 20:00-23:00  
Jardines de la Hacienda

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**CONTRIBUTED PAPERS. Wednesday, 08:30 B-9 STU**

**Determiniation of critical regions for toxicity of the Vip3A protein in European, American, African and Asian pests**  
*Gomis-Cebolla, J.*, Bel, Y.; Ferreira Dos Santos, R.; Wang, Y.; Caballero, J.; Caballero, P.; He, K.; Jurat-Fuentes, J.L.; Ferré, J.*  
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The bacterium *Bacillus thuringiensis* (*Bt*) produces Vip3 proteins with toxicity against several lepidopteran pests during the vegetative growth phase. These Vip3 proteins do not share binding sites with Cry proteins (also from *Bt*), establishing Vip3 proteins as a valuable tool to develop pyramided insect-resistant crops such as corn, cotton, etc. To date, three different Vip3 protein families have been identified based on sequence identity: Vip3A, Vip3B and Vip3C. These different Vip3 protein families display distinct insecticidal spectrum, with Vip3A proteins being active against several insect species, while the Vip3B and Vip3C proteins show a narrow host spectrum. In this study we report the construction of chimeras by exchanging the N-terminal, C-terminal and the region between the first and second protein processing sites (PPS1 and PPS2) of the Vip3Aa and Vip3Ca toxins. Furthermore, the proteolytic activity, tetrameric conformation, protein stability and insecticidal activity of the parental (Vip3Aa and Vip3Ca) and Vip3 chimeric proteins were evaluated in European, North American, African and Asian lepidopteran pests. Results from this study extend our understanding of the critical regions responsible for stability and toxicity of Vip3 proteins.

**CONTRIBUTED PAPERS. Wednesday, 08:45 B-10 STU**

**Characterization of vip3 positive *Bacillus thuringiensis* isolates and toxicity of Vip3Aa65 against lepidopteran pests**  
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The Vip3 proteins produced by *Bacillus thuringiensis* (*Bt*) show toxicity against a wide range of lepidopteran pests. In this study, we sequenced the Vip3 genes of 18 *Bt* isolates previously selected from a *Bt* collection (80 *Bt* isolates) based on theirvip3 gene content. The sequence results indicated that the 18 *Bt* isolates harbored a vip3-like genes with a similarity of 94-100% to either vip3Aa, vip3Af or vip3Ag. Then, the Vip3 expression was determined in the 18 *Bt* isolates by dot blot assay. Four of the isolates had similar expression level to the reference strain, HD1. *Bt* isolate 6A showed the highest Vip3 expression level and toxicity against Spodoptera spp. Also, we selected the vip3-like gene harbored by the *Bt* isolate 6A, Vip3Aa65, to express in the heterologous system *Escherichia coli*.
Helicoverpa armigera and Vip3Aa65 was tested against five lepidopteran species (Spodoptera exigua, Spodoptera littoralis, Spodoptera frugiperda, Grapholitha molesta and Helicoverpa armigera). The Vip3Aa65 was highly toxic against G. molesta and H. armigera, while the Vip3Aa65 protein was less toxic than Vip3Aa16 against three species from the genus Spodoptera.

CONTRIBUTED PAPERS. Wednesday, 09:00 B-11

Empirical test of spatiotemporal alternation strategy of multiple single-gene events for delaying insect resistance to Bt crops

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Although Bt Crops have revolutionized pest control, the benefits of this approach have been reduced by rapid evolution of pest resistance. High-dose refuge strategy plus 'pyramid strategy' for delaying resistance have been widely adopted, especially in the developed countries. However, multiple single-gene events developed by different companies are usually deployed simultaneously in the small landscape farms in developing countries such as China and India, especially in the initial stage of Bt crops commercialization. Therefore, two different events expressing different toxins are planted in simultaneously across (neighbors) fields and/ or in subsequent seasons, which could be considered as "toxin rotation" across generations, i.e. spatiotemporal alternation strategy of multiple single-gene events. Hereby we set out laboratory selection experiments to examine resistance evolutionary trend in Ostrinia furnacalis under alternation of multi-toxins by mixing individual toxins (Cry1Ab, Cry1F, or Cry1Ie) in artificial diet to emulate single-gene Bt maize plants. Under the conditions evaluated, we found that resistance evolution to each toxin was virtually independent in O. furnacalis under multi-toxins alternation scenarios.

CONTRIBUTED PAPERS. Wednesday, 09:15 B-12

Role of Sigma54 on sporulation in Bacillus thuringiensis

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The transcriptional RNA polymerase regulator protein "Sigma54" is reported to control the use of various nitrogen and carbon sources, metabolic pathways and plays a role in virulence and biofilm formation in many bacterial species. We previously constructed a Bacillus thuringiensis HD73 mutant with a deletion of the sigL gene encoding the Sigma54 factor and demonstrated that the Sigma54 factor controlled the g-aminobutyric acid shunt, sarcosine utilization, and lysine metabolic pathway. Here, we show that deletion of sigL gene decreased the sporulation level in vitro and in dead larvae. DNA microarray data revealed that 17 sporulation related genes were downregulated in sigL mutant. Sigma54-dependent transcription strictly requires activation by bacterial enhancer binding proteins (bEBPs). Eight bEBPs with Sigma54 activator domains were identified in the Bt HD73 chromosome. The phenotypic analysis of eight bEBPs mutants showed that motility of six bEBPs mutants was delayed compared to that of the HD73 wild-type strain and five bEBPs mutants decreased sporulation. These results suggest that Sigma54 controls different metabolic pathways, which through the binding of specific bEBPs, influences B. thuringiensis sporulation.

CONTRIBUTED PAPERS. Wednesday, 09:30 B-13 STU

The stationary phase regulator CpcR controls cell differentiation and cry gene expression in Bacillus thuringiensis

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A challenge in bacterial developmental biology is to understand the mechanisms underlying cell fate decisions. Cell differentiation within an isogenic population allows specialization of subpopulations and an efficient division of labor which contributes to the survival of a group of bacteria under unfavorable conditions. We recently reported that strain Bacillus thuringiensis (Bt) LM1212 presents the unique ability to differentiate into two subpopulations during stationary phase: spor-formers and crystal-producers. Here, we identified and characterized the transcriptional regulator CpcR responsible for this differentiation and the expression of the cry genes. This regulator belongs to a family of two-component response regulators. We showed that cpcR transcription is autoregulated. The alignment of LM1212 cry gene promoters revealed the presence of a conserved DNA sequence upstream from the -35 box, which was also found in the promoter of cpcR. Electrophoretic mobility shift assays suggested that CpcR directly controls the transcription of its target genes by binding to this consensus sequence. We showed that CpcR was able to direct the production of a crystal consisting of the heterologous insecticidal Cry1Ab protein in non-sporulating cells of a typical Bt kurstaki strain. Moreover, cpcR expression induced a strong reduction in sporulation. The involvement of CpcR in the sporulation regulatory networks may provide interesting data for a better understanding of the differentiation processes occurring during the stationary phase of Bacillus species.

CONTRIBUTED PAPERS. Wednesday, 09:45 B-14 STU

Bip, a protein required for the integration of planktonic bacteria inside a biofilm

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Bacillus thuringiensis produces a floating biofilm in glass tubes when cultured in static conditions. This biofilm is composed of a pellicle that covers the whole liquid surface and a ring that sticks to the tubes walls. A massive recruitment of planktonic bacteria, located in the culture medium beneath the biofilm, occurs during biofilm formation. Using a built-up device and molecular biology techniques, we were able to quantify the recruited population in the biofilm ring, and to determine the molecular mechanisms involved in this process. Screening of a transposon insertion mutant library for severe recruitment defects led to the discovery of the gene bip (for Biofilm Integration Protein). A prevalence study showed that bip is found in more than 50% of B. thuringiensis strains isolated from Lebanese soils. Deletion of bip by allelic exchange gravely affects the recruitment capacity and complementation of the mutant strain restores
Midgut is the primary site of infection for pathogenic bacteria integrating host resident biofilms. By using immunofluorescence methods we confirmed 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a larval 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a 21 kDa protein which displays two C-terminal transmembrane domains in the wild type phenotype. Located on a 8.5 kb plasmid, bip encodes for a
CONTRIBUTED PAPERS. Wednesday, 08:45 V-18 STU

Bombyx mori nucleopolyhedrovirus Bm8 protein (BV/ODV E-26) suppresses viral gene expression and regulates viral virulence in Bombyx mori larvae

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Bombyx mori nucleopolyhedrovirus (BmNPV) infection in Bombyx mori larvae is tightly regulated in a spatiotemporal manner. Our previous studies identified BmNPV Bm8 protein as a factor regulating viral infection both spatially and temporally. We demonstrated that deletion of Bm8 results in abundant occlusion body production in larval middle silk glands, in which wild type virus rarely propagates. Bm8 deletion virus also exhibits fast-killing phenotype in B. mori larvae. At present, however, it is unclear how Bm8 deletion induces these phenotypes. Here, we first examined the effect of Bm8 deletion on viral gene expression in the tissues other than middle silk glands and found that the Bm8 protein suppresses viral gene expression in the brains and prothoracic glands. We next generated a recombinant virus, Bm8OE, which possesses an additional Bm8 with its endogenous promoter in the upstream of the poly locus and examined its phenotype. The larvae infected with Bm8OE exhibited longer survival time and reduction of occlusion body release in the hemolymph compared with those infected with wild type virus. We further measured V-CATH activity in the larval hemolymph, which is known as one of the viral factors determining host survival time. The hemolymph of larvae infected with Bm8-deletion virus exhibited higher V-CATH activity compared with those from the infected larvae with wild type virus, whereas those from the Bm8OE-infected larvae did not show detectable activity even at the late stage of infection. Taken together, these results suggest that the Bm8 protein regulates the viral factors which have adverse effect on host larvae in order to optimize viral virulence.

CONTRIBUTED PAPERS. Wednesday, 09:00 V-19 STU

Study of host effect in two broad host range alphabaculoviruses after serial passaging

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Mamestra brassicae multiple nucleopolyhedrovirus (MbmNPV) and Helicoverpa armigera multiple nucleopolyhedrovirus (HearMNPV) are alphabaculoviruses that infect a relatively wide range of lepidopteran host species. Genetic and biological studies suggested that both alphabaculoviruses are variants of a single virus species (Rovesti et al., 2000; Choi et al., 2013; Hou et al., 2016). The aim of this study was to investigate whether these alphabaculoviruses had a similar behaviour after serial passage in a permissive host (Spodoptera exigua) and a semipermissive host (Spodoptera littoralis). MbmNPV and HearMNPV were both subjected to six successive passages in S. exigua and S. littoralis. For each host-pathogen combination, three independent infection lines were set up and considered as replications of the experiment. After every passage, DNA was subjected to restriction endonuclease analysis in order to detect possible genetic modifications. After the third and sixth passages biological activity was analysed in terms of pathogenicity (median lethal concentration) and virulence (mean time to death). In three out of four host-pathogen combinations analysed both alphabaculoviruses showed a reduction in biological activity but remained unchanged in the other combination. Slight changes in the genetic structure were detected by restriction enzyme analysis but no correlation with biological modifications were observed. According to these results, changes at the genetic and phenotypic characteristics differed for each host-pathogen system. We conclude that the adaptation ability of baculoviruses to novel hosts is not necessarily predictable and should be analysed independently for each host-pathogen system.

CONTRIBUTED PAPERS. Wednesday, 09:15 V-20

The Two Prevalent Genotypes of an Emerging Infectious Disease, Deformed Wing Virus, Cause Equally Low Pupal Mortality and Equally High Wing Deformities in Host Honey Bees

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Deformed wing virus (DWV) is an emerging infectious disease of the honey bee (Apis mellifera) that is considered a major cause of elevated losses of honey bee colonies. DWV comprises two widespread genotypes: the originally described genotype A, and genotype B. In adult honey bees, DWV-B has been shown to be more virulent than DWV-A. However, their comparative effects on earlier host developmental stages are unknown. Here, we experimentally inoculated honey bee pupae and tested for the relative impact of DWV-A versus DWV-B on mortality and wing deformities in eclosing adults. DWV-A and DWV-B caused similar, and only slightly elevated, pupal mortality (mean 18% greater mortality than control). Both genotypes caused similarly high wing deformities in eclosing adults (mean 60% greater wing deformities than control). Viral titer was high in all of the experimentally inoculated eclosing adults, and was independent of wing deformities, suggesting that the phenotype ‘deformed wings’ is not directly related to viral titer or viral genotype. These viral traits favor the emergence of both genotypes of DWV by not limiting the reproduction of its vector, the ectoparasitoid Varroa destructor mite, in infected pupae, and thereby facilitating the spread of DWV in honey bees infested by the mite.

CONTRIBUTED PAPERS. Wednesday, 09:30 V-21

Identification of genetic loci associated with virulence in Spodoptera litura nucleopolyhedrovirus isolates using deep sequencing approaches and analyses

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Two genetically distinct Spodoptera litura nucleopolyhedrovirus (Splt-NPVs) isolates from Pakistan were investigated by deep sequencing of their full genomes. Previously we reported that isolate SpltNPV-Pak-TAX1 kills S. litura (leafworm) larvae significantly faster than SpltNPV-Pak-BNG (Ali et al., 2018, J. Invertebr. Pathol 153: 2019). Here we report that the genome consensus sequences of these two virus isolates is 99% identical at the nucleotide level, suggesting they are closely related. The major difference between these two isolates is the absence of hr17 (putative enhancer of transcription and origin of replication) in SpltNPV-Pak-TAX1
and the absence of ORF125 with unknown function in SpltNPV-Pak-BNG. Analysis of the rates of nonsynonymous and synonymous single-nucleotide substitutions (dNdSs and dS/dS analysis) showed that strong purifying selection predominates, although for a small number of genes there was neutral or positive selection. The most striking case is ORF122, which encodes a putative viral fibroblast growth factor (FGF), known to be involved in the passage of virus from the midgut to the interior of the larva and linked to virus virulence in other baculoviruses. We found very little polymorphism within both virus isolates, a result at odds with observations for other baculoviruses, possibly suggesting recent dispersal of the virus in Pakistan. We have therefore identified two loci possibly linked to virus virulence in other baculoviruses, possibly suggesting recent dispersal of the virus elsewhere.

**CONTRIBUTED PAPERS. Wednesday, 09:45 V-22**

Investigating the importance of Sindbis virus replication in overcoming the *Aedes aegypti* midgut escape barrier using midgut-restricted viruses  
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Transmission of arboviruses by arthropod vectors exhibits a high degree of specificity. This specificity is due to a number of factors, one of which is the presence of tissue barriers that must be overcome for the virus to be transmitted in the saliva. The midgut escape barrier is one such obstacle which can limit virus dissemination. We aim to elucidate how viral replication in midgut cells contributes to overcoming this barrier using Sindbis virus and *Aedes aegypti*. We have exploited natural midgut-specific miRNAs in *Aedes aegypti* to design viruses that are predicted to have reduced ability to replicate in midgut cells, but should replicate normally in other tissues. These modified viruses contain insertions of complementary target sequences to two midgut-specific miRNAs or control scrambled inserts. When BHK21 cells were transfected with mimics of the midgut-specific miRNAs and subsequently infected with a midgut-restricted virus at a MOI of 0.1, the viral titer was decreased more than 10-fold at 14 hours post-infection (hpi) and nearly 100-fold at 24 hpi compared to cells transfected with a control mimic. The differences in titer were larger and longer lasting when the MOI was reduced to 0.01. No significant differences were observed between the mimic treatments in cells infected with the control scrambled insert virus. We are currently assessing the ability of these viruses to replicate in mosquito midgut and cause disseminated infection. We expect that our results will help us to better understand the nature of the midgut escape barrier and inform arbovirus control strategies.

**CONTRIBUTED PAPERS. Wednesday, 10:00 V-23**

The effect of light signal on the climbing behavior of cotton bollworms infected with NPV  
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The long-term co-evolution of parasites and hosts forms a mechanism for mutual utilization. For example, lepidopteran larvae are manipulated by baculovirus to produce climbing behavior to the top of the host plant and liquefy to die, which is beneficial to the spread of virus, but the reasons and mechanisms of this phenomenon are still unclear. Here, we investigate the effect of light signal on the climbing behavior of *Helicoverpa armigera* larvae infected with *H. armigera* single nucleopolyhedrovirus (HaSNPV). The climbing death height of the infected cotton bollworms increased with the increase of the light position by different light position treatments. The different photoperiods treatments showed that the climbing death height of infected cotton bollworms in the continuous light or normal photoperiod (14L:10D) treatment was significantly higher than continuous dark treatment. Different wavelengths of light treatment showed that the climbing death height in green, blue, or violet treatment were significantly higher than the red or dark treatment. These results indicate that the light signal is the key factor affecting the climbing behavior and has a guiding effect.

**CONTRIBUTED PAPERS. Wednesday, 10:15 V-24**

Characterization of Novel RNA viruses isolated from tsetse fly  
**Glossina morsitans morsitans**  
Meki, I.K. 1, 2; Karithi, H.M. 2; Rezapannah, M. 3; van der Vlugt, R.A.A. 4; Abd-Alla, A.M.M. 5; van Oers, M.M. 6; and Vlak, J.M. 7  
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Two single stranded RNA viruses were detected from G. m. morsitans (tsetse flies) originating from a colony maintained at the Insect Pest Control Laboratory in Seibersdorf, Austria. The genome organisation and the phylogenetic analysis based on the RNA-dependent RNA polymerase revealed that the two viruses belong to the *Iflavirus* and *Negevirus* taxon, respectively. The names proposed for the two viruses are *Glossina morsitans morsitans* *illavirus* (GmmIV) and *Glossina morsitans morsitans* *negevirus* (GmmNV). The GmmIV genome is 9,685 nucleotides long positive-sense single-stranded RNA flanked by 5’ and 3’ untranslated regions, with a 3’ poly(A) tail and encodes a single polyprotein that codes for virion and non-structural proteins. The GmmNV genome consists of 8,140 nucleotides and contains two major overlapping open reading frames (ORF1 and ORF2). ORF1 encodes the largest protein, which includes a ribosomal RNA methyltransferase domain, a helicase domain and a RNA-dependent RNA polymerase domain. In this study, a selective RT-PCR assay to detect the presence of the negative RNA strand for both GmmIV and GmmNV viruses proved that both viruses replicate in G. m. morsitans flies A host range study indicated that both viruses are present in other tsetse species with different prevalence, with the exception of G. pallidipes which show no infection with iflavirus and only 20% infection with Negevirus. Preliminary results on the quantitative PCR analysis indicated that the midgut and carcass showed a high level of virus infection compared to other tissues such as salivary gland, fat bodies, and ovary. The role of these two new viruses in the fly is enigmatic.

**CONTRIBUTED PAPERS. Wednesday, 08:30-10:30 Multispace CD**

Entomopathogenic fungi as endophytes  
**Chairs:** Enrique Quesada-Moraga / Stefan Vidal

**CONTRIBUTED PAPERS. Wednesday, 08:30 F-17**

Direct and indirect effects of exposure to *Metarhizium*-colonized plants on the cotton leafworm Spodoptera littoralis  
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It has been recently reported by several researches that exposition of lepidopteran larvae to entomopathogenic fungi-endophytically colonized plants may lead to medium to low mortality rates not related to fungal
outgrowth, with the underlying cause of death not well understood. The aim of the current work was to investigate the effects of the ingestion of *M. brunneum* EAMb 09/01-Su strain colonized melon plants on *S. littoralis* survival and reproduction. The fungus was able to colonize melon plants (*Cucumis melo* var. *Galia*), with 45.0% leaf colonization at 48 hours post-inoculation. Larvae feeding on fungus-colonized leaves showed a 53.3% mortality and 7.3 d average survival time (AST) that differed significantly from the 16.7% mortality and 6.5 d AST detected in the controls. It was noteworthy that neither signs of fungal outgrowth nor apoptosis related to caspases 3/7 and 8 activities were detected in the cadavers. Meanwhile, fecundity of adult females coming from fungus challenged surviving larvae, 71.2 eggs laid per female, was significantly lower than that of the controls, 219.7 eggs laid per female. These results were discussed in terms of their significance to the mode of action of entomopathogenic fungi when they are offered via colonized plant tissues and on the impact of such colonization of the control of the pest.

**CONTRIBUTED PAPERS. Wednesday, 08:45 F-18 STU**

**Effects of entomopathogenic fungi as wheat endophytes on plant growth, aphid reproduction and regulation of plant enzyme systems**

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Recent research has revealed that entomopathogenic fungi can be natural endophytes and inoculations of plants can cause growth promotion and affect herbivore-plant interactions, most likely by altering plant physiological responses. However, the relationship between plant inoculations using entomopathogenic fungi and plant physiological effects has not been investigated. In the present study, effects of seed treatments by three entomopathogenic fungal isolates, *Beauveria bassiana* (KVL 13-39, obtained from BotanicGard) *Metarhizium brunneum* (KVL 04-57) and *Metarhizium robertsi* (KVL 16-38) on plant growth and reproduction of the bird-cherry oat aphid (*Rhopalosiphum padi*) in wheat were evaluated in greenhouse trials over 20 days. At the end of the experiments, plant physiological responses as variability in selected carbohydrate and antioxidant enzymes were also investigated. The three fungal isolates were able to colonize roots and stems of inoculated wheat plants, and the application of the *M. brunneum* isolate significantly increased plant height and biomass compared to plants of the control and *B. bassiana* treatments. Aphid reproduction was also highest in *M. brunneum* inoculated plants. The profile of degrading carbohydrate and specific antioxidant enzymes in relation to observed plant growth promotion and enhanced aphid reproduction will be discussed. The study provides a link between ecological effects and the physiological responses of wheat plants caused by inoculations with entomopathogenic fungi.

**CONTRIBUTED PAPERS. Wednesday, 09:00 F-19**

**Endophytic *Metarhizium robertsi* Affects Maize Growth and Gene Expression and Growth of Black Cutworm**

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We evaluated V4 maize grown from seed exposed to conidia of *M. robertsi* for endophytic leaf and root colonization, and effects on plant height, chlorophyll content, above-ground biomass, defense gene expression and relative growth rate of second instar black cutworm, *Agrotis ipsilon*. We recovered *M. robertsi* from 91.06 ± 4.05 % (n=116) of maize plants grown from treated seed. Detection was more frequent in root sections (49.66 ± 2.33 %) compared with leaf sections (33.33 ± 2.43 %) of endophytically colonized plants. Height and above-ground biomass of endophytically colonized maize plants was significantly greater when compared to control plants. Chlorophyll content did not differ among treated and control plants. In insect feeding bioassays, the relative growth rate of 2nd instar black cutworm was lower when fed on maize leaves from endophytic plants compared to control plants. Plant defence genes involved in jasmonic acid and salicylic acid pathways were upregulated. In summary, endophytic *M. robertsi* had growth promotive effects on maize plants, growth suppressive effects on black cutworm larvae, and altered the gene expression of key defence genes in maize, suggesting a potentially important role in tri-trophic interactions.

**CONTRIBUTED PAPERS. Wednesday, 09:30 F-21**

**Interaction between *Beauveria bassiana* and the nitrogen fixing bacterium *Sinorhizobium meliloti***

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*Beauveria bassiana* is an entomopathogen that can form intimate relationship with plants, as an endophyte and/or as rhizosphere-competent fungus. When associated with plants, *B. bassiana* antagonizes fungal pathogens via competition for space and nutrients within the plant. This research focuses on the interaction between *B. bassiana* and another beneficial symbiont, the nitrogen fixing bacteria *Sinorhizobium meliloti*. We have examined the interaction between these two microorganisms in vitro, via cross-streak assays, and in vivo using the legume *Medicago sativa* to evaluate the effects of *B. bassiana* on the formation of root nodules by *S. meliloti*. Cross-streak assays showed no signs of inhibition between the bacteria and the fungus. In addition, co-inoculation of *M. sativa* roots with *S. meliloti* and *B. bassiana* not only did not suppress root nodulation but resulted in a greater number of root nodules per plants compared to *S. meliloti* application alone. The potential mechanisms by which *B. bassiana* application increases *S. meliloti* nodulation opportunities and the fitness benefits for *B. bassiana* and *S. meliloti* provided by the tripartite association are discussed.
when studies on in vitro production and formulation were conducted in 2005, but expectations to develop a product arose only after 2015, with the aid of symbiotic bacteria. EPNs have been tested against this insect pest of sugarcane belowground. An alternative for the management of this pest could be the use of biological control agents, especially entomopathogenic fungi, Metarhizium anisopliae, and Steinernema feltiae, which attack larvae and pupae. In this regard, 15 fungal isolates were screened for their endophytic property in tomato and nightshade host plants. Among them, 12 were found endophytic to both host plants with varying colonization rates. Hypocreales lixii F3ST1, Trichoderma asperellum M2RT4 and Trichoderma atroviride F2S21 colonized all the parts of the two host plants, while M. anisopliae isolates ICIPE7, ICIPE30 and ICIPE69 failed to colonize the two plant species. Assessment of pathogenicity and induced systemic resistance to T. absoluta revealed that both tomato and nightshade endophytically-colonized by F3ST1, M2RT4 and F2S21 significantly reduced adult oviposition, leafmining and pupation of T. absoluta as compared to other treatments and the control. Adult emergence from pupae was suppressed by 1.4 times in M2RT4 compared to the control on tomato, while no significant differences were observed between the treatments and control on nightshade.

Tomato is an economically important vegetable crop that is extensively cultivated and consumed in Africa. The invasive tomato leafminer, Tuta absoluta, is a devastating pest of tomato in Africa that causes yield losses of up to 100%. As a quarantine pest, it hinders trade and loss of lucrative export markets for tomato and other solanaceous vegetables. Currently, synthetic insecticides are widely used in its management with negative impacts on human and environment. Entomopathogenic fungus, Metarhizium anisopliae, and Steinernema feltiae, which attack larvae and pupae. In this regard, 15 fungal isolates were screened for their endophytic property in tomato and nightshade host plants. Among them, 12 were found endophytic to both host plants with varying colonization rates. Hypocreales lixii F3ST1, Trichoderma asperellum M2RT4 and Trichoderma atroviride F2S21 colonized all the parts of the two host plants, while M. anisopliae isolates ICIPE7, ICIPE30 and ICIPE69 failed to colonize the two plant species. Assessment of pathogenicity and induced systemic resistance to T. absoluta revealed that both tomato and nightshade endophytically-colonized by F3ST1, M2RT4 and F2S21 significantly reduced adult oviposition, leafmining and pupation of T. absoluta as compared to other treatments and the control. Adult emergence from pupae was suppressed by 1.4 times in M2RT4 compared to the control on tomato, while no significant differences were observed between the treatments and control on nightshade.

Brazil
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Brazil is the world’s largest producer of sugarcane (> 10,000,000 ha). Among the pests that attack the crop, the billbug Sphenophorus levius (Coleoptera: Curculionidae) has gained prominence due to its increasing dissemination and the immense damage the insect inflicts on sugarcane by boring the rhizome belowground. An alternative for the management of this pest could be the use of biological control agents, such as entomopathogenic nematodes (EPNs) that kill the insect with aid of symbiotic bacteria. EPNs have been tested against this insect since 2005, but expectations to develop a product arose only after 2015, when studies on in vitro production and formulation were conducted in the USDA-ARS, SEFTNRL, Byron, GA, USA. Currently, the selected nematode Steinernema rarum is being produced by solid and liquid fermentation processes. The EPNs are being applied in sugarcane fields by an implement that cuts the straw (with a disc) over the ground, and sprays the EPNs directly to the soil. Various dosages are yet to be tested, but some satisfactory results were obtained with 2 x 10^8 IJs/ha (> 50% control). Other more suitable implements to apply EPNs were initially developed to discard the vinasse (alcohol residue) on the sugarcane field, containing 6-8 pipes that deliver the vinasse over the 6-8 cane rows, at the flow rate of 30 m³ of vinasse/ha (3 liter/m). The results presented thus far indicate substantial promise for widespread implementation of EPNs to control billbugs in sugarcane; the outcome will likely have applicability in other crop/pest systems as well.

Acknowledgements: To São Paulo Research Foundation FAPESP (grant 2017/11021-0).

Entomopathogenic nematode application: tools left in the box
Hiltpold, I.
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When it comes to the application of entomopathogenic nematode to control insect pests, scientists are never short of innovative ideas. This contribution will discuss some very original ways to optimally release these beneficial organisms in various environments. Explored techniques will span from application in the furrow together with seeds at planting to the use of drip systems or even the release of previously infected insects in the environment. Benefits and disadvantages of these approaches will be discussed and ways to extend their field of application explored.

Enhancing the aboveground efficacy of entomopathogenic nematodes
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2USDA-ARS, Cattle Fever Tick Research Laboratory, Edinburg, TX, USA.

Entomopathogenic nematodes (EPNs) in the genera Steinernema and Heterorhabditis are used as biocontrol agents to suppress a wide variety of economically important arthropod pests. The nematodes are primarily used to control soil dwelling pests. Aboveground applications have been limited due to the nematode’s sensitivity to UV radiation and desiccation. However, novel approaches have been developed to improve EPN survival and efficacy in aboveground arenas. Nematode strain improvement programs have been developed to enhance UV and desiccation tolerance, e.g., via hybridization and directed selection. The improved strains can be stabilized by relying on homozygous inbred lines to fix beneficial traits. Advanced formulations such as gels, polymers and other mixtures also facilitate prolonged nematode survival and efficacy. These approaches will be discussed along with a focus on case studies involving the control of wood-boring insects and ticks. Further advances in technology to enhance aboveground efficacy will lead to substantial expansion of EPNs as biocontrol agents in a mostly untapped market.
developed against the maize-root feeding larvae of the western corn rootworm (Diabrotica virgifera virgifera, Coleoptera: Chrysomelidae). Commercial products based on Heterorhabditis bacteriophora (Rhabditida: Heterorhabditidae) are available in many world regions. They are also highly effective against D. v. virgifera larvae, and already registered for this insect in several countries. However, whilst laboratory and plant-scale field experiments have shown high control efficacies by those nematodes, larger field-scale trials lead to variable results. We herewith summarise 22 field-scale trials implemented with farmer machinery analysing the dose-efficacy response and its variability under such conditions. Nematodes were applied as fluid stream sprays into the furrow after seed placement, being the most common application method. Nematodes usually appeared as effective as standard pesticides at reducing pest populations; and usually similarly effective or slightly less effective at preventing root damage. Regression models showed that the recommended commercial dose of 2 billion nematodes per hectare appears likely enough for pest management in most cases; this is, keeping the pest and root damage below thresholds. Findings support a nematode-based solution for the biological control of D. v. virgifera larvae in maize fields as one among the alternative options to replace synthetic insecticides.

The studies were funded through the Ministry for Rural Areas and Consumer Protection of Baden-Württemberg, the Bavarian State Ministry of Food, Agriculture and Forestry, and by e-nema, Germany.

SYMPOSIUM. Wednesday, 10:06 NS-5 STU

A novel strategy to control fall armyworm with entomopathogenic nematodes

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Native to the Americas, fall armyworm (FAW; Spodoptera frugiperda, Lepidoptera: Noctuidae) recently invaded most of Africa and large parts of Asia. Its caterpillars cause tremendous damage to a large variety of crops, but most importantly to maize. Therefore, sustainable control measures are urgently needed. This project explores the possibility of using entomopathogenic nematodes (EPN) to control FAW caterpillars. We intend to use locally well-adapted EPNs that have been isolated from soils in Rwanda in 2014 and 2018. To assess the potential of Rwandan EPNs in infecting and killing FAW, we conducted a comparative screening of Rwandan, Mexican and commercially available EPNs. Results show that different steinernematid and heterorhabditid species and strains can effectively infect and kill FAW larvae. The most promising EPNs are currently being incorporated into a carrier that will protect them from desiccation and UV radiation. Carriers considered are alginate beads, alginate gels and sand. To increase the efficacy and specificity of the control measure, we aim to identify attractants and feeding stimulants that could be added to the carrier in order to encourage FAW to feed on the EPN-containing substrate. We plan to apply the carrier into the whorl of maize plants, where FAW caterpillars are mostly found. First laboratory trials showed that this approach has potential to control FAW. R

Coffee Break

Wednesday, 10:30-11:00

Foyer

SYMPOSIUM. Wednesday, 11:00 MCS-1

Realisling the potential: Ecological benefits of microbial biocontrol

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Microbial biocontrol products continue to rapidly increase in use and importance for pest management. However, there is still a tendency to assess the benefits of biopesticides using a traditional pesticide paradigm, that effect is measured through pest mortality and crop production. This ignores the growing evidence that using biopesticide approaches provides numerous ecological and societal benefits. The compatibility of biopesticides in integrated management systems, for example, leads to many long-term advantages and their broader contribution to crop production systems can be underestimated if single crop data is solely evaluated. In this talk and the accompanying presentations in this symposium we will explore the evidence of wider benefits derived from biological approaches, their compatibility other with pest control options (natural and applied), and integration with other emerging crop management strategies.

SYMPOSIUM. Wednesday, 11:30 MCS-2

Entomopathogens as endophytes: Their broader contribution to IPM

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Traditionally, the soil has been considered the main reservoir of entomopathogenic fungi (EPF) as well as the insect populations that they regulate, whereas mostly during the XXI century, it has been highlighted the association of EPF with the plants playing additional ecological roles, in the rhizosphere, in phylloplane, and as plant endophytes. The endophytic behaviour of EPF has altered the rationale behind its use as biocontrol agents in agriculture providing new approaches and delivery methods to pest’s management and crop production. There have been several studies aimed at using these endophytic strains as microbial control agents for the systemic protection of the plant against cryptic pests, whose life cycle seriously limits the effectiveness of chemical insecticides and other control methods. Noteworthy, endophytic EPF may allow not only the systemic protection of the plant against boring, chewing and sucking pests, but even improving the plant response to other biotic (i.e. plant diseases) and abiotic stresses (i.e. nutritional), with promotion of plant health and growth also demonstrated. Compared to conventional mycoinsecticides, the use of EPF as artificial endophytes have the advantage of targeting the pest within the plant at reduced application costs because little inoculum is required in cases were colonization is systemic. Furthermore, the endophytic fungus is protected inside the plant from detrimental abiotic and biotic factors that would limit its use as an epiphyte.
Autographa california multiple nucleopolyhedrovirus (AcMNPV) IE2 was first identified as a transcription factor that increased gene expression from baculovirus reporter genes. Subsequently, it was shown in transient assays to be a factor that increased viral origin dependent DNA replication. In addition IE2 has also been implicated in cell cycle arrest in three different cell types, including Si21, TN-368 and Hz-A1. Viruses containing partial deletions of IE2 had reduced viral DNA synthesis, gene expression, budded virus (BV) production and occlusion body formation in Si21 cells but not TN-SB1-4 cells suggesting it may play a role in virus host range. Plutella xylostella nucleopolyhedrovirus-CL3 (PxyN-CL3) is essentially identical to AcMNPV except for IE2, which retains only 37% amino acid identity PxyN-CL3 has been reported to be over 1000x more virulent for the economically important global pest, Plutella xylostella (diamondback moth) than other AcMNPV wildtype strains thus also implicating IE2 in host range determination. To further investigate the host range properties of IE2 we generated an extensive number of ie2 knockout and repair viruses to further define the function of IE2 and also determine if gene exchange with PxyN-CL3 ie2 can alter AcMNPV host range both in cell culture and in vivo. Unlike previous reports we find that deletion of ie2 cripples virus replication and suggests that ie2 is critical for AcMNPV replication.

CONTRIBUTED PAPERS. Wednesday, 11:15 V-26 STU

The baculovirus Ac108 protein is a per os infectivity factor and a component of the ODV entry complex.

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Baculoviruses orally infect caterpillars when these consume viral occlusion bodies (OBs). These consist of a crystalline protein matrix in which the occlusion-derived viruses (ODVs) are embedded. The protein matrix dissolves in the alkaline environment of the midgut lumen and the liberated ODVs then infect midgut epithelial cells through the action of at least nine different ODV-envelope proteins, called per os infectivity factors (PIFs). These PIFs mediate ODV oral infectivity, but are not involved in the systemic spread of the infection by budded viruses (BVs). Eight of the known PIFs form a multimeric complex, named the ODV entry complex. In this study, we show for Autographa californica multiple nucleopolyhedrovirus that mutation of the ac108 open reading frame abolishes the ODV oral infectivity, while production and infectivity of the BVs remains unaffected. Furthermore, repair of the ac108 mutant completely restored the ODV oral infectivity. With an HA-tagged repair mutant, we demonstrated by western assays to be an accessory factor that increased viral origin dependent DNA replication.  In addition IE2 has also been implicated in cell cycle arrest in three different cell types, including Si21, TN-368 and Hz-A1. Viruses containing partial deletions of IE2 had reduced viral DNA synthesis, gene expression, budded virus (BV) production and occlusion body formation in Si21 cells but not TN-SB1-4 cells suggesting it may play a role in virus host range. Plutella xylostella nucleopolyhedrovirus-CL3 (PxyN-CL3) is essentially identical to AcMNPV except for IE2, which retains only 37% amino acid identity PxyN-CL3 has been reported to be over 1000x more virulent for the economically important global pest, Plutella xylostella (diamondback moth) than other AcMNPV wildtype strains thus also implicating IE2 in host range determination. To further investigate the host range properties of IE2 we generated an extensive number of ie2 knockout and repair viruses to further define the function of IE2 and also determine if gene exchange with PxyN-CL3 ie2 can alter AcMNPV host range both in cell culture and in vivo. Unlike previous reports we find that deletion of ie2 cripples virus replication and suggests that ie2 is critical for AcMNPV replication.
The cysteine-rich region of a baculovirus VP91 protein contributes to the morphogenesis of occlusion bodies

Zhong, Fengqiao1; Kuang, Wenhua1; Wang, Xi2; Hou, Dianhai1; Huang, Huachao1; Sun, Xiailian1; Deng, Fei1; Wang, Huailin1; van Oers, Monique M.4; Wang, Manli1; Hu, Zhihong1

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The core proteins of baculoviruses play an important role(s) in different processes of virus infection. In this study, we dissected the function of a core protein VP91 (HA76) in Helicoverpa armigera nucleopolyhedrovirus (HearNPV). HA76 was expressed at the late stage of HearNPV infection; deletion of ha76 showed that the gene is required for budded virus production. A series of recombinants with truncated ha76 was constructed and analyzed in vitro and in vivo. The results showed that the region encoding the C-terminus of HA76 was essential for nucleocapsid assembly, whereas the N-terminal cysteine-rich region was responsible for oral infection. This is in agreement with previous findings for VP91 of Autographa californica multiple nucleopolyhedrovirus (AcMNPV), the prototype of baculovirus that the protein is essential for nucleocapsid assembly and consequent BV production, as well as oral infectivity. However, electron microscopic analyses further showed that the cysteine-rich region contributed to morphogenesis of occlusion bodies (OBs), with amino acids 136–223 (namely CR1) of HA76 being critical for this function. The results revealed a novel function of VP91 and suggested that the impact on OB morphogenesis is partially related to oral infectivity. Together, these findings suggest VP91 is a key protein with multiple functions for baculovirus infection.

Virus biology of Euscelidius variagatus iflavirus 1: towards the production of an infectious viral clone

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Euscelidius variagatus Kirschbaum (Hemiptera Cicadellidae) is a well-known leafhopper vector of phytoplasmas. A new member of a new species of the genus Iflavirus was discovered during transcriptomic analyses of a laboratory population of E. variagatus, and named E. variagatus virus 1 (EV1V). EV1V infection was asymptomatic, and the virus was constantly detected both in phytoplasma exposed and not exposed vectors. Interestingly, virus load was significantly lower in the former category. Two other E. variagatus populations from France and US were found to be virus free (EV1V-), and one virus free population was therefore established in our lab starting from the French one. EV1V distribution was assessed by PCR on total RNAs extracted from dissected organs and insects at different life stages. The virus was present in all tested organs and life stages, with different loads. Vertical transmission through the ovary was confirmed. EV1V was also detected in the honeydew of infected individuals, but viral transmission could not be obtained by feeding on artificial medium containing virus-contaminated honeydew. Horizontal transmission through co-feeding of EV1V-infected and healthy insects, resulted in low infection rates. Plants exposed to infected insects
could be contaminated on the plant surface, but were generally virus-free, therefore horizontal transmission through the plant was, at best, extremely inefficient. An infectious clone was constructed and its ability to infect and replicate in virus-free insects was demonstrated. The application of the infectious clone for virus-induced gene silencing to interfere with insect ability to transmit the phytoplasma is ongoing.

CONTRIBUTED PAPERS. Wednesday, 12:45 V-32

Development of autofluorescent baculoviruses to follow infection in living cells
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The ANCHORTM technology has been adapted to the model baculovirus, AcMNPV, as a TM system. The virus obtained (Ixodida: Ixodidae), is widespread in Korea, China as well as Australia, and has spread to the USA and European countries. Longhorned tick is one of the vectors of severe fever with thrombocytopenia syndrome virus in human. The tick occurs in mostly grass fields, and the use of pyrethroid insecticides induced pest resistance and environmental residual toxicity. Particularly the use of chemicals near residential areas where persons live become a big issue. In this work, our interest was given to the selection of highly virulent fungal isolates against longhorned tick. A total of 101 fungal pathogens were assayed by a dipping the nymph stage of ticks into a conidial suspension (1×107 spores/ml). Interestingly of the several species, showed high virulence and mycosis were observed in 7-15 days. Highly virulent strains were selected, and semi-field experiments were conducted. As a result, the control efficacy of M. anisopliae was over 80% at 30 days of treatment. Additionally, we analyzed whole genome sequence of the highly virulent M. anisopliae and genetic diversity with other M. anisopliae isolates. This work suggests that entomopathogenic fungi, particularly Metarhizium anisopliae could be used to control longhorned ticks as an environmentally sound way.

CONTRIBUTED PAPERS. Wednesday, 11:15 F-26 STU

e-Biopesticide: Management of silverleaf whitefly, Bemisia tabaci using entomopathogenic Beauveria bassiana
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Silverleaf whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae) is a major agricultural pest that is distributed worldwide and cause serious damage to crops. Among the various ecological types, B and Q are the most problematic. Especially, biotype Q mediates more than 40 kinds of viruses, including tomato yellow leaf curl virus (TYLCV). Whitefly is resistant to various chemical agents including neonicotinoid insecticides. Therefore, we tried to use entomopathogenic fungi for eco-friendly management of this pest. These fungi are a natural pathogen of their invertebrate host and contribute to the regulation of their host population in the environment. In this study, we screened pathogenic fungi against the nymphal stage of whitefly. A total of 72 entomopathogenic fungi were collected from soil in Korea and virulence assay was conducted with conidial suspension (1×107 spores/ml) in laboratory conditions. As a result, the characteristics of 10 isolates showing 80-100% virulence at 5 days after treatment were compared, and finally, 2 isolates were selected. Lastly, we confirmed the conidia productivity and thermotolerance of the selected isolates when different substrates were used in cultures. We have a plan to evaluate the control of the isolates under semi-field conditions.

CONTRIBUTED PAPERS. Wednesday, 11:30 F-27 STU

isScreening Potential Entomopathogenic Fungi for the Control of the Greenhouse Whitefly (Trialeurodes vaporariorum)
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Greenhouse whitefly (Trialeurodes vaporariorum) (GHWF) is a globally important pest, causing significant damage to >250 plant species by consuming sap, secreting honeydew and transmitting viral plant diseases. Entomopathogenic fungi (EPF) are being developed for biological control of whitefly in integrated pest control strategies which include reducing chemical applications and increasing the use of sustainable alternatives. Whitefly have piercing-sucking mouthparts, therefore EPF are the most promising microbial natural enemies to be exploited for whitefly control as they can infect directly through the integument. Temperature conditions
with temperate greenhouses vary from 10 to 35°C which presents a challenging abiotic environment for EPF and impacts greatly on pest mortality. EPF used as biological control for GHWF need to be highly virulent and work across a range of temperatures, therefore, we compared temperature profiles of eighteen EPF originating from temperate, subtropical or tropical regions. In vitro germination, growth and spore production experiments were conducted as well as an investigation into the pathogenicity of these EPF isolates to third instar greenhouse whitefly using a novel standardised bioassay design, with a bespoke benchtop sprayer. Using the data collected, we were able to rank isolates based on growth rate, germination and spore production at a range of temperatures to select those with distinctive temperature profiles. The methods employed in this study could be utilised in the selection of isolates for microbial control of whitefly and in particular, selecting pathogens to be co-applied with the potential to improve virulence or cause an increase in workable climatic conditions.

CONTRIBUTED PAPERS. Wednesday, 11:45 F-28
Development of a biological tick control agent based on an innovative attract-and-kill strategy
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Ticks are vectors for a multitude of pathogens, causing, e.g., Lyme disease and tick-borne encephalitis. 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 work is the development of a novel biological control agent against ticks based on an innovative attract-and-kill approach. The basis of the control agent is the attractive effect of CO₂ together with further tick-specific attractants and combined with an entomopathogenic fungus (*Metarhizium spp.*) as kill component. In olfactometer trials with *I. ricinus* nymphs, the attractive effect of CO₂ released by encapsulated baker’s yeast was investigated and for the first time distinct attractive concentration ranges were identified. Beside CO₂ more specific volatile attractants and aggregation pheromones were identified. In order to find a suitable kill component, several *Metarhizium* spp. isolates were screened not only for their virulence against *I. ricinus*, but also for biotechnological criteria. An isolate of *M. pemphigi* isolated from a dead German tick showed the highest virulence (>65% dead nymphs after 11 days) combined with a high blastospore (BS) concentration in liquid cultivation. When co-encapsulated in calcium alginate with a substrate, *M. pemphigi* was able to sporulate on the bead surface resulting in 1.6x10⁷ conidia/bead. The newly formed aerial conidia were highly virulent against *I. ricinus* nymphs with 100% of dead nymphs after 10 days. Altogether, this work will pave the way for novel tick control strategies.

CONTRIBUTED PAPERS. Wednesday, 12:15 F-30
Are phytopathogenic fungi capable of producing insecticidal metabolites?
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In recent years some evidence of production of insecticidal metabolites by necrotrophic and hemibiotrophic plant pathogenic fungi was accumulated. However, their production in vitro and in planta, chemical structure, spectrum of biological activity were not well studied. Techniques used in invertebrate pathology were applied to study insecticidal properties of secondary metabolites produced by some fungal pathogens of cereals and weeds. The cereal pathogens (*Bipolaris sorokiniana*, *Gibellina cerealis*, *Pyrenophora tritici-repentis*, *Parasatagonospora nodorum*) demonstrated high potential to produce insecticidal metabolites when tested against the cereal aphid, *Schizaphis graminis*. Aphidicidal activity of 0.5% extracts from the fungal cultures in some treatments reached 90–100% (e.g., some extracts from *P. nodorum*). Despite some fungal extracts being phytotoxic when tested on wheat leaf segments, no significant correlation was found between aphidicidal and phytotoxic activity. This can indicate that metabolites produced by the cereal pathogens can act directly on the aphid. Substrate composition had considerable impact HPLC/UV spectra-profiles of the extracts. Comparative analysis found differences in composition of extracts with different levels of insecticidal activity. Using the model system «B. sorokiniana-wheat-cereal aphid» the production of insecticidal metabolites in planta by the phytopathogenic fungus was demonstrated. Screening some phytopathogens from the genus *Alternaria* showed high potential of this fungus to produce insecticidal metabolites. The research was supported by Russian Fund of Basic Research (project # 17-04-01445).

CONTRIBUTED PAPERS. Wednesday, 12:00 F-29 STU
Effectiveness of entomopathogenic *Beauveria pseudobassiana* on *Corythucha arcuata* in laboratory conditions
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Invasive pest oak lace bug (*Corythucha arcuata*), (Hemiptera: Tingidae) was first recorded in eastern Croatia in 2013 in lowland stands of pedunculate oak (*Quercus robur* L.), from where it expanded to the west causing summer yellowing and intensive chlorotic damages of oak leaves. In 2018 entomopathogenic fungus *Beauveria pseudobassiana* Rehner et Humber (*Hypocreales Cordycipitaceae*) was isolated from several dead *C. arcuata* adults found in moss, where one part of population usually overwinters. Two isolates were used for testing of fungus virulence against *C. arcuata* under laboratory conditions. After preparation of conidial suspensions with desired 1 x 10⁷ concentrations healthy adults of *C. arcuata* were treated by spraying the moss where they were previously put on. Results showed no difference in mortality rate between isolates (96-97%), but isolate BBCN02 produced mycosis value of 49 % which was higher than mycosis value of 32 % produced by isolate BBC10, with 4 % of mycosis value in control. In nature these results indicate possible rise of entomopathogenic *B. pseudobassiana* natural inoculum up to 12 X. Moreover, we estimated the number of overwintering adults in 1 m² of moss by counting them and calculating their average number in 6 m² of moss collected in six different locations in infested oak forest. Results demonstrated high rate of dead overwintering individuals, with approximately 15 % of them infested by various entomopathogenic fungus. The results presented here will be beneficial for future investigations of effectiveness of naturally occurring *B. pseudobassiana* on *C. arcuata* in field conditions.
Thiourea as polyphenoloxidase inhibitor accelerate the *Galleria mellonella*’s infection by entomopathogenic nematode (*Heterorhabditis beicherriana*)

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Entomopathogenic nematodes (EPN) are commonly used as biocontrol agents. Infective juveniles (IJ) seek out insects in soil. These IJs are resistant to stress and can survive without feeding for prolonged periods, especially at lower temperatures. Storage temperature has drastic effects on EPN IJs’ behaviour and lifespans, and the molecular basis for this is not well understood.

In the present study, *Steinernema carpocapsae* and *Heterorhabditis megidis* IJs were stored at 9°C for up to 12 weeks or at 20°C for up to 6 weeks, and protein expression was examined at specific intervals. Proteins were identified and quantified using a label-free, proteomic quantitative approach. Out of the thousands of proteins identified, statistically significant (p<0.05) and differentially abundant proteins were selected for further analysis. There is a paucity of data regarding the change in the somatic proteome of nematode dauer/IJs in relation to temperature and time, and the extent to which the somatic proteome of these two parasites conform and differ are of interest. The differential protein expression of IJs may assist in understanding the effect of storage temperature on their lifespan, and on their behaviour over time. This may enhance EPN IJs properties as biopesticides.

**Contribution papers.** Wednesday, 11:00 N-17

**Cancelled**
Influence of *Photorhabdus luminescens* density on the life history traits of *Heterorhabditis bacteriophora* and bacterial exchange on virulence and reproduction

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Life history traits (LH ts) of *Heterorhabditis bacteriophora* were assessed at 25°C in a hanging drop technique using *Photorhabdus luminescens* in a semi-solid nematode growth medium at cell densities ranging from 2.5-20 × 10⁹ cells ml⁻¹. Increasing food density had a significant positive influence on the body volume of hermaphrodites. The offspring production ranged from 50-269 juveniles/hermaphrodite. The lifespan of hermaphrodites, which was predetermined by the beginning of the *endotokia matricida*, remained similar (8 days). In another investigation six different *P. luminescens* strains/species were combined with two *H. bacteriophora* strains in liquid culture and the effect on dauer juveniles (DJ recovery, yield and virulence was recorded. Exchange of symbiotic bacteria affected DJ recovery that ranged from 20-88%. Similarly, DJ yield was significantly influenced by the type of bacterial strain. It was found that native bacteria are not necessarily more virulent or provide the highest yield. However, variation between the two nematode strains in DJ recovery and yield was higher compared with variations obtained with exchanging the bacterial strains. The virulence of a nematode-bacterium combination seems to be governed more by the nematode strain than the associated bacterial strain.

CONTRIBUTED PAPERS. Wednesday, 12:15 N-22 STU

Isolation, identification and nematcidal activity of secondary metabolites produced by the entomopathogenic bacterium *Photorhabdus luminescens sonorenensis* (Enterobacteriaceae) against the root knot nematode, *Meloidogyne incognita* (Tylenchidae)

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The screening of several chemical nematicides has prompted the need for new and environmentally-friendly methods to enhance current management of plant parasitic nematodes. Insect pathogenic *Photorhabdus* bacteria, the natural symbionts of *Heterorhabditis* entomopathogenic nematodes, are considered a goldmine for the discovery and application of biologically active secondary metabolites (SMs) with antibacterial, anti-fungal, insecticidal, and nematicidal activities. In this study, we evaluated three metabolites that were isolated and purified from culture filtrates of *Photorhabdus luminescens sonorenensis* (strain CaboCorca). The chemical identification of active SMs was done by bioassay-guided fractionation. Spectral analyses identified two of these compounds as phenylpropanoids (AK1 and AK2) and one alkaloid (AK3). In vitro assays were carried out to assess the nematiccidal activity of these SMs on the infective stage (second juvenile stage or J2) of the root-knot nematode, *Meloidogyne incognita*. The activity of these SMs was also tested on four non-target nematode species: *Caenorhabditis elegans* (free-living bacterivore) and three entomopathogenic species, *Steinernema carpocapsae*, *H. bacteriophora*, and *H. sonorenensis*. These compounds revealed different inhibitory activity ranging from a transient quiescence to death. AK1 and AK2 exhibited nematicidcial activity to *M. incognita*. The LC₅₀ for AK1 was 64 µg/ml and 45 µg/ml for AK2. AK3 showed nematicidal activity to *M. incognita* and *C. elegans* at the two highest concentrations tested (300 and 400 µg/ml). At 60 to 200 µg/ml, AK3 induced reversible quiescence in both nematode species. All entomopathogenic species tested were resistant to AK3. This work sheds light on ascertaining the potency of the *Photorhabdus*-derived SMs as nematicicides.

CONTRIBUTED PAPERS. Wednesday, 12:30 N-23

Improving virulence and post-application longevity of *Heterorhabditis bacteriophora* dauer juveniles through selection and breeding

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Entomopathogenic nematodes to control the Western Corn Rootworm (WCR) are applied into the soil together with the maize seeds at sowing time, when eggs are still in diapause. Therefore, genetic improvement of persistence is crucial as nematodes must survive and remain infective for 2 to 6 weeks until insect larvae hatch. Wild-type strains were phenotypically characterized for virulence towards WCR larvae and larvae of *Tenebrio molitor*. LD₅₀ for *T. molitor* ranged between 1.4 to 30.5 dauer juveniles (DJs) per insect and for WCR from 10.3 to 218 DJs. Nematode persistence was assessed by subsequent baiting the soil using *T. molitor* as trap insect. Nematodes survived and were able to infect the target pest after six weeks of incubation at 17°C. A wild type strain with best performance in virulence and persistence was selected over several cycles in sand bio-assays with WCR larvae and resulted in an increased virulence in four consecutive selection cycles. Results of persistence from sand assays correlate with longevity and oxidative stress resistance assays. Nematode persistence at a commercial application dosages of 2 billion DJs ha⁻¹ and the target dose of 1 billion DJs ha⁻¹ did not significantly differ. These results support the hypothesis that more persistent and more virulent strains enable the reduction of the application dosage and consequently reduce nematode application costs. Phenotypic data are being combined with genotypic data to find molecular markers to assist in nematode breeding.
formed in many important issues by reading unsubstantiated information in printed and online news. Furthermore, misinformation is widely and quickly spread via social media networking platforms. I will discuss how communicating science to the public, starting with our local communities and expanding beyond, furthers our leadership and builds our public professional image but, more importantly, fosters a civic sense of trust in science, educates the public, leads to job creation, increases federal research funding, and ensures wise policymaking and citizen decisions. Effective science communication curtails fake news and presents facts rather than “alternative facts” in truthful communication exchanges. I will provide examples of how to engage the public with storytelling, improvisation, theatrical techniques, and personable messages using different venues and distribution modes. As each of us effectively advocates for the value of science, misconceptions that support political agendas or anti-science groups will taper, augmenting public confidence in the scientific process and engaging the next generation of well-informed young scientists and citizens.

### Analysis of *Bacillus thuringiensis* diversity from different environments

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The *hag* gene has been established as a classification tool for *B. thuringiensis* strains in one of the 71 known H serotypes. This gene has two conserved regions and a central highly variable region, which presumably harbors the epitope responsible for eliciting the immunological reaction in H serotyping. The Bt communities from two animal farms and from an open field agroecosystem were studied using the variability of the *hag* gene as criterion (Xu and Côté, 2008). Nucleotide sequences related to known flagellin sequences were found in Bt strains, some of them with an identity lower than 85% but keeping the two conserved regions on both sides of *hag* gene. Particularly, these sequences with low identity are more abundant in Bt strains from animal farms. The comparison of Bt diversity shows differences among these ecosystems, obtaining greater diversity of serotypes from the open field agroecosystem. Crystal proteins of the families Cry1, Cry2, and Cry9 were found in Bt strains isolated from both kind of environments. Other novel proteins closely related to Cry5 (nematocidal) or to the anti-coleopteran Cry8 and Cry34/Cry35 were present in Bt strains from animal farms. For the Bt strains included in this study, it was possible to establish a correlation among flagellin nucleotide sequences, predicted H serotypes and crystal protein compositions. We discussed the possibility of using the diversity of the variable part of the *hag* gene as a criterion to analyze the serological diversity of Bt and on this basis predict the presence of Bt strains carrying new insecticidal genes.

### Whole-genome phylogeny and taxonomy of *Bacillus thuringiensis* strains by composition vector analysis

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The goal of this study was to find a suitable method for *Bacillus thuringiensis* (Bt) strains typing using whole genome sequence information that could be used as a reliable approach for typing new Bt strains. In this work we compared four typing methods, including multi-locus sequence typing (MLST), single-copy core genes phylogenetic analysis (SCCGPA), dispensable genes pattern analysis (DGCPA) and composition vector typing (CVT) to analyze genomic variability of 23 Bt strains that are standards from Bt serovars aizawai, kurstaki, israelensis, thuringiensis, and morrisoni. We found that CVT method is the best option to be used for typing new Bt strains since it uses all coding protein data from each strain resulting in in a high-level strain resolution, in contrast to MLST, SCCGPA and DGCPA that use partial sequence data. Furthermore, the CVT is the fastest algorithm since it uses k-mer to analyze composition vector being significantly much faster than the other two whole genome-based methods, SCCGPA and DGCPA. In addition, CVT proved to be highly valuable since it also allows characterization of the content of virulence and insecticidal genes in the Bt strains. Then, we performed a CVT analysis of 190 Bt strains from 86 different serovars, and chose boundaries for defining new typing nomenclatural ranks of these Bt strains, based on dissimilarity coefficients (DC) values. We propose that the CVT method could be used for typing Bt strains since is a fast reliable method that allows distinguishing and clustering Bt strains using whole genomic information.
Identification and functional analysis of two novel Cry proteins from Paenibacillus popilliae ATCC14706

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Paenibacillus popilliae is an obligate pathogen of scarab beetles, first isolated from milky diseased larvae of Japanese beetle (Popillia japonica). This bacterium enters into the alimentary tract of the host perorally and transfers from the midgut to hemocoel. It propages in body fluids for several days, and eventually kill host because of septicemia. However, the factors regulating translocation of bacterial cells from the host midgut to hemocoel has not been elucidated. In previous studies, it was reported that P. popilliae forms parasporal body containing Cry18Aa1 upon sporation, yet to be mentioned about its function or relation to the mechanism of infection. In this study, we searched for proteins necessary for P. popilliae infection based on the whole genome sequence of the bacterium, and confirmed two novel Cry proteins. In order to determine whether the two novel Cry proteins exert their activity in the host midgut, recombinant proteins were expressed in Escherichia coli and treated with a digestive fluid extracted from the host midgut of Japanese beetles. These proteins were processed by gut juice, indicating some activity in the host midgut. In order to investigate the influence of these two novel Cry proteins on P. popilliae infection to host insects, bioassays should be conducted using primary cell culture of the host midgut as well as the larva of the scarab beetles.

Novel mosquitocidal toxins from Paraclostridia

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New biological insecticides with different mode of actions are needed in order to broaden the range of susceptibility and also to avoid mosquito resistance to presently used biologicals. We recently reported the identification of novel toxin from two Paraclostridium bifermentans mosquitocidal strains that are active primarily against Anopheles species. The toxin is encoded in a megaplasmid, and we show that this toxin has very high activity being an enzyme and cleaves an intracellular target in the nervous system.

Drosophila suzukii bacterial interaction and their potential in biological control

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The invasive insect pest Drosophila suzukii causes considerable economic damage to thin-skinned ripening fruits in Europe and the Americas. Novel, environmentally safe approaches will be needed to control the spread of this species and entomopathogenic bacteria offer a possible solution for the use in biological control. We have collected and analyzed various fruits infested with D. suzukii larvae and have sampled a selection of moribund larvae from three independent locations in Hesse, Germany during two separate field seasons. Next, we have studied the associated bacteria of moribund D. suzukii larvae and created a library of analyzed and sequenced bacteria species. We infected D. suzukii flies with the strains to test their potential insecticidal activity and could categorize the strains in beneficial and detrimental bacteria. Mixing experiments with detrimental and beneficial bacteria in combination indicate a complex interaction between D. suzukii and the bacteria. Thus, our study provides an insight in the host-pathogen interaction of the invasive species D. suzukii and may lead to the isolation and identification of bacteria suitable for the development in pest control.

Histopathology of Anticarsia gemmatalis strains susceptible and resistant to Cry1Ac protein and their susceptibility to bioinsecticides based on Bacillus thuringiensis

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The velvetbean caterpillar, Anticarsia gemmatalis Hubner, can cause severe damage to soybean in Brazil. Studies of the susceptibility of these insects to commercial products that have different Cry toxins are necessary due to factors that may be related to the development of resistance to Bt-soybean already used in their control. Our objectives were: 1) to determine the response of one A. gemmatalis strain resistant to Cry1Ac (382x) and one susceptible to biological insecticides based on Bacillus thuringiensis strains; and 2) to quantify cellular alterations in the midgut of both A. gemmatalis strains after inoculation with B. thuringiensis spp. kurstaki HD-73, a Cry1Ac producer strain. Insects were exposed to the commercial products Agreen®, Dipel®, and Xentari® incorporated into the artificial diet. Mortality data were analyzed using Weibull-2 model to determine statistical differences between treatments. To quantify differences between midgut cells, both strains of A. gemmatalis were inoculated with HD-73. The biological product that showed the greatest activity against both strains of A. gemmatalis was Xentari®. The product with the lowest biological activity was Dipel®, exhibiting 42-fold lower activity against the resistant population compared to the susceptible strain, followed by Agreen® and Xentari®, which displayed approximately 18- and 11-fold lower activity, respectively. Significant differences were observed in the number of columnar, goblet, and regenerative cells from the midgut in susceptible insects challenged with HD-73. No alterations in the number of cells (columnar, goblet, or regenerative) were observed in the resistant strain after bacterial challenge.

Gene interaction networks in Helicoverpa zea challenged with Cry1Ac toxin from Bacillus thuringiensis

Perera, O.P.; Polpitiya, A.D.
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Biological systems use regulatory pathways to transmit signals and coordinate multiple processes. Gene transcription networks exhibit an approximately coordinate multiple processes and to efficiently respond to internal and external stimuli. Gene transcription networks exhibit an approximately coordinate multiple processes and to efficiently respond to internal and external stimuli. Gene transcription networks exhibit an approximately coordinate multiple processes and to efficiently respond to internal and external stimuli.
study, third instar larvae of a Cry1Ac tolerant strain of *H. zea* was challenged with a discriminating dose of Cry1Ac toxin and gene expression profiles were generated using RNA-Seq. Approximately 20 million reads from each replicate were mapped to the transcriptome and weighted co-expression networks that exhibit a scale-free topology were identified. The interactive modules obtained from this analysis identified transcripts co-regulated with some of the genes associated with *Bt* resistance and/or mode of action.

CONTRIBUTED PAPERS. Wednesday, 14:30-16:30
Multispace AB

Immunity and host response

Chair: Bergmann Ribeiro / Sassan Asgari

CONTRIBUTED PAPERS. Wednesday, 14:30 V-33

Mitochondrial and Innate Immunity Transcriptomes from *Spodoptera frugiperda* Larvae Infected with the *Spodoptera frugiperda* ascovirus

Heba A. H. Zaghloul1, Robert Hice2, Peter Arensburger3, and Brian A. Federici3,4

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Ascoviruses are large, enveloped DNA viruses that induce remarkable changes in cellular architecture during which the cell is partitioned into numerous vesicles for viral replication. Previous studies show these vesicles arise from a process resembling apoptosis, yet differ after nuclear lysis that mitochondria are not degraded but modified by the virus, changing in size, shape, and mobility. Moreover, infection does not provoke an obvious innate immune response. Thus, in the present study we used *in vivo* RNA-sequencing to determine whether infection by the *Spodoptera frugiperda* ascovirus (SfIAV-1a) modified expression of host mitochondrial, cytoskeletal and innate immunity genes. We show that while expression of many mitochondrial genes was similar to uninfected controls, others were upregulated, especially ATP8 synthease during vesicle formation, indicating the importance of conserving these organelles for virus replication. Of 106 genes that code for cytoskeleton proteins only three gene orthologs of kinesin and a dynein, major mitochondrial motor proteins, were upregulated more than two-fold. Similar moderate increases also occurred in expression of many Toll, melanization and phagocytosis immunity genes and their negative regulators. However, genes for the antimicrobial peptides moricin and golverin were upregulated more than 32-fold, and those for lebocin-1, lebocin-2 and Hdd23-like proteins, also antimicrobial, were upregulated more than 15-fold, as was a phenoloxidase inhibitor gene. SfIAV-1a destroys most fat body cells by seven days after infection, so expression of these innate immunity genes apparently occurs in remaining intact cells in this tissue and possibly others such as the epidermis and tracheal matrix.

CONTRIBUTED PAPERS. Wednesday, 14:45 V-34 STU

Polydnavirus regulates the extracellular adenosine levels in *Spodoptera litura* to suppress its immune system

Yuan Chang; Yueh-Lung Wu

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Immune systems require enormous amounts of energy, so organisms tend to redistribute energy from storage and development functions when infected by pathogens. Recent studies in *Drosophila melanogaster* also indicated that increased extracellular adenosine upon wasp or bacterial infection induces the metabolic switch between developmental tissues and immune cells, which slows down the development of infected larvae but benefits the immune system against pathogens. Extracellular adenosine is a energy signal that mediates the metabolic switch to increase cellular glycolysis, allowing the fat body to convert glucose to trehalose, which is then released to the circulation systems to facilitate immune cell differentiation and activate immune responses. Polydnaviruses (PDVs) are parasitoid symbionts that only replicate in calyx cells of the wasp. When the parasitoid *Snelleinius manila* infects its eggs into a host, PDVs are also spread into *Spodoptera litura*. Thus, the immune system of the host is suppressed by the PDV. We found that PDV affects adenosine levels and immune responses. Moreover, after PDV infection, gene expression levels of adenosine receptors and adenosine deaminase-related growth factors in *S. litura* were significantly reduced. Additionally, carbohydrate metabolism was affected by adenosine signaling, leading to significant decreases in immune responses, proving that PDV affects host immune responses through regulation of adenosine content. Injection of artificial adenosine increased host immunity and significantly lowered parasitism rates. Our study showed that PDV can inhibit immune responses through inhibition of adenosine signaling. This result might bring a breakthrough for enhance biological control by using adenosine inhibitors to suppress immune responses of pests.

CONTRIBUTED PAPERS. Wednesday, 15:00 V-35 STU

The role of baculovirus P26 in suppressing the insect melanization response

Yin, Mengyi1,2; Kuang, Wenhua3; Wang, Qianran1,2; Yuan, Chunfei4; Lin, Zhe4; Gong, Peng4; Zou, Zhen4; Hu, Zhihong4; Wang, Manli4;5

1State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P. R. China; 2University of the Chinese Academy of Sciences, Beijing 100049, P. R. China; 3Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, P. R. China; 4State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.

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Melanization is a prominent humoral immune response widely found in arthropods. The process is initiated by a modular serine protease (SP), which sequentially activates downstream proteases. Finally, a clip domain SP (cSP) converts prophenoloxidase (PPO) into its active form, leading to the formation of melanin that protects insects from invading pathogens. We previously showed that melanization inactivated the Helicoverpa armigera nucleopolyhedrovirus (HearNPV) in vitro; while HearNPV infection could efficiently antagonize host melanization reaction. However, the underlying molecular mechanism regarding the interplay between baculovirus and host melanization system remains poorly understood. In this study, we demonstrated the viral protein P26 as a crucial suppressor against host melanization response both *in vitro* and *in vivo*. In addition, by reconstituting PPO activation cascades, some key host factors in the melanization cascades were identified as the targets for HearNPV P26. Moreover, the crystal structure of HearNPV P26 has been solved and the molecules assemble into dimers with distinct properties of electrostatic surface representation. Interestingly, the structure of P26 shares high homology with the recently reported crystal structure of the poxvirus immune nuclease (poxin), further suggesting a crucial role of P26 in suppressing host immune response. Taken together, our results revealed a novel mechanism whereby baculovirus P26 suppresses host melanization response to facilitate virus propagation.
The role of outbreak-associated factors in activation of covert nucleopolyhedrovirus infection in *Lymantria dispar* L.


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The causes of mass epizootics during insect’s outbreaks are one of the important questions in the population dynamics. Here we study the most common outbreaks-associated stress factors: starvation and high larvae density. The main idea of our experiments was to show how insect innate immunity characteristics assist with the activation of covert to overt infection by the stress factors. The most vivid result we obtained are negative consequences for the insect host. This study was supported by Russian scientific foundation (grant # 17-46-07002).
arthropods.

To date (March 2019), there are eleven described species belonging to the genus *Phasmahhabditis*. They have a worldwide distribution, and are soil-dwelling facultative parasitoids of slugs and snails that parasitize many mollusc families e.g. Limacidae, Agriolimacidae, Arionidae, Milacidae, Vaginicinellidae, Helicidae etc. The only species that has been developed as a commercial product for slug management is *P. hermaphrodita* under the trade name of Nemaslug® by BASF. Within this study we tested the effect of three *Phasmahhabditis* species on mortality of target and non-target molluscs, influence on feeding activity, the ability to grow on different organic substrates and the ability to grow on solid and in liquid medium. In our research, our aim was to demonstrate that other species (e.g. *P. bonaquaense*, *P. apuliae*), isolated in the Czech Republic and Italy from cadavers of *M. sowerbyi* and *Milax gagates*, are also effective against target species. Our results also prove that nematodes that are orally ingested contain feeding stimulants. We also showed that these extracts did not have a negative impact on the fitness of the nematodes contained within most nematodes die before they can get into contact with their slug hosts. Our beads solve this problem by acting as small hydric enclosures ensuring the prolonged survival of the nematodes, thus increasing the chances of them infecting the slugs. Our original bead formulation was not readily ingested by *A. vulgaris* slugs. The aim of this research project was to ensure that the slugs would feed on the beads under field conditions. We achieved this by enhancing them with natural extracts that sensitive to desiccation, which is a problem with the current application method, where the nematode is spraying over plants or field soil. Indeed, most nematodes die before they can get into contact with their slug hosts. Our beads solve this problem by acting as small hydric enclosures ensuring the prolonged survival of the nematodes, thus increasing the chances of them infecting the slugs. Our original bead formulation was not readily ingested by *A. vulgaris* slugs. The aim of this research project was to ensure that the slugs would feed on the beads under field conditions. We achieved this by enhancing them with natural extracts that contain feeding stimulants. We also showed that these extracts did not have a negative impact on the fitness of the nematodes contained within the beads. The slugs were not put-off by the presence of nematodes inside the beads; they were readily ingested despite containing deadly predators. Our results also prove that nematodes that are orally ingested are at least as effective at killing slugs as nematodes sprayed over the plants. Together these results show the potential for our nematode-filled beads to be an efficient biocontrol method.

**CONTRIBUTED PAPERS. Wednesday, 14:30 SS-1**

*Phasmahhabditis hermaphrodita* is not the only slug killing nematode

**Nermut, J.; Holmes, M.; Púza, V**

1Biological Centre CAS, Institute of Entomology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic

2University of South Bohemia, Faculty of Agriculture, Studentská 1668, 370 05 České Budějovice, Czech Republic

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To date (March 2019), there are eleven described species belonging to the genus *Phasmahhabditis*. They have a worldwide distribution, and are soil-dwelling facultative parasitoids of slugs and snails that parasitize many mollusc families e.g. Limacidae, Agriolimacidae, Arionidae, Milacidae, Vaginicinellidae, Helicidae etc. The only species that has been developed as a commercial product for slug management is *P. hermaphrodita* under the trade name of Nemaslug® by BASF. Within this study we tested the effect of three *Phasmahhabditis* species on mortality of target and non-target molluscs, influence on feeding activity, the ability to grow on different organic substrates and the ability to grow on solid and in liquid medium. In our research, our aim was to demonstrate that other species (e.g. *P. bonaquaense*, *P. apuliae*), isolated in the Czech Republic and Italy from cadavers of *M. sowerbyi* and *Milax gagates*, are also effective against target species. Our results also prove that nematodes that are orally ingested contain feeding stimulants. We also showed that these extracts did not have a negative impact on the fitness of the nematodes contained within most nematodes die before they can get into contact with their slug hosts. Our beads solve this problem by acting as small hydric enclosures ensuring the prolonged survival of the nematodes, thus increasing the chances of them infecting the slugs. Our original bead formulation was not readily ingested by *A. vulgaris* slugs. The aim of this research project was to ensure that the slugs would feed on the beads under field conditions. We achieved this by enhancing them with natural extracts that contain feeding stimulants. We also showed that these extracts did not have a negative impact on the fitness of the nematodes contained within the beads. The slugs were not put-off by the presence of nematodes inside the beads; they were readily ingested despite containing deadly predators. Our results also prove that nematodes that are orally ingested are at least as effective at killing slugs as nematodes sprayed over the plants. Together these results show the potential for our nematode-filled beads to be an efficient biocontrol method.

**CONTRIBUTED PAPERS. Wednesday, 14:45 SS-2 STU**

Parasites associated with terrestrial slugs of the Arionidae family in Europe, with emphasis on the invasive *Arion vulgaris*

**Filipiak, A.1; Haukeland, S.2; Zając, K.; Lachowska-Cierlik, D.; Antzéee-Hylseth, H.; Trandem, N; Hatteland, B.A.6,7**

1Institute of Plant Protection - National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland.

2Norwegian Institute of Bioeconomy Research (NIBIO), Plant Health and Mollusc Behaviour and Mollusc Biology, Chairs: R Rae / Solveig Haukeland

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The invasive slug *Arion vulgaris* (Gastropoda: Arionidae) is an agricultural pest and a serious nuisance in private and public gardens in Central and Northern Europe. We investigated and compared the occurrence and prevalence of nematode and trematode parasites in *A. vulgaris* to three native gastropod species in Norway. *A. vulgaris* turned out to have the highest prevalence of both parasite groups, indicating that the enemy release theory (hypothesis) is not a valid explanation for the success of *A. vulgaris* in Northern Europe. We extended this work and conducted a survey of parasites associated with *A. vulgaris* and the native *A. ater* in France Germany, Netherlands, Norway and Poland. More than 600 slugs were collected from 18 sample sites and identified by means of morphological and molecular analysis. The parasites found included four nematode species; *Allionema appendiculatum* Angiostoma margaretae, *Phasmahhabditis hermaphrodita*, *Entomelas* sp., two trematode species; *Brachylaima mosostoma*, *Eurytrema* sp., and one tapeworm species; *Skrijabinia* sp. *Allionema appendiculatum* was the most common parasite in the slug populations investigated in this study. Furthermore, we found higher prevalence of trematodes in the invasive *A. vulgaris* compared with the native *A. ater*, while differences in the prevalence for nematodes are not yet clear.

**CONTRIBUTED PAPERS. Wednesday, 15:00 SS-3 STU**

Finding feeding stimulants to improve the efficiency of a newly-developed slug biocontrol product against *Arion vulgaris* slugs

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1Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

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The slug *Arion vulgaris* is an important pest in European agriculture. In order to limit its impact on plant cultures, we (the Functional and Applied Research in Chemical Ecology laboratory in Neuchâtel, Switzerland) are currently developing a new biocontrol product: beads containing the slug-killing nematode *Phasmahhabditis hermaphrodita*. While *P. hermaphrodita* is already commercially available as bio-control agents, it is extremely sensitive to desiccation, which is a problem with the current application method, where the nematode is spraying over plants or field soil. Indeed, most nematodes die before they can get into contact with their slug hosts. Our beads solve this problem by acting as small hydric enclosures ensuring the prolonged survival of the nematodes, thus increasing the chances of them infecting the slugs. Our original bead formulation was not readily ingested by *A. vulgaris* slugs. The aim of this research project was to ensure that the slugs would feed on the beads under field conditions. We achieved this by enhancing them with natural extracts that contain feeding stimulants. We also showed that these extracts did not have a negative impact on the fitness of the nematodes contained within the beads. The slugs were not put-off by the presence of nematodes inside the beads; they were readily ingested despite containing deadly predators. Our results also prove that nematodes that are orally ingested are at least as effective at killing slugs as nematodes sprayed over the plants. Together these results show the potential for our nematode-filled beads to be an efficient biocontrol method.

**CONTRIBUTED PAPERS. Wednesday, 15:15 SS-4**

Biological and area-specific slug control for farming

**Slotsbo, S.; Krogh, P. H.; Wendelboe, K.; Smedegaard, J.; Tirado, J.; Cordsen Nielsen, G.**

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The slug *Arion vulgaris* is an important pest in European agriculture. In order to limit its impact on plant cultures, we (the Functional and Applied Research in Chemical Ecology laboratory in Neuchâtel, Switzerland) are currently developing a new biocontrol product: beads containing the slug-killing nematode *Phasmahhabditis hermaphrodita*. While *P. hermaphrodita* is already commercially available as bio-control agents, it is extremely sensitive to desiccation, which is a problem with the current application method, where the nematode is spraying over plants or field soil. Indeed, most nematodes die before they can get into contact with their slug hosts. Our beads solve this problem by acting as small hydric enclosures ensuring the prolonged survival of the nematodes, thus increasing the chances of them infecting the slugs. Our original bead formulation was not readily ingested by *A. vulgaris* slugs. The aim of this research project was to ensure that the slugs would feed on the beads under field conditions. We achieved this by enhancing them with natural extracts that contain feeding stimulants. We also showed that these extracts did not have a negative impact on the fitness of the nematodes contained within the beads. The slugs were not put-off by the presence of nematodes inside the beads; they were readily ingested despite containing deadly predators. Our results also prove that nematodes that are orally ingested are at least as effective at killing slugs as nematodes sprayed over the plants. Together these results show the potential for our nematode-filled beads to be an efficient biocontrol method.
Winter survival of the invasive slug *Arion vulgaris* in agricultural fields in Norway

_Hatteland, B. A. 1,2; Roth, S. 3 and Andersen, A. 4_

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The invasive slug *Arion vulgaris* has spread across Europe appearing in great abundance in many countries including Norway. The slug inflicts considerable damage in gardens, strawberries, vegetables, cereal fields and grasslands in many parts of Europe. Management of these slugs is mainly restricted to the use of molluscicides, although nematodes as biological control agents are also used. There is a lack of knowledge concerning the overwintering abilities of these slugs in various habitats, although it is crucial to know to what degree slugs are able to overwinter in fields and what conditions that cause higher or lower mortality. We investigated the overwintering abilities of *A. vulgaris* in various agricultural fields as well as surrounding vegetation. In addition, data loggers were used to track temperatures both in the ground surface as well as in the soil. In addition to the field surveys we also tested survival of juvenile *A. vulgaris* subjected to low temperatures in climate chambers. Mortality was found to be higher for slugs experiencing freeze-thaw cycles compared with slugs kept at constant low temperatures.

**CONTRIBUTED PAPERS. Wednesday, 15:45 SS-6 STU**

_A story of the beta pore forming toxins in the fresh water snail Biomphalaria glabrata_

_LASSALLE, D. 1; Galinier, R. 1; Gourbal, B. & Duval, D. 1_

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_Biomphalaria glabrata* is the vector snail of Schistosomiasis, a neglected tropical disease affecting over 230 million people worldwide. Only one efficient chemotherapy treatment is currently available and cases of resistance arise. In that way, WHO recommend focussing on new ways to fight and control the disease by paying attention to the vector snail. In this context, we managed to characterize the immunobiological interactions between the snails and Schistosome parasites. Sometimes, the snail’s immunity succeeds in eliminating the parasite (i.e., incompatible interaction) but sometimes the snail’s immune system fails, and the parasite succeeds to infect (i.e., compatible interaction). The characterization of the molecular basis of compatibility between the host and the parasite appears relevant in identifying new potential ways of control, or new therapeutic targets.

Recently, we have identified some beta pore forming toxin family members, like Aerolysins or Epsilon toxins in the genome of *Biomphalaria glabrata* snails. *Biomphalaria glabrata*’s Aerolysin, named Biompthersin, turned out to be a key molecule in the snail’s immunity. In fact, this protein had the ability to bind and kill the parasite. Twenty-three Biompthersins have been characterized, rising some hypothesis concerning their acquisition, diversification, subfunctionalisation and potential role in the snail immunity. Epsilon toxins have been discovered by structural homology with Biompthersins. The function of these toxins, named Glabralsins, remains largely unknown. These results raise many transversal questions on the acquisition and diversification of these toxins, but also their expression and function following pathogen infections of snails.

**CONTRIBUTED PAPERS. Wednesday, 16:00 SS-7**

Morphological indicators of reproduction in the garden snail *Cornu aspersum*

_Ortega-Hidalgo, M.M. 1; Rodriguez-Zaldúa, I. 1; Txurruka, J.M. 1_

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A wild population of *Cornu aspersum* (Leioa, Biscay, 43°19’N, 2°58’W) was surveyed along a period of 16 months (20 sampling episodes) in a long-term study resulting in the identification of morphological indicators of reproductive status. Parameters were chosen according to readiness compatible with fieldwork: live weight (portable balance 0.01 gr) and shell features such as shell and peristome diameters (SD and PD determined with vernier calliper 0.01 mm), as well as reflected lip and growth marks presence. A sample of animals was taken to the laboratory in every occasion to be dissected in order to determine gonadal index (GI) on mass terms: Gonad Dry wt/Total soft body wt. Peristome diameter relates lineally to shell diameter in snails below 24 mm of SD whereas dispersion appears for larger presumingly breeding individuals. Presence of both, reflected lip (ANCOVA: F1, 527 = 22.056, p <0.0001) and GI higher than 10% (ANCOVA: F1, 527 = 50.623, p <0.0001) significantly dissociate the population into two groups of morphometric ratios of the shell (MI = DP/DS): a transition related to the progressive development of the genital, is evidenced by the high negative correlation between morphometric and gonadal indexes (Z52 = -24.010; p <0.0001), with breeding snails exhibiting values below 0.65 and juveniles increasing between 0.7 to 0.9. Exponential equations used to analyse this trend result in highly significant predictors of mean maximal GI (34.3%) associated to minimum MI (0.56). Linear equations relating DP to DC for juvenile and reproductive snails show a significant correlation.

**CONTRIBUTED PAPERS. Wednesday, 16:15 SS-8**

Physiological and biochemical responses of mussels _D. polymorpha and D. bugensis_ to exposure to hypoxia

_Klimova Y.S. 1; Chuko G.M. 1; Sharov A.N. 1,2; Khodolevich S.V. 1,3_

1 Papanin Institute for Biology of Inland Waters Russian Academy of Sciences, Russian;
2 Saint-Petersburg Scientific Research Center for Ecological Safety, Russian Academy of Sciences, Russian;
This study deals with the adaptation of two invasive mussel species D. polymorpha and D. bugensis to hypoxia. The mussels were exposed to hypoxia (0.56–1.12 mg/l) for 96 hours. The mortality of mussels, behavioral reactions, cardioactivity (heart rate) lactate content, antioxidant enzyme activities and the level of low-molecular antioxidant – reduced glutathione (GSH) and lipid peroxide oxidation (LPO) in the whole soft tissues were observed. Being subjected to the hypoxia, 5% and 95% of D. polymorpha died after 72 and 96 hours respectively whereas D. bugensis didn’t. When the concentration of dissolved oxygen in water was ≥ 2 mg/l, D. polymorpha increased heart rate, and D. bugensis decreased heart rate (bradycardia). The catalase activity decreased in D. polymorpha all exposed to hypoxia for 48 h. The activity of glutathione reductase, glutathione-S-transferase, content GSH and the LPO levels significantly increased in this species in the 72h hypoxia. The increased lactate concentrations were found only in D. polymorpha, also. The most of the studied parameters of D. bugensis did not change, but only short-term increase of GST activity was found after 48 h exposure to hypoxia. D. bugensis has shown more effective strategy of adaptation to anoxia than D. polymorpha as the level of studied parameters of antioxidant defense system of this species was higher. Thus both of them are important diagnostic indices as the level of studied parameters of antioxidant defense system of this GST activity was found after 48 h exposure to hypoxia.}

**CONTRIBUTED PAPERS. Wednesday, 14:45 DBI-2**

**Addressing the health of Macrobrachium rosenbergii in Bangladesh aquaculture**

*Hooper, Ch.; Bateman, KS.; Ross, S.; Stentiford, GD.; Rahman, MM.; Basak, SK.; Bass, D.*

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Bangladesh aquaculture produced in excess of 2,000,000 tonnes, valued at 1.9 billion USD in 2016 and was the world’s second largest producer of freshwater prawns (Macrobrachium rosenbergii). M. rosenbergii is an attractive species for culture due to its increasing demand and value at international market. Since 2011, hatcheries have been experiencing high levels of larval mortality, depleting the number of active hatcheries and reducing the number of animals surpassing the larval stage. Poor hatchery management may contribute as a factor to the low survival of larva, however the hatcheries were still functioning well until 2010 despite large inconsistencies in water quality, biosecurity and feeding practices, suggesting that a new factor had been introduced to account for the mortalities. Histological samples taken from hatchery M. rosenbergii have identified intracytoplasmic inclusion bodies in the hepatopancreas suggestive of an RNA virus. Similar inclusions have also been seen in the hepatopancreas of M. rosenbergii sampled from a wild population. We carried out histopathological and electron microscopy screens, and have generated metagenomic sequence libraries and in order to characterise this RNA virus and to develop primers to screen for it in larval and post-larval populations. Screening for this virus as well as other diseases known to cause issues in larvae, such as M. rosenbergii nodavirus and the ciliate Metanophrys sinensis, provides a multi-agent risk assessment toolkit to determine a possible cause for the mortalities and may lead to changes in practices to prevent spread of infectious agents both in and between hatcheries.

**CONTRIBUTED PAPERS. Wednesday, 15:00 DBI-3**

**Diseases of the Caribbean spiny lobster, Panulirus argus**

*Atherley, NAM.*; Dennis, MM.; Freeman, MA.

Ross University School of Veterinary Medicine, Island Main Road, Saint Kitts, West Indies

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The Caribbean spiny lobster, Panulirus argus, is important to the economy of several countries in the Caribbean, including Saint Kitts and Nevis. According to the IUCN red list, the P. argus population is currently decreasing and is believed to be exploited throughout its geographical range. As a result, it is important to investigate the factors affecting population health and commercial yield. Although few lobster diseases and parasites have been reported, surveillance is important since diseases may affect yield and marketability of this species. Two hundred and sixty-four lobsters were screened for pathogens and parasites which can impact lobster fisheries, such as microsporidians and nemertean worms. Microsporidian infections in P. argus are rare and documented events have been restricted to Florida. However, using histological and molecular techniques, one infected lobster was found in Saint Kitts, which is indicative of geographical expansion. The isolate found in both areas is a microsporidian belonging to the genus Metanophrys sinensis, providing a multi-agent risk assessment toolkit to determine a possible cause for the mortalities and may lead to changes in practices to prevent spread of infectious agents both in and between hatcheries.

**CONTRIBUTED PAPERS. Wednesday, 14:30-16:30 Commission R8**

**Important diseases of beneficial invertebrates; from cockles to crickets**

Chairs: Mark Freeman / Helen Hesketh

**CONTRIBUTED PAPERS. Wednesday, 14:30 DBI-1 STU**

**A bacterial insect pathogen as a threat to cricket farming in East Africa**

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Various cricket species are reared in several countries around the world as a source of protein. Particularly in countries of the Global South, small holders are engaged in cricket farming as a mean for income generation and food security. However, one of the main problems in cricket farming is the development of insect diseases. Recently, an outbreak of a bacterial disease was detected in a number of cricket species (Family Gryllidae) reared in farms in Kenya and Uganda. Screening of diseased crickets resulted in the identification of a bacterium belonging to the genus Rickettsiella with high taxonomic similarity to Rickettsiella gyrtlii. This genus contains several species described as being highly pathogenic to insects. The characteristic symptoms of the disease were abnormal swelling of the crickets’ abdomen which turned viscous, liquefied and yellowish inside. Crickets lost mobility, some of their limbs broke eventually, egg production decreased or ceased entirely and the colony collapsed over some months, compromising the entire project due to all the farms presenting the same problem. Measures to control the disease were drafted and ideas for future strategies such as egg-surface disinfection are being developed.
merites conanobrienii, an egg-predator found in lobsters from Florida and the only one known to be associated with *P. argus*. The second isolate from Saint Kitts has not been fully identified to date and is not closely related to *Carcinonemertes* spp.

**Contribution Papers. Wednesday, 15:15 DBI-4**


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*Marteilia* is caused by paramyxid parasites of the genus *Marteilia*, a serious disease of bivalve molluscs which is linked with mass mortalities and the collapse of commercially important shellfish populations. Until recently, common cockle (*Cerastoderma edule*) populations, including major fisheries. Phylogenetic analysis of ribosomal RNA gene sequences from this parasite indicates that it is closely related to *M. cochillia*, a parasite linked to mass mortality of *C. edule* fisheries in Spain, but suggests that it may represent a different strain or species. Light and transmission electron microscope (TEM) observations support this conclusion, indicating that the parasite from Wales is located primarily within haemocyte aggregates associated with the gill as well as the sinuses of the adductor muscle, whereas *M. cochillia* is found mainly within the epithelium of the digestive diverticula. Future work will investigate the impact of *Marteiliosis* on UK cockle fisheries and interactions with climate change.

**Contribution Papers. Wednesday, 15:430 DBI-5**

Characterization of a novel mutant of *Vibrio parahaemolyticus* that carries binary toxin genes, pirA and pirB but does not cause acute hepatopancreatic necrosis disease (AHPND) in Pacific white shrimp (*Penaeus vannamei*). Dhar, Arun K.; Aranguren, F. L.; Mai, H. N.; Kanrar, S.; Cruz-Flores, R.

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Acute hepatopancreatic necrosis disease (AHPND), caused by *Vibrio parahaemolyticus* carrying binary toxin genes, pirA and pirB is an emerging disease in shrimp aquaculture that has caused enormous losses in Asia and has now spread to the Americas. During a routine screening of *V. parahaemolyticus* isolates by PCR an isolate, R14, was identified that contains binary toxin genes but failed to cause any mortality when bioassay was conducted using Specific Pathogen Free (SPF) *Penaeus vannamei* shrimp. Histopathological examination of challenged animals also did not reveal any pathognomonic lesion that are characteristic of AHPND. Whole genome sequence of R14 isolate revealed that R14 isolate contained both *pirA* and *pirB* genes and there was a 1000 bp insertion upstream of the *pirA* locus. The insertion appeared to disrupt the transcription of the cognate genes. Western blot analysis using anti-PirA protein failed to detect the toxin. *Vibrio parahaemolyticus* isolates characterized by Biostar downstream analysis using anti-PirA protein failed to detect the toxin. *Vibrio parahaemolyticus* isolates characterized by Biostar downstream analysis using anti-PirA protein failed to detect the toxin. *Vibrio parahaemolyticus* isolates characterized by Biostar downstream analysis using anti-PirA protein failed to detect the toxin. *Vibrio parahaemolyticus* isolates characterized by Biostar downstream analysis using anti-PirA protein failed to detect the toxin. *Vibrio parahaemolyticus* isolates characterized by Biostar downstream analysis using anti-PirA protein failed to detect the toxin.

**Contribution Papers. Wednesday, 16:00 DBI-7 STU**

Understanding the molecular basis of susceptibility to white spot syndrome virus infection in shrimp *Penaeus vannamei*. Millard, RS.; Verbruggen, B.; Bickley, LK.; Bateman, KS.; Stentiford, GD.; Tyler, CH.; van Aerle, R.; Santos, EM.

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White spot syndrome virus (WSSV) is the major pathogen of shrimp culture, causing global annual losses in the region of $1bn USD. This study aims to identify the molecular pathways associated with susceptibility test(s) for AHPND screening.
to WSSV in the hugely economically important Pacific whiteleg shrimp (*Penaeus vannamei*). To do so, the temporal transcriptional and microRNA changes in response to WSSV infection are being investigated, and compared with an existing dataset documenting these changes in the European shore crab, *Carcinus maenas*, an allied crustacean that is highly resistant to the disease. This provides an opportunity to uncover the key pathways responsible for resistance which could be utilised in the development of disease treatments. In this study, shrimp were divided into two groups and injected with either specific pathogen-free or WSSV-shrimp homogenates. The gills from four shrimp from each treatment were sampled from 3-36h post-injection. Total RNA was extracted (n=48) and mRNA libraries (Illumina) and small RNA libraries (Nextflex) were prepared and sequenced. Initial findings demonstrate strong similarity in the responses of both crustaceans to WSSV infection. However, in shrimp, immune responses are enriched during late stage infection (following virus replication) whereas in crabs this occurs prior to virus replication. In addition, we show that crabs enrich novel immune responses associated with miRNA processes during early infection. From these findings we hypothesise that these may play a key role in resisting disease. These findings provide a promising platform to seek identification of novel targets for disease prophylactics in the future.

**CONTRIBUTED PAPERS. Wednesday, 16:15 DBI-8 STU**

Comparative genomics analysis of White Spot Syndrome Virus (WSSV) isolates from different geographical regions.

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White Spot Syndrome Virus (WSSV; the sole member of the Nimaviridae family within the genus Whispovirus) was first discovered in farmed Japanese tiger prawn (*Penaeus japonicus*) in Taiwan in the 1990s. The virus has spread globally throughout shrimp farming regions and has had a devastating impact upon shrimp aquaculture. It has a double stranded DNA genome of approximately 300kbp and is one of the largest known animal viruses. WSSV was fully sequenced starting with the earliest isolates reported in Asia; Thailand (WSSV-Th), Taiwan (WSSV-Tw) and China (WSSV-Cn), these three isolates sharing overall nucleotide identity of 99.32%. More recently, additional viral isolates have been reported in other areas such as America, Europe, Africa and Australia, all of which share high homology with the Asian isolates yet found to have smaller genomes. Comparative genomics approaches were applied to study the genetic variation of 23 WSSV isolates from different geographical locations in detail, based on extracted data from published literature and data available in the National Center for Biotechnology information (NCBI) sequence database. Bioinformatics tools were used to examine variations such as deletions, SNPs, recombination events as well as phylogenetic analysis. By identifying the most evolutionarily labile elements in these closely related genomes, and standardising WSSV genome annotation we aim to facilitate robust comparative genomic analyses in this rapidly advancing field. The study revealed the presence of more than 500 SNPs among the isolates and recombination events were detected in several countries including Taiwan, India, Mexico and Australia. However, the full impact of these genomic variations is yet to be investigated.
Vip3Aa induces apoptosis through lysosomal-mitochondrial axis in Spodoptera frugiperda Sf9 cells

Xiaoyue-Hou,¹; Lu, Han,¹; Baojun, An,¹; Zhanglei, Cao,¹; Yanli, Zhang,¹; Xia, Cai,¹; Yunda, Zhan,²; Bing, Yan,¹; Jun, Cai,¹,²,³
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Vip3Aa, which is secreted by some Bacillus thuringiensis strains during vegetative growth, has been known to induce apoptosis in Sf9 cell. However, the apoptosis mechanism triggered by Vip3Aa is unclear. In this study, we investigate the possible molecular mechanism of Vip3Aa-induced apoptosis. We found that in Sf9 cells, Vip3Aa impaired mitochondria as evidenced by signs of mitochondrial membrane permeabilization, such as reactive oxygen species production and cytchrome C release. Western blot analysis and caspases activity assay further confirmed that apoptosis occurred through caspase-9 and caspase-3 activation following mitochondrial damage. Additionally, Vip3Aa induced lysosomal membrane permeabilization, which was evidenced by AO staining. Western blot analysis verified cathepsin D and cathepsin L translocated from lysosomes into the cytosol several hours before mitochondrial dysfunction. Treatment Sf9 cells with Pepstatin (a cathepsin D inhibitor) or Z-Phe-Tyr-CHO (a cathepsin L inhibitor) could decrease the activity of caspase-9 and caspase-3 and increase cell viability. Taken together, it suggested that Vip3Aa could induce apoptosis in Sf9 cells by regulating lysosomal-mitochondrial axis.

POSTER SESSION. Wednesday, 16:30 PB-3

Microencapsulation of Bacillus thuringiensis: preparation and its process optimization

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Bacillus thuringiensis (Bt) strains are used worldwide to protect crops and forest against insect pests. The bacteria act orally and the main larvicidal activity relies on the pore forming Cry toxins. Despite the successful application of Bt for pest control, which presently is approximate 2% of the total insecticidal market, the short persistence of Bt agents after application has become an important influencing factor for its further development. Previous report demonstrated that microencapsulation can dramatically increase resistance of microorganisms to environmental stresses. Microcapsule of Bt Cry1Ac parasporal crystals using biopolymer materials has been developed which give protection from high temperature and desiccation. In this study, microcapsule of Bt bacteria were successfully prepared with non-toxic biopolymer chitosan and sodium alginate as wall materials through layer by layer self-assembly technology. The size of microcapsules is 2.5×1.2 μm which is suitable for spray application and larval feeding. Microencapsulated bacteria were protected from environmental stresses such as high temperature and desiccation. In addition, innovative flocculation precipitation method was used in this study during bacteria collection. The optimization of the preparation process reduced centrifugal equipment utilization which can effectively decrease final cost.

POSTER SESSION. Wednesday, 16:30 PB-5

Identification and characterization of a new cry gene of Bacillus cereus sensu lato.

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**Bacillus thuringiensis** (Bt) is known as the most successful microbial insecticide against different pests. Its activity is attributed to the Cry proteins that are expressed during its sporulation phase. Although cry genes are highly diversified in Bt strains, there are reports where cry genes have been identified in bacteria other than Bt. In this work, 223 strains of non-Bt bacillaceae isolates were analysed by their cry-gene content. Thirteen putative cry-gene amplicons were obtained, which were cloned and sequenced; however, only six amplicons tested positive for cry genes, showing identical sequences. Once analysed, the six strains belonged to the same isolate (LBIC-004) and was identified as *B. cereus sensu lato* by sequencing of 16S rRNA, gyrB and hag genes. Additionally, a 1,953 bp cry gene was cloned and sequenced which codes for a 651 amino acid protein (74.9 kDa), and is expressed in the wild-type strain, but is unable to form a crystal. According to the Phyre2 software, the typical Cry three-domain structure of the protein was predicted. This protein showed its highest hit at 41% identity with the Cry8Ca protein, which indicates that the gene of strain LBIC-004 might be considered a new cry holotype. This cry-like gene was transferred into acrystalliferous strains of *Bt* but, in spite of being abundantly expressed, no crystal was formed. The transformed strains as well as LBIC-004 were bioassayed against *Aedes aegypti*, *Manduca sexta*, *Phyllophaga* sp., and *Caenorhabditis elegans*. No activity was detected.

**POSTER SESSION.** Wednesday, 16:30 **PB-6**

**Mosquicidal activity of a Cry1C toxin of Bacillus thuringiensis and its synergy with Cyt1A**

**González-Villarreal, S.E.; García-Montelongo, M.; Ordoñez-Acevedo, L.G.; Luévano-Borroel, J.; Ibarra, J.E.**

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The Cry1C protein family of *Bacillus thuringiensis* form bipyrimal crystals, which are commonly associated with toxic activity against lepidopteran species; however, some members of this family may also be toxic to dipterans. In the present work, a Cry1C protein synthesized by the *B. thuringiensis* LBIT-1217 strain was analysed. The gene coding for this protein was amplified and sequenced. The gene was cloned into the pSTAB vector and electro-transferred into the acrystalliferous *B. thuringiensis* 4Q7 strain. The recombinant strain showed bipyrimal crystal morphology (identical to the original LBIT-1217 strain), which showed toxicity against larvae of *Manduca sexta* (Lepidoptera) and *Aedes aegypti* (Diptera). Crystals from the recombinant strain were purified by NaBr gradients and used in bioassays against *Ae. aegypti*. An LC_{50} of 4.61 μg/ml was estimated. Additionally, a *B. thuringiensis* recombinant strain carrying the plasmid pWF45, which encodes the Cyt1A protein, was used to carry out joint-action tests with the Cry1C crystals. An LC_{50} only for pure Cry1A crystals was estimated at 1.2 μg/ml, while an LC_{50} of 0.61 μg/ml was estimated when both toxins were tested together. Data from these bioassays was analysed using several joint-action tests such as the Tames-Bakuniak graphical method and the formula proposed by Tabashnik. All tests clearly showed a synergistic effect between these two toxins.

**POSTER SESSION.** Wednesday, 16:30 **PB-7**

**Microbial protein toxins that are toxic to apple nails and mosquito larvae**

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Cry46Ab produced by the soil bacterium *Bacillus thuringiensis* (Bt) is a protein that exhibits food toxicity to apple nails and mosquito larvae. Apple snails are regarded as rice pests in East Asia and Southeast Asia. Mosquitoes are pests that mediate infections such as dengue fever and zika fever. The half lethal doses of Cry46Ab against apples nail larvae and *Culex pipiens* second instar were 4.2 μg/ml and 4.6 ng/ml, respectively. On the other hand, Cry46Ab did not show a lethal effect on Japanese killifish nor nematode. Unlike 3-domain insecticidal Cry toxins (3dCry) used as BT agents, Cry46Ab has a structure rich in β strands and belongs to the same group of β-pore forming toxins (β-PFT) as Aerolysin and Lysenin. β-PFTs recognize a specific receptor on target cells and assemble in oligomers. They form β barrel and insert it into the cell membrane to form pores. The pores of the toxin are thought to disrupt cell membrane permeability, or trigger signaling cascades, leading to cell death. Many toxins belonging to β-PFT have been isolated, and their three-dimensional structures are similar. It has also been proposed that the oligomerization and membrane insertion necessary for the toxicity of β-PFT be associated with partial structures such as domains, motifs and patterns. Therefore, we are investigating the relationship between partial structure and toxicity mechanism using various mutants of Cry46Ab.

**POSTER SESSION.** Wednesday, 16:30 **PB-8**

**Isolation and characterization of the insect juvenile hormone antagonists from Streptomyces sp.**


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Secondary metabolites from actinomycetes species are considered as potential pesticides due to their insecticidal and growth inhibitory activities. In order to investigate novel insect growth regulators (IGRs) from actinomycetes, 363 isolates of actinomycetes from Korean soil samples were tested for their IGR activities using yeast two-hybrid β-galactosidase assays. Among them, *Streptomyces* sp. AN120537 showed the highest juvenile hormone antagonist (JHAN) activity and significant insecticidal activities against larvae of *Aedes albopictus* and *Flutella xylostella* with 100% mortality at a concentration of 10 ppm. In addition, the dead larvae of *A. albopictus* and *P. xylostella* treated with the AN120537 extracts showed morphological deformities such as contraction of body segments or pigmentation of all body parts. Through liquid chromatography and bioassay-guided fractionation, 9 IGR compounds were identified from the crude hexane extract of AN120537. These results suggested that *Streptomyces* sp. AN120537 could be useful resources for development of eco-friendly insecticidal agents.

**POSTER SESSION.** Wednesday, 16:30 **PB-9 STU**

**Mosquicidal activities of actinomycetes with insect growth regulatory activities**


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Mosquitoes are medically important insect pests that act as vectors of various diseases when they feed on humans. Insect growth regulators (IGRs) could become an effective alternative to control mosquitoes because of their high specificity and relatively low toxicity to environment. Recently, we have developed high-throughput juvenile hormone antagonist (JHAN) screening system based on yeast-two hybrid assay. Actinomycetes have been reported to produce various bioactive compounds including insect growth regulators (IGRs). In this study, culture filtrates and mycelia extracts of 2,875 actinomycetes isolates were screened for their IGR activities and mosquicidal activities. Among 75 culture filtrates and 17 mycelia extracts with JHAN activities, 19 culture filtrate and 3 mycelia extracts...
showed high level of mosquitocidal activities against 3rd instar larvae of *Aedes albopictus*. These results suggested that secondary metabolites of actinomycetes could be used as environmentally benign mosquitocidal agents.

**POSTER SESSION. Wednesday, 16:30 PB-10**

**Suppression of Sacbrood virus by virus-derived dsRNA produced from *Bacillus thuringiensis* toxic to *Galleria mellonella***

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Honeybees play an important role in the global economy by assisting in the pollination of food crops and by producing honey, and other hive products. However, honeybees are inevitably threatened by various pathogens including Sacbrood virus (SBV) and *Galleria mellonella*. Recently, RNA interference (RNAi) has been suggested as a promising strategy for suppression of honey bee viruses. Also, *Bacillus thuringiensis* (Bt) has been widely applied for the control of lepidopteran pests such as *G. mellonella*. In this study, it was intended to develop dsRNA production platform using Bt. For this, the pHT1K-SBV vp1 vector which transcribes sense and anti-sense SBV vp1 gene under the control of Cry1Aa sporulation-dependent promoter with STAB-SD sequence was constructed. This vector was introduced into Bt strain NTO423 expressing Cry1-toxins types. SBV replication was suppressed in the worker *A. cerana* ingested dsRNA produced from the Bt transformant. Crystal proteins from the Bt transformant showed high level of insecticidal activity against 4th instar larvae of *G. mellonella*. These results demonstrated that Bt-based dsRNA producing system could be exploited for the control of both SBV and *G. mellonella* simultaneously.

**POSTER SESSION. Wednesday, 16:30 PB-11**

The presence and diversity of insecticidal proteins in metagenomes from various environments

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Insecticidal microbes are an effective and environmentally safe solution against agricultural pests and insects that vector human pathogens. The increasing rate of insect resistance to commonly used toxin proteins, e.g. in Cry families, necessitates the search for novel insecticidal proteins. Historically, insecticidal bacteria have been discovered by selective isolation of insecticidal species from dead insects, grain dust, and soil. Here we used metagenomics to access genetic information from insecticidal bacteria that escaped isolation and to enable efficient mining of a wide variety of environmental sources. However, insecticidal bacteria outside of an insect host are often found at low abundances making assembly of their genes from complex metagenomes especially challenging. In the present study, we evaluated the presence and diversity of insecticidal proteins in various environments including soil, grain, and plant surfaces. To improve the recovery of assembled insecticidal genes from complex environmental samples, we developed a set of enrichment approaches facilitating the growth of insecticidal bacteria. The enriched metagenomes contained hundreds of insecticidal-like proteins identified using the Second Genome discovery platform based on both homology and machine learning methods. The results of this study showed that insecticidal genes can be successfully assembled from enriched metagenomes and the occurrence of insecticidal protein classes varies by environmental source and enrichment approach.

The results have implications for agriculture and healthcare and for better understanding the ecology of insecticidal bacteria.

**POSTER SESSION. Wednesday, 16:30 PB-12**

*Bacillus thuringiensis*’s plant disease control effect and plant growth promotion effect

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*Bacillus thuringiensis* (Bt) is a bacterium used as a microbial insecticide (BT agent). However, some strains with plant disease control effect and plant growth promoting effect has been reported. In addition, it has been reported that B. thuringiensis forms a biofilm on the root of tomato and induced systemic resistance to plants. Therefore, in this research, we focused on such function of B. thuringiensis and aimed to develop usage as biofertilizer and biostimulant in existing BT strains and BT agents. We investigated the growth promoting effect using BT treated seed. In addition, we investigated the disease control effect and PGPR effect by applying BT to the root of tomato in the greenhouse. Two BT strains (BT - 17, BT - 20) and Jackpot which are effective in suppressing plant diseases and plant parasitic nematodes in the pot test were used as the test strain. (Experiment 1) Tomato seeds were immersed in a BT suspension for 24 hours and the roots were elongated using a roll towel method and measured. (Experiment 2) The BT suspension was periodically applied at the root of the tomato in the greenhouse. Then, Phytophthora infestans incidence rate, plant height, weight per bunch, weight per fruit, and number of fruits per bunch were investigated. In Experiment 1, the roots of BT treated seeds significantly increased compared to the control plot. In Experiment 2, the disease control effect, plant height, second bunch weight and weight per fruit in the second bunch were significantly higher in the jackpot treated plot than in the control plot.

**POSTER SESSION. Wednesday, 16:30 PB-13**

*Photorhabdus* lectins disrupt the activity of insect and human immune system

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Bacteria of the genus *Photorhabdus* are known as potent entomopathogens that produce a variety of toxins, proteases and other virulence factors to overcome host immune system and successfully establish the infection. Although lectins with their carbohydrate-binding abilities are not the typical example of molecules causing direct damage to host organism, they are indispensable in the processes such as attachment to cells, immune evasion and immunosuppression. Bacteria of the genus *Photorhabdus* are not an exception as they produce lectins that could help them to interact with nematode symbionts, other bacteria or host immune system. Recently, we focused on lectins produced by *P. laumondii* (formerly classified as *P. luminescens* subsp. *laumondii*) that are not only able to bind to insect haemocytes, but also to disturb cellular and humoral immune response. Lectin treatment of insect haemolymph induced melanisation.
Sex-specificity in innate immunity of insect larvae

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Percentage of females in population is one of the important determinants of its growth ability. Focus on females in pest control leads to significant advantages. An example of this is SIT. In the last decade, there is evidence that activity of innate immunity parameters of female larvae are different from males. The question arises: is it possible to use this data for development of new progressive pest control methods? In the studies in this area only a few immune parameters were evaluated separately in females and males. Accordingly, this question remained completely open. For our research we chose one of the important parameters of innate immunity against bacterial infection – lysozyme-like activity. We measured sex-specificity of this parameter in a midgut tissue and lymph of Lymantria dispar larva in native conditions and after challenge of Bacillus thuringiensis. We also evaluated sex-specific mortality induced by B. thuringiensis infection. Lysozyme-like activity of the females midgut tissue was higher than activity of males one day after bacteria challenge. Females mortality from bacteria was higher also. Thus sex-specificity is present in this system. Consequently, artificial decrease of larvae lysozyme can lead to two effects: increase of susceptibility to bacteria of whole population and decrease percentage of females in population.

Specificity determination in Bacillus thuringiensis Cry2A toxins

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The Cry2A toxin family from Bacillus thuringiensis consists of a set of highly similar proteins, but which demonstrate quite specific toxicity spectra. As a result we have been using them as a model system to help us understand the nature of toxin specificity. It has been known for many years that while Cry2Aa is toxic to both Lepidopteran and Dipteran species Cry2Ab lacks the activity that Cry2Aa has against the mosquito Aedes aegypti. Previous experiments have suggested that the region responsible for Aedes activity resides within Domain II of the toxin, however we have shown that mutating just a few residues at the N-terminus of Domain I of Cry2Ab can give it activity against A. aegypti. We wanted to extend these studies to the Lepidopteran Plutella xylostella but found that data in the literature on which Cry2A toxins were active against this insect lacked consistency. We will present data showing that the activity of Cry2A toxins against P. xylostella is dependent on the population of insect tested, and also that regions in both Domain I and Domain II are important for specificity, albeit not the same region of Domain I required for Aedes activity.

Mode of action of the Cry41Aa parasporin against human cancer cells

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We have previously shown that the three-domain Cry41Aa toxin from Bacillus thuringiensis acts like its insecticidal counterparts in that its primary mechanism of action is via pore formation. Although it has only been reported as showing activity against two human cancer cell lines we have found that it can affect a number of other lines to a greater or lesser extent. In this poster we will present a hypothesis speculating on why activating the toxin with different proteases can significantly affect its toxicity. We will also present data examining the association between toxin binding and activity of Cry41Aa and its mutants.

Type II toxin-antitoxin system regulates the pathogenicity of Bacillus thuringiensis during its infection

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Toxin-antitoxin (TA) systems are widely distributed in bacteria, which play important roles in many processes. Recent studies found that type II TA systems are involved in the pathogenesis of many pathogenic bacteria. However, how TA systems were activated and the detailed molecular mechanism of its regulation in host is still unclear. Bacillus thuringiensis (Bt) is the pathogen of insects and nematodes, which produces a variety of insecticidal active substances. It has been developed the worldwide most successful and safety microbial insecticide, and plays important roles in controlling the agriculture and forest pests. We found that the type II TA system also plays an important role in Bt infecting nematodes and insects, for example the transcription of type II TA system was rapidly increased when Bt infecting hosts; TA system influences the colonization and virulence of Bt inside host and the transcription of spore cortex-lytic enzyme as well as the germination related genes; the toxin in TA system is involved in the regulation of multiple levels of Bt interaction with the host like germination, immune escape, transcription regulatory factors and essential growth genes. The mechanism of how TA system regulating the pathogenicity of Bt during infection will enrich our deep understanding about the function and mechanism of TA system of bacteria, and also will provide new ideas for the creation of high and/or fast efficiency Bt preparations.

Type I-C CRISPR-Cas system mediates Bacillus thuringiensis pathogenicity and environmental adaptation

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CRISPR-Cas is the microbial adaptive immune system that help prokaryotic cells to prevent the invasion of foreign genetic elements such us virus or exogenous plasmids. Research on their molecular mechanisms has led to the development of genome-editing and CRISPR interference techniques based on several systems. Recently works demonstrated that such systems involved in bacterial virulence regulation. However, the CRISPR-Cas systems in Bacillus thuringiensis has been scarcely researched. Here, we analysed the CRISPR systems in reported genomes of Bacillus thuringiensis, we found most of CRISPR systems showed a genetic

catalysed by phenoloxidase; this increase was not observed when we used the lectin pre-treated with saccharides selected according to its specificity. Although, P. launodontii is not considered to be a human pathogen (unlike closely related P. asymbiotica), its lectin is able to inhibit production of reactive oxygen species in human blood induced by neutrophil activator zymosan A, whereas it is not able to supress the action of other activators such as phorbol 12-myristate 13-acetate or fMLF. Our results suggest that the lectin interferes with Toll-like receptor 2 and thus can impair the production of reactive oxygen species by host phagocytes.

The work was supported by the Czech Science Foundation (grants no. 17-03253S and 18-18964S).
degradation phenomenon, mainly display as the deletion of Cas proteins that responsible for new spacers acquiring. We then characterized a functional Type I-C CRISPR-Cas system, which is capable of efficient interference with plasmid DNA, and utilizes a TTN protospacer-adjacent motif. The bacterial that harbour such Type I-C CRISPR-Cas system got resistance to phage infection. We also demonstrated that this Type I-C system can be used as effective gene editing tool in B. cereus group strains. What’s more, it’s interesting that such CRISPR-Cas system reduce virulence and adaptability both under host and environmental conditions of B. thuringiensis. We suspected the CRISPR-Cas system would prevent horizontal gene transferring during it function as bacterial immune system, so the host cannot gain new components to improve its virulence and environment adaptability.

POSTER SESSION. Wednesday, 16:30 PB-19

Insights on the immune response of Colorado potato beetle larvae challenged with Bacillus thuringiensis, arising from an hemolymph proteomic analysis

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Bacillus thuringiensis (Bt) toxins constitute effective, environmentally safe biopesticides. Nevertheless, Bt insects’ tolerance is influenced by key proteins involved in metabolic processes, gut regeneration and innate immunity, which might be particularly relevant upon ingestion of low toxin doses. Therefore, we obtained the proteomic profile of hemolymph in Colorado potato beetle (CPB) non-treated control larvae and larvae challenged with a non-lethal dose of a spore-crystal mixture containing the coleopteran specific Cry3Aa toxin, 24 h following intoxication. The repertoire of hemolymph proteins involved in immunity and defense remarkably increased upon intoxication, whereas among downregulated proteins in CPB larvae ingesting the spore-crystal mixture containing Cry3Aa toxin compared to non-treated control larvae there were proteins reported to be involved in insect growth, molting and metamorphosis, which is in accordance with the delayed growth exhibited by challenged larvae. The microRNA miR-8, which has been proposed to regulate genome homeostasis in Drosophila, might also participate in CPB regulatory network involved in activating the immune response to innate immune homeostasis in Drosophila, which might also participate in CPB regulatory network involved in activating the immune response to Cry3Aa toxin since, correlating with the downregulation of Lde-miR-8 in CPB larvae intoxicated with a non-lethal dose of the Cry3Aa spore-crystal mixture, hemolymph PO enzyme activity significantly increased and the AMPs acaloleptin A-like and attacin-B-like proteins accumulated in the hemolymph of intoxicated larvae. Interestingly, acaloleptin A-like and attacin-B-like genes expression 24 h after challenge was not significantly different in control and CPB larvae intoxicated with a Cry3Aa spore-crystal mixture, suggesting a transient nature of AMPs gene expression to avoid detrimental effects to the host and development of resistance.

POSTER SESSION. Wednesday, 16:30 PB-20

Identification and localization of insecticidal genes through the genomic analysis of a new strain of Bacillus thuringiensis

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Bacillus thuringiensis is an entomopathogenic bacterium capable of producing insecticidal proteins broadly used in the biological control of pests. The genes responsible for the expression of these proteins are located in genomes and plasmids of the strains, and their correct identification and localization allows the isolation of their sequences for use in biotechnological innovations, such as transgenic plants. The objective of this study was to analyze the nucleotide sequence corresponding to the genome of a new Bt strain with toxic activity to lepidopteran pests of agricultural importance to identify candidate genes present. The genomic DNA of strain 1608A isolated in Brazil was sequenced in paired-end by the Illumina HiSeq 4000 system and the resulting Scaffolds were analyzed by BLASTn against a specific database containing nucleotide sequences of Bt toxins named and deposited in the NCBI. Sequencing generated a total of 8,514,870 high quality reads, with a total size of 5,893,234 bp, a N50 value of 55,012 and a 35% G + C content. The analyzes provided an output report containing score, e-value, gap, mismatch, gene location and direction on the tape. Ten cry genes have been identified (cry1Ab, cry1Bd, cry1Cb, cry1Db, cry1Fb, cry1Ga, cry1Gb, cry1Ib, cry2Ac, cry9Aa) and three vip genes (vip1Ac, vip2Ae, vip3Aa). The complete genome sequence of B. thuringiensis 1608A will be deposited in GenBank and further studies are being carried out from that work, including analysis of gene expression by qPCR, cloning and expression of the proteins in E. coli.

POSTER SESSION. Wednesday, 16:30 PB-21

Development of a Bacterial Pesticidal Protein Resource Center

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To date over 700 cry genes sequences that code for crystal (Cry) proteins have been formally identified. Most of these have been isolated from strains of Bacillus thuringiensis although an increasing number are being found in other bacterial species. The goals of this project were to review the existing Bt toxin nomenclature and to establish an online database of bacterial pesticidal proteins along with and an accompanying website offering information and further analyses. Following extensive consultation with academics, industry scientists and regulators the decision was made to revise the nomenclature to incorporate the following principles: 1) the nomenclature to be renamed bacterial pesticidal proteins rather than Bt toxins 2) the system to be as automated as much as possible 3) pesticidal proteins to be sub-classified according to sequence/structure homology groups. Different mnemonics have been chosen for the different homology groups eg Mpp for the MTX/ETX type toxins, Tpp for the Toxin-10 (Bin-like) proteins. In this poster we will present the full new nomenclature and associated database, as well as the current status of the interface developed for user access. This will allow users to compare their own proteins with those in the database. We will also highlight future plans for this resource center.

POSTER SESSION. Wednesday, 16:30 PB-22 STU

Molecular taxonomic characterization of Bacillus thuringiensis isolates from Kazakhstan

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The Gram-positive, spore-forming bacterium Bacillus thuringiensis (Bt) is the economically most important entomopathogen for insect biocontrol. Systematically, B. thuringiensis forms a sub-species within the Bacillus cereus sensu lato complex. In addition to further toxin types, the bacterial pathogen kills its host (e.g. Lepidopteran, Coleopteran, and Dipteran insects) through the action of highly specific, crystal-forming Cry pro-
tein toxins (Crickmore et al. 2016, http://www.btnomenclature.info/). In the present study, the molecular taxonomy of monosporic cultures of B. thuringiensis collected from south and south-east region of Kazakhstan was investigated.

A partial sequence of the pyruvate carboxylase encoding pycA gene and the glyceral uptake facilitator protein glpF gene were sequenced as molecular-taxonomic markers to determine the systematic position of the isolates with respect to the Bacillus cereus sensu lato complex as part of the multi locus sequence analysis (MLSA) scheme introduced by Priest et al. (2004, J. Bacteriol 186: 7959-7970). Phylogenetic reconstruction located all isolates within the Bacillus cereus complex and with firm bootstrap support in the clade representing the B. cereus sub-species Bacillus thuringiensis. Importantly, isolates were thereby unambiguously differentiated from human both pathogenic B. cereus and B. anthracis. Moreover, Kazakh isolates were shown to belong to the Bt lineages “sotto” and “kurstaki”.

Poster Session. Wednesday, 16:30 PB-23

Molecular characterization of a new Bacillus thuringiensis strain from Argentina toxic against Lepidoptera and Coleoptera base on its whole-genome analysis

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The insecticidal proteins of Bacillus thuringiensis are used as formulations of spore-crystal complexes and their genes have been incorporated into several crops, which has provided a model for genetic engineering in agriculture. Despite the variability of the Cry proteins described so far, it is still necessary to look for toxins with a broad spectrum of action, since a significant number of pests are not controlled with the available Cry proteins. It is also important to provide alternatives to address the problem of insect resistance, which has already appeared with the use of formulations and in transgenic plants that express cry genes that code for insecticidal proteins. We report the characterization of a novel B. thuringiensis isolate native to Argentina (FCC7) toxic against lepidopteran and coleopteran insects. The strain shows a rounded crystal harboring mainly a protein of about 130 kDa. Through the whole-genome sequencing by Illumina Miseq 1500 platform we detected two crystal protein genes with cry8-like genes homology, three vegetative insecticidal protein (Vip) genes and multiple virulence factors such as phospholipases, proteases, enhancers, chitinases, among others. The two cry8-like genes, homologous to the sequences Cry8Ac1 and Cry9Qa1 with 73.4 % and 88.9 % identity respectively, were cloned and expressed into the 4Q7/88,9 % identity respectively, were cloned and expressed into the 4Q7/3 strain and the larvicidal activity was tested against Spodoptera frugiperda and Tenebrio molitor. Two of the Vip genes were identified as Vip1-like with an identity of 74.9% and 70.3% respectively while the third Vip gene was 82% identical to Vip2 sequences. Supported by ANPCyT (PICT No 2015-0575) and Universidad Nacional de Mar del Plata (15/E793 EXA840/17).

Poster Session. Wednesday, 16:30 PB-24

The impact of the absence of the Bacillus cereus siderophore Bacillibactin (BB) on iron acquisition and in insect virulence

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The ability of B. cereus and B. thuringiensis to colonize hosts is linked to several adaptation factors, including iron acquisition from host iron sources like hemoglobin, ferritin. Previously, we showed that the surface protein IlsA is expressed in the insect haemocoeel and takes part in virulence. IlsA binds host ferritin and the iron stored in this molecule can be mobilized for bacterial growth by the combined action of IlsA and the siderophore Bacillibactin (BB) encode by “Ent” operon. To enter the bacteria the BB/Iron complex binds to a Siderophore Binding Proteins (SPB) at the cell surface. To get insight into the role of the SPB “FeuA” in iron uptake from ferritin a Bc ATCC 14579 mutant ΔfeuA and a ΔfeuAΔentA double mutant along with complemented strains were analysed. The results indicate that the ΔfeuA mutant is affected in vitro almost similarly to the entA-mutant with ferritin as the sole iron source. Interestingly, in vivo injection (injection into the hemocoeel of G. mellonella) shows that the feuA mutant and particularly the double mutant ΔfeuAΔentA were as virulent as the wildtype strain, while the FeuA complemented double mutant resulted in the less virulent EntA mutant phenotype. In addition, a in trans plasmid born transcriptional ilsA fusion was analyzed in the various strains and in different growth media. IlsA was significantly expressed in the double mutant background but absent from the wild type and the single mutants when ferritin was the only iron source. This suggests, that in the absence of BB/FeuA B. cereus can modulate expression of iron acquisition and or virulence factors to maintain host colonisation.

Poster Session. Wednesday, 16:30 PB-25

Populational and Genetic Analysis of Wolbachia Symbionts in some Pests of Russia

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Maternally-inherited symbionts of Wolbachia genus are extremely widespread among insects. These symbionts can affect biology of their hosts in different ways, including sex ratio shift, contribution to essential metabolite synthesis, suppress host mutations, protection against viruses and etc. However, there are few information of Wolbachia effect on the most infected hosts. Indeed such experiments are rather laborious and should imply multidimensional and integral design. Here we present data on Wolbachia prevalence and its genetic diversity in populations of different pests. In particular, we studied native and invasive populations of Polygraphus proximus (Coleoptera; Curculionidae: Scolytinae), Siberian and European populations of Aporia crataegi (Lepidoptera; Pieridae), Far-Eastern and Siberian populations of Dendrolimus sibiricus and Dendrolimus pini (Lepidoptera; Lasiocampidae), East European populations of Ostrinia nubilalis and Ostrinia scapulalis (Lepidoptera; Crambidae), and other species. As a result, we observed cases of high Wolbachia prevalence in populations, recent Wolbachia horizontal transmission, and Wolbachia loss. High Wolbachia prevalence in pest populations over broad territory is likely to indicate an important role of these symbionts in host biology. Finally, we aim to confer the ways of experimental tests of Wolbachia role in certain species and applying Wolbachia infection as an agent for pest control.

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Poster Session. Wednesday, 16:30 PB-26 STU

No synergism of Cry1Ca and Vip3Aa by Lepidoptera and Coleop- tera fragments of cadherin in Spodoptera exigua and Grapholita molesta

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Bacillus thuringiensis (Bt) is a gram positive bacteria used as a biope-
sticide worldwide. During its life cycle, Bt produces different insecticidal proteins, among them Vegetative Insecticidal Proteins (Vip) and Crystal proteins (Cry) are the most commercially used. Although large number of Bt toxins have been reported, only few of them (such as Cry1Ca and Vip3Aa) showed toxicity to the cosmopolitan pest Spodoptera exigua. Several strategies have been developed to enhance the toxicity of Bt toxins and to delay the arise of resistant populations, including the addition of synergistic agents, such as different cadherin fragments that enhance Bt toxicity. Singly, synergistic effect of cadherin fragment from S. exigua (rSeCad1bp) on Cry1C toxicity in S. exigua has been reported. However, our results were in disagreement with the previous study. In addition, we observed no synergistic effect of rSeCad1bp in Grapholita molesta, another lepidopteran species susceptible to Cry1C. Moreover, the cadherin fragment from Tenebrio molitor (rTmCad1p) does not increase the susceptibility of Cry1C to either of these insect species. Additionally, the effect of both cadherin fragments in combination with Vip3 protein in S. exigua and G. molesta were tested, showing no enhancement. Remarkably, no antagonistic effect was observed in S. exigua or in G. molesta in all combinations of toxins-cadherin fragments used. Our results confirm previous observations in which synergistic effect of cadherin fragments could not be considered universal, as well as point out the importance of understanding the basis of synergistic effect between Bt toxins, peptide receptors, and insect populations.

**POSTER SESSION. Wednesday, 16:30 PB-27**

Potentially pathogenic microbiota in bacterial communities of the aquatic invertebrates from freshwater waterbodies of different trophic states

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The structure of bacterial communities associated with the different aquatic invertebrates was estimated from three sites in Russia: Lake Teleskoye (51°27’N; 87°30’E), Chany Lake (54°36’N, 78°12’E), and the Kolyma River (68°27’N, 160°48’E). The collected invertebrates belonged to different orders of Insecta (Coleoptera, Diptera, Hemiptera, and Trichoptera), Crustacea (Amphipoda, Argolida, Cladocera, and Copepoda), two different families of Gastropoda (Lymnaeidae and Planorbidae), one subclass of Annelida (Hirudinea), and one family of Arachnida (Hydrachnidae). The associated microbial communities were studied using high-throughput sequencing of the V3-V4 region of the 16S rRNA gene. Our data showed that the relative abundances of bacteria associated with the aquatic invertebrates were varied according to the environmental habitats of the host and phylogenetic position. At the genus level, Wolbachia was the dominant species in Corixidae sp. (42.0%), Notonectidae sp. (25.01%), and Hydrachnidae sp. (35.9%). The presence of other intracellular symbionts that could regulate host reproduction, such as Spiroplasma, and “Candidatus Cardinium”, were presented in low abundance (<1%). Ricetta was only presented in Gammarus sp. (2.97%). The microbiota of parasitic crustacea (Argulus sp. and Lernaea sp.) were represented by Flavobacterium, Aeromonadaceae sp., Corynebacterium and Streptococcus. The dominant microbiota associated with Mollusca were Arcobacter, Rhodobacter and bacteria from family Comamonadaceae. These results could be of value of interest in invertebrate pathology, providing insights into the relationships between host and their associated bacteria.

**POSTER SESSION. Wednesday, 16:30 PB-28**

Occurrence of entomopathogenic bacteria in fruit flies reared in laboratory condition

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Fruit flies are insect pests that attack a wide range of grown plants, have short life cycles and high reproductive rates, occurring in several regions of the planet. The rearing of Ceratitis capitata, Anastrepha fraterculus and A. grandis have been maintained by the Laboratory of Economic Entomology of the Biological Institute, Brazil, for about three decades. The flies have been used for the development of researches aiming to study the behavior, biology, resistance to pesticides, biological control and management of these pests. Recently, a high mortality rates (> 90%) of these flies were observed in the laboratory rearing, demanding new studies to find out the causes of such large mortality. Microbiological analysis showed the occurrence of bacterial contamination, being the agents isolated and molecularly identified as Bacillus subtilis and Brevisbacillus sp. These same bacteria were isolated as endophytic from the fruits of pumpkins used for the A. grandis rearing, explaining how these contaminants entered in rearing and started kill the insects. These bacteria were tested against C. capitata, providing mortalities of 66% and 94% for pupae, 99% and 95% for larvae, respectively. The mixture of the two bacteria was highly virulent for both stages (95% for pupae and 93% for larvae). These data highlight the importance of using fruits free from endophytic bacteria for the rearing of flies. In addition, these data suggest the bacteria are potential agents for the biological control of fruit flies.

**POSTER SESSION. Wednesday, 16:30 PB-29**

Characterization of Bacillus thuringiensis isolates from Jamaica

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Bacillus thuringiensis is a microorganism which has proven to be beneficial to the agricultural sector. Its unique ability to produce (cry) proteins that have insecticidal properties has been pivotal in the control of several insect pests which are detrimental to agriculture. As such this study sought to isolate and characterize Bacillus thuringiensis from Jamaican soil. Bacillus thuringiensis was isolated from soil samples using the acetate selection method as described by Martin et. al (1989). The Bt index was determined to be 0.87. The parasporal crystal morphology associated with the isolates included cuboidal, bi-pyramidal, round, rectangular as well as hour-glass. 16S rRNA analysis showed the native B. thuringiensis strain from soil samples using the acetate selection method as described by Martin et. al. (1989).

**POSTER SESSION. Wednesday, 16:30 PB-30 STU**

Critical structural and functional amino acids of Vip3A f from Bacillus thuringiensis

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The Vip3A proteins, produced during the vegetative phase of growth of Bacillus thuringiensis, show insecticidal activity against Lepidoptera, while the details of the mode of action are poorly elucidated. According to our previous results from an alanine scanning of Vip3A, several residues have been found to be critical regarding the stability and function of Vip3A. In
this study we have focused on these Ala-mutants. The insecticidal activity against Spodoptera frugiperda has been compared between the proteox and activated toxin forms. Mutants T167A, E168A, F229A, W552A and G727A totally lost insecticidal activity, both as proteox or activated toxin. However, E483 was moderately toxic as proteox, but when tested after in vitro trypsin activation, it completely lost the insecticidal activity. Gel filtration chromatography has been used to determine the effect of the Ala substitutions on oligomer formation. F229A and E483 proteoxins behaved as dimer and monomer, respectively. However, upon trypsinization, the chromatogram only showed small fragments, revealing the instability of the toxin. Trypsin proteolysis revealed different proteolytic patterns depending on the residue substitution, suggesting cleavage sites between different domains of the Vip3A protein.

POSTER SESSION. Wednesday, 16:30 PB-31 STU

Characterization of two highly insecticidal endophytic strains of Bacillus thuringiensis

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Bacillus thuringiensis (Bt) is known as the most successful microbial insecticide against different orders of insect pests in agriculture and medicine. It is known that isolates of Bt can be found in a wide and diverse number of habitats. The recent discovery of endophytic Bt strains opened new perspectives for studies aimed at the control of insects that feed on internal tissues of plants. Therefore, the search for Bt as an endophytic bacterium is a promising technique to find new strains. In this work, leaves of 30 plant species (trees and weeds) were selected. The leaves were surface sterilized and the sap was extracted. The sap was inoculated on LB agar plates and incubated at 30°C for 96 h. Colonies with typical morphology of Bt and presence of parasporal bodies were selected. Parasporal inclusions with amorphous structure in the sap of Lavandula angustifolia (lavender) and bipyrimal structure in the sap of Euphorbia pulcherrima (poinsettia) plant were identified. Interestingly, the lavender strain was toxic to Aedes aegypti and the poinsettia strain was toxic to Manduca sexta. Quantitative bioassay with the spore-crystal complex and pure crystals showed that both strains are highly toxic. Flagellin gene sequencing was carried out and sequence analysis indicated that the lavender strain is 99% similar to the israelensis serotype and the poinsettia strain is 89% similar to the kurstaki serotype.

POSTER SESSION. Wednesday, 16:30 PB-32

Identification of antimicrobial peptides in wheat stink bug, Aelia rostrata (Hemiptera: Pentatomidae)

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Antimicrobial peptides (AMPs) are small cationic peptides characterized by hydrophobicity and amphipathicity and produced mainly by fat body and hemocytes upon microbial challenge. They are well-characterized in lepidopterans, dipterans and coleopterans; however, poorly-known in hemipterans. In the current study, we report three classes of AMPs, defensin, Acalo Defnin and Attacin from the hemipteran wheat stink bug, Aelia rostrata, a major pest of grains in Turkey and Middle East. The A. rostrata defensin 1 (ArDef1) cDNA has a length of 433 bp encoding a 120 amino acid mature protein with a predicted molecular weight (mw) of 13.8 kDa. A 435 bp partial cDNA encoded an incomplete 145 amino acid protein, denoted A. rostrata Acalo Defnin 1 (ArACL1), had a predicted mw of 16.5 kDa. Six cDNAs (492-561 bp) encoding attacins (ArATT1-6) with a predicted mw of 15.8 kDa were also defined. Transcriptome analyses of the fat body indicated that ArDEF1 expression in feeding females was 100-fold more than that in males. ArACL1 is expressed 3-fold more in the estivating females than that of hibernating females and 32-fold more in estivating females than that in males. ArATT1 is expressed 32 fold more in estivating females than that of hibernating females and 127-fold more in estivating females than that in males. ArATT1 expression in females dramatically upregulated 1,150-fold by estivation. Overall, the higher expression of the AMP genes in estivating stage and more in females in general is a mysterious finding that requires further investigation.

POSTER SESSION. Wednesday, 16:30 PB-33

Identification of novel hemiptericins in sunnpest, Eurygaster mauro (Hemiptera: Scutelleridae)

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One defence mechanism in insects includes the humoral responses primarily composed of antimicrobial peptides (AMPs). AMPs are small, hydrophobic, glycine-rich peptides and produced exclusively by haemocytes and fat body. Midgut is also a site for AMP synthesis as it is the principal target for initiation of infection for per os infecting agents. In this study, we report the first time AMPs from the midgut of the hemipteran sunnpest, Eurygaster mauro (Hemiptera: Scutelleridae), the major pest of wheat and barley in Turkey and the Middle East. A scan of a midgut cDNA library generated from the feeding E. mauro adults in the field resulted in eight novel AMPs in BLAST analysis. These denoted E. mauro hemiptericins 1-8 (EmHEM1-8). The full length E. mauro hemiptericin (EmHEM3, EmHEM4, EmHEM5, EmHEM6, and EmHEM7) cDNAs have a length of 408-477 bp encoding a 104-122 amino acid mature protein with a predicted molecular weight (mw) of 11.9-15.2 kDa. The partial length E. mauro hemiptericin cDNAs (EmHEM1, EmHEM2 and EmHEM8) cDNAs have a length of 300-324 bp encoding a 77-107 amino acid mature protein with a predicted molecular weight (mw) of 8.3-11.7 kDa. Besides, three defensins, denoted as E. mauro defensin 1-3 (EmDEF1-3) have been identified as well. The E. mauro defensin cDNAs have a length of 300-372 bp encoding a 79-101 amino acid mature protein with a predicted molecular weight (mw) of 8.8-11.5 kDa. Proteomic analysis revealed that EmHEM4 was the only predominant AMP present at protein level in the midgut among 11 identified AMPs.

POSTER SESSION. Wednesday, 16:30 PB-34 STU

Cultivation of entomopathogenic fungi in orbitally shaken bioreactors – investigation of respiratory activity in scale-up experiments

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Scale-up considerations are an everlasting challenge in industrial scale production processes of all biotechnological applications. Bioengineering parameters are consulted to determine important scale-up factors and in addition, process control utilizing on-line, at-line or off-line measurements are performed. Analysis of cellular respiration allows improved understanding of cell metabolism and direct determination of cultivation conditions. In this study, batch cultivations with Metarhizium brunneum using complex medium supplemented with ground barley in 500 mL Erlenmeyer flask and in the single-use OrbShake system SB10 X (Kuhnner Shaker) were performed. Working volume and cultivation parameters were set to values which result in similar theoretical oxygen-transfer-rates (OTR). Data concerning the respiratory activity and sugar consumption of Metarhizium brunneum using off-line sugar and on-line exhaust gas measurements were obtained. High reproducibility in shake flasks and a
comparable respiratory and sugar consumption profile in the OrbShake system was observed. A maximum carbon dioxide-transfer-rate (CTR) of 3.89 mmol·L⁻¹·h⁻¹ at 113.9 h and 5.96 mmol·L⁻¹·h⁻¹ at 51.9 h was achieved in shake flasks and the OrbShake system, respectively. The sum of carbon dioxide production resulted in 563.0 mmol·L⁻¹ and 532.9 mmol·L⁻¹, respectively, after 240 h of cultivation. Sugar depletion occurred after 163.5 h and 189.5 h of cultivation, respectively, with changing respiratory quotients due to a metabolic change in starch hydrolysis.

We present a reliable process monitoring strategy which is easily applicable across lab- and pilot-scale cultivation processes, with simplified process monitoring and contamination detection. The understanding of the respiratory activity of entomopathogenic fungi during cultivation enables further improvement of production processes and makes the commercialisation of these biological pest control agents economically more attractive.

Application of polyamine carbon quantum dots (CQDs) to aquatic viral disease control: taking shrimp white spot syndrome (WSS) as an example

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White spot syndrome virus (WSSV) is the causative agent of white spot syndrome (WSS) that has led to severe mortalities of cultured shrimps all over the world. WSSV is an enveloped, ellipsoid, large, double-stranded DNA virus and it has a wide host range among crustaceans. Currently, the mainly antiviral methods are to block the receptor of host cell membrane by recombinant viral protein or virus antisera. In addition to interfering with the ligand-receptor binding, disrupting the structure of the virus envelope may also be a means of combating viral infection. Carbon quantum dots (CQDs) are carbonaceous nanomaterials and have many advantage characteristics, including small size, low cytotoxicity, ease of production and modification. Polyamine CQDs have been identified with strong antibacterial ability. They are very small and have highly positive charge which cause severe disruption of the bacterial membrane. In this study, polyamine CQDs are identified to destroy WSSV envelope. Polyamine CQDs can interfere WSSV to inhibit the virus infection and show dose-dependent effect. The results in this study also show that polyamines CQDs can induce several immune genes in shrimp and reduce the virus envelope may also be a means of combating viral infection. Carbon quantum dots (CQDs) are carbonaceous nanomaterials and have many advantage characteristics, including small size, low cytotoxicity, ease of production and modification. Polyamine CQDs have been identified with strong antibacterial ability. They are very small and have highly positive charge which cause severe disruption of the bacterial membrane. In this study, polyamine CQDs are identified to destroy WSSV envelope. Polyamine CQDs can interfere WSSV to inhibit the virus infection and show dose-dependent effect. The results in this study also show that polyamines CQDs can induce several immune genes in shrimp and reduce the virus envelope may also be a means of combating viral infection.

First record of entomopathogenic fungus Entomophaga aulicae in the populations of browntail moth in Bosnia and Herzegovina

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Tetrahydrobiopterin (BH4) is an essential coenzyme for aromatic amino acid hydroxylases and nitric oxide synthase (NOS). NOS catalyze the production of nitric oxide (NO), which is an important cellular signaling molecule. Therefore, BH4 controls the levels of monoamine neurotransmitters of dopamine and serotonin in vivo and plays a key role in many biological processes. BH4 deficiency is associated with numerous metabolic syndromes and neuropsychological disorders. Our previous studies have shown that, in the silkworm Bombyx mori, genetic mutation of sepiapterin reductase (SPR, a key enzyme in the BH4 synthesis pathway) caused activity loss is responsible for the lemon lethal (lemr) mutant phenotypes. In order to know whether NOS/NO contribute to lethality of the 2nd instar larvae, in this study, we performed several experiments and found that both NOS activity and NO concentration in lemr were significantly lower than those in wild type silkworms while the content of superoxide anion was extremely higher on the contrary. When the expression of BmSpr was knocked down in BmN cells, similar results were repeatedly obtained. Whatmore, addition of L-NNAME, an effective inhibitor of NOS, clearly induced the decrease of NO and increase of superoxide anion in BmN cells, which at a result, directly facilitated the decline of cell proliferation ability. Our findings give a first description on the involvement of NOS/NO in the silkworm mutant lemr and suggested that due to serious lack of BH4, uncoupling of NOS resulted in a set of downstream oxidative stress responses, which are harmful to cell and body and promote the lethality of lemr larvae.
Brownail moth, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Erebidae), is a well-known pest of broadleaf forests of Bosnia and Herzegovina. Although it is extremely polyphagous, it prefers to consume the leaves of various species of oaks. Brownail moth occurs periodically in high numbers (outbreak). Entomopathogenic fungus *Entomophaga aulicae* (Reichardt and Hail) Humber (Zygomycotina: Entomophorales, Entomophoraceae) is widespread Holarctic species, with many host insects from order Lepidoptera, where are some of the most economically harmful, outbreaks species of forest defoliators. In sessile oak forests of eastern Bosnia and Herzegovina, the population density of brownail moth was determined by using route measurement during the growing season in the period 2015-2016. Brownail moth newly litters (40) were collected in four oak stands located in the region of Foča, Višegrad and Rogatica (PE Forests of the Republic of Srpska, Forest Estates Maglić, Panos and Šjemeć). The evaluation of *E. aulicae* infections was recorded as positive when hyphal bodies, primary conidia, or resting spores were detected on the surface of cadavers and puparia or in their tissues. The species identification was based on the size, shape and structural characteristics of different life forms of the fungus. By the microscopical studies of the causes of the mortality of the brownail moth larvae and pupae, the presence of hyphal bodies, primary conidia and resting spores of the *E. aulicae* were confirmed in them. As entomopathogenic fungus on two development stages of the host, larvae and pupae, presented results indicate that *E. aulicae* is naturally regulating brownail moth populations and should be considered for conservation microbial control.

**POSTER SESSION. Wednesday, 16:30 PF-3**

*New Beauveria bassiana* strain (Bals.-Criv.) Vuill., pathogenicity against weevil pests and physiological characterization

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Entomopathogenic fungi have shown promising results as agents for insect pest control. Isolation and characterization of local highly virulent strains is one of the most important steps towards development and large-scale application of microbial control agents. *Beauveria bassiana* CNMN-FE-01, a new strain isolated in the Republic of Moldova, was tested against two insect pest species, pea leaf weevil *Sitona lineatus*, and alfalfa weevil *Hypera postica*. A concentration of 9.7 × 10⁴ conidia/ml of *B. bassiana* CNMN-FE-01 strain caused 100% mortality of *S. lineatus* adults on the day five after treatment. LC₅₀ value was equal to 1.127 × 10⁴ conidia/ml. The mortality and LC₅₀ values obtained for *H. postica* were not significant. The effect of different parameters (temperature, salinity, pH, UV radiation) on colony growth, sporulation, and germination rate, has been evaluated to proceed with biopesticide formulation. For each parameter, four Petri dishes were used, measurements being recorded daily. Results were taken as mean ± standard error (S.E.) of N observations taken in four replicates (n = 4). Data sets were examined by one-way analysis of variance (ANOVA). P-value of less than 0.05 was considered significant. Local *B. bassiana* CNMN-FE-01 strain has manifested tolerance and adaptability to variable conditions with encouraging physiological features for biopesticide formulation.

**POSTER SESSION. Wednesday, 16:30 PF-4**

Evaluation of entomopathogenic fungi to control *Stenoma cecropia* (Lepidoptera: Elachistidae), insect pest of oil palm in Colombia


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*Differential response of Beauveria bassiana isolates to growth on hydrocarbons and its potential association with virulence to the soybean pest Piezodorus guildinii*

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The hydrocarbon layer of the insect epicuticle represents the first barrier to infection by entomopathogenic fungi *Beauveria bassiana*. We hypothesize that fungal genes involved in hydrocarbon assimilation and the resulting oxidative stress are key factors in pathogenesis. We studied the effect of hydrocarbon nutrition on phenotype and gene expression of two *B. bassiana* strains, showing different virulence towards *Piezodorus guildinii*, an hemipteran highly resistant to fungal entomopathogens. Both strains were grown on minimal media (MM) supplemented with different hydrocarbons (*n*-tridecane, *n*-pentadecane and *n*-octacosane) as sole carbon source and on complete media (CM) with glucose as carbon source. After 15 days of incubation, conidigenous ability, germination rate and radial growth were evaluated. The expression of genes involved in either hydrocarbon assimilation (cytochrome P450s) or oxidative stress (superoxide dismutases, catalases, and glutathione peroxidase) was also evaluated at different time periods. Both strains were able to grow in hydrocarbon-supplemented MM and exhibited high germination rates, although ILB 299 showed significantly lower sporulation in MM than in CM. Two cytochrome P450 genes were induced at day 3 in alkane-grown ILB308, the same as two catalases, one superoxide dismutase and glutathione peroxidase genes at day 6. The strain ILB299 also showed an induction of these genes starting at day 6. In conclusion, gene induction was much higher in ILB308 and started earlier in time. These data correlate well with colony growth, sporulation and mortality rates of both isolates and might help to explain, at least partially, the different virulence exhibited by both *B. bassiana* isolates.
Spanish mycoviral population of the entomopathogenic fungus *Beauveria bassiana*  

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Currently there is a resurgence of interest in the use of microbial insecticides as an alternative to chemical control or as part of Integrated Pest Management programs. Entomopathogenic fungi in particular have a unique mode of action and can control effectively a great variety of insect pests. Recently, fungal virulence was found to be increased in *Beauveria bassiana* by the presence of a double stranded (ds) RNA virus, a rare occurrence since most dsRNA infections in fungi are asymptomatic or reduce virulence. The aim of the current work is to investigate the occurrence since most dsRNA infections in fungi are asymptomatic or reduce virulence. The current work is to investigate the occurrence and diversity of mycoviruses in large panels of entomopathogenic fungal populations, mostly from Spain and Denmark. Following screening of 151 B. bassiana and *Metarhizium* spp. isolates for dsRNA elements, 12 Spanish B. bassiana isolates were found to harbor mycoviruses, previously described as *Beauveria bassiana* victorivirus 1 (BbVV-1). *Beauveria bassiana* partitivirus 2 (BbPV-2) and *Beauveria bassiana* polymycovirus 1 (BbPmV-1). The Spanish mycoviral population structure may be a result of both vertical and horizontal mycovirus transmission as suggested by phylogenetic analysis. Additionally, the virulence of the 12 fungal isolates against the Mediterranean fruit fly *Ceratitis capitata* did not show direct correlation with the presence of specific mycoviruses. Future experiments should be focused on curing B. bassiana isolates from the mycovirus infection and comparing the phenotype of the isogenic lines.

**POSTER SESSION. Wednesday, 16:30 PF-6**

**Down regulation of chitin synthase 1 gene elevates insecticidal activity of Beauveria bassiana ANU1 against Solenopsis invicta**  

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Entomopathogenic fungi have been studied to develop for biological control agents as an alternative to chemical control agents in insect pest management. A fungal isolate collected from a dead *Spodoptera exigua* larva in onion field and identified as *Beauveria bassiana* by sequencing analysis on ITS and β-tubulin and morphological characteristics under microscope. The fungi named *B. bassiana* ANU1 as Korean isolate and test insecticidal effect against a red imported fire ant, *Solenopsis invicta* workers in laboratory condition. A LC50 was determined as 1x10⁸ conidia/ml by spray application. *S. invicta* chitin synthase 1 gene (SICS-1) obtained by NGS analysis on *S. invicta*. Feeding dsRNA, which is specific to SICS-1, decreased expression level of target gene and elevated insecticidal activity of *B. bassiana* ANU1. This result suggests that RNAi will apply to control *S. invicta* in the field with entomopathogenic fungi.

**POSTER SESSION. Wednesday, 16:30 PF-8**

**Differential susceptibility towards UV-B radiation among *Metarhizium* spp. isolates from different ecosystem compartments**  

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Differential susceptibility towards UV-B radiation among *Metarhizium* spp. isolates from different ecosystem compartments is an important factor in relation to this niche differentiation is coping with solar radiation, which can be detrimental to fungal propagules. The objective of this study was to evaluate conidial survival of *Metarhizium robertsi* (n=3), *Metarhizium brunneum* (n=3) and *Metarhizium anisopliae* Mani 2 (n=6) isolates exposed to UV-B radiation. Hypothesis is that the latter species tolerates better the exposure. Isolates were cultivated in Potato dextrose agar plus yeast extract (PDAY) for 10 days. Then inocula (150 µL; concentration: 1x10⁶ conidia mL⁻¹) were prepared, applied in plates containing PDAY placed under fluorescent lamps (313 nm, equivalent to UV-B light) and exposed for up to 8h; every 2h one plate of each isolate was transferred to an incubator (25 °C, 12h photophase) for 48h, allowing DNA repair and conidia germination. Control plates, not exposed, were incubated for 24h. Viabilities were then evaluated. After 8h, most of the isolates had viabilities below 5%, while one *M. robertsi* isolate was the most tolerant, with viability higher than 50%. There was considerable within-species variability for *M. robertsi* and *M. anisopliae*. Tolerance to UV radiation therefore varies infra- and interspecifically. This knowledge improves the understanding of fungal adaptations to the environment and can assist in better selection of strains for use in biocontrol programs.
Novel biocontrol product, Nomu-Protec, developed through solid state fermentation of the entomopathogenic fungus *Metarhizium (Nomuraea) rileyi*

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The entomopathogenic fungus *Metarhizium rileyi* (previously *Nomuraea rileyi*) targets lepidopteran pests, especially from the Noctuidae family. This group includes economically important pests such as the fall armyworm (*Spodoptera frugiperda*) and African bollworm (*Helicoverpa armigera*). These pests infest a range of field crops such as maize, soybean and wheat, many of which are staple foods in Africa and other parts of the world. *Metarhizium rileyi* has been studied previously for its potential as a biological control agent against insect pests. However, the fastidious nature of the fungus makes it difficult to grow in culture and mass produce. Scientists at Plant Health Products have developed unique methods using solid state fermentation to overcome these challenges and in so doing can successfully produce *M. rileyi* fungal spores for use as the active ingredient in the product Nomu-Protec. Although there are other products based on *M. anisopliae* that are available globally, this will be the first product to use *M. rileyi* in Europe. Nomu-Protec is a wettable powder which makes it easy to use and apply in the field as a cover spray. Its unique formulation prevents the fungal spores from drying out and enhances the ability of the spores to penetrate and infect the pest. In several field trials, Nomu-Protec proved to be very effective against bollworm, revealing a significant decrease in the pest population as well as effective reduction in feeding damage comparable with other registered chemical standards.

**POSTER SESSION. Wednesday, 16:30 PF-10**

**Effect of Lecanicillium spp. against eggs of greenhouse whitefly, Trialeurodes vaporariorum and sweetpotato whitefly, Bemisia tabaci**

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Whiteflies are one of important pests on crop production worldwide. Entomopathogenic fungus *Lecanicillium* spp. is the well-known biological control agents against whiteflies. *Lecanicillium* spp. has been reported to have high pathogenicity to larvae and adult whitefly, but there is very little information about effect to whitefly’s egg. We evaluated effect of *L. longisporum* and *L. muscanarium* against egg of *Trialeurodes vaporariorum* and *Bemisia tabaci*. The hatching rate of *T. vaporariorum* eggs decreased by inoculation of *Lecanicillium* spp., whereas there was no significance in *B. tabaci* egg. Fungal invasion to egg on both whitefly species was confirmed. Egg invasion rate corresponded with reduction of hatching rate, and that the decline in the hatching rate may be mainly caused by the direct invasion of *Lecanicillium* spp. into egg. Furthermore, *Lecanicillium* spp. showed high mortality rate (50-90%) against hatching larvae of both whiteflies. Meanwhile, hatching rate and invasion rate of *Lecanicillium* spp. inoculated whitefly eggs were higher in immature yellow eggs than mature blackish eggs. This is because immature yellow eggs don’t have a hard-outer protective shell that can inhibit *Lecanicillium* spp. invasion. Likewise, the mature blackish egg was able to resist infection because of the outer hard shell. In conclusion, *Lecanicillium* spp. can infect to the both whitefly’s egg, and the hatching rate will be decreased. Also, there is possibility that whitefly egg become new target developmental stage in microbial control.

**POSTER SESSION. Wednesday, 16:30 PF-11**

**Virulence of entomopathogenic fungus Beauveria bassiana ARP14 against egg of Trialeurodes vaporariorum (Homoptera: Aleyrodidae)**

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*Trialeurodes vaporariorum* is an important pest of various crops worldwide. We compared the virulence of a new *Beauveria bassiana* strain (ARP14) with commercialized one (GHA) against egg of *T. vaporariorum*. The eggs were exposed to a concentration of 1x10^8 conidia/mL using a leaf dipping method. Mycosis rate of the eggs was not statistically different between ARP14 (13.5%) and GHA (35.3%). Also mycosis rate of 1st instar hatched was also similar between ARP14 (95.6%) and GHA (90.9%). Interestingly, the period from egg to 1st instar nymph was shortest in ARP14 (7.3 d) followed by GHA (7.8 d) and untreated control (8.2 d) at 25°C with unknown reason. These results suggest that the new *B. bassiana* strain (ARP14) would be another potential entomopathogenic fungus to be developed for whitefly management.

**POSTER SESSION. Wednesday, 16:30 PF-12**

**Comparison of infection dynamics using two strains of Beauveria bassiana which has different virulence in Anopheles stephensi**

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Entomopathogenic fungi kill insects by “invasion to host tissue/organ and multiplication” and “production of secondary metabolites in hemocoel”. In our previous study, we detected high virulence strain *Beauveria bassiana* 60-2 and low virulence strain *B. bassiana* 2112. *B. bassiana* 2112 showed low virulence on percutaneous infection, but it expressed high virulence on conidia micro-injection to hemocoel. There was possibility to be suppress pathogenicity of *B. bassiana* 2112, despite this strain intrinsically has virulence. In this study, infection dynamics of these two different strains was compared by observing paraffin section of fungus infected Anopheline to declare the factors making difference of virulence. As a result, intense hyphal growth in hemocoel and other host tissues were observed in *B. bassiana* 60-2 infected mosquitoes, but only agglomerated fungal propagules were detected in *B. bassiana* 2112 infected individuals. *B. bassiana* 2112 formed mass-structures of hyphal body-like propagules and there was no hyphal growth at haemocoel. It is known that after invasion to host haemocoel, hyphal body of *B. bassiana* is once encapsulated by host immune system, then they will break this immunity by hyphal extension. In the case of *B. bassiana* 60-2, this strain can express pathogenicity by breaking of host immune system. On the other hand, *B. bassiana* 2112 cannot break these host defense systems and inhibited fungal growth and/or multiplication. These results show that virulence of *B. bassiana* is related to the factors which can overcome host immune system or not.

**POSTER SESSION. Wednesday, 16:30 PF-13**

**Effect of artificial media and temperature on the growth and development of bee brood pathogen Ascosphaera apis and optimization its cultivation in vitro**

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Ascosphaera apis is a causative agent of chalkbrood, which belongs to the most widespread bee brood diseases. It can significantly reduce bee

**POSTER SESSION. Wednesday, 16:30 PF-14**
numbers and colony productivity, especially in humid and stress-related areas. It is closely adapted to bee brood; therefore, it is not possible to use many well-known cultivation methods for common entomopathogenic fungi. In this paper there were compared influence of several artificial media and cultivation under different temperatures on the growth and development of both, female (+) and male (-) mating types of local strains. In the first experiment, radial growth of separated mating types was measured daily. In the second one spore cysts, spore balls and ascospores were counted simultaneously in a Neu-bauer haemacytometer chamber. This technique does not allow counting real number of spores, but it suits very well for assessment of A. apis development characteristics in different conditions. The last experiment was focused on a morphometry of spore cysts and spore balls. There was found a correlation pattern between reproductive structures size and temperatures, which was very useful for overall assessment. As a result of these experiments, the best temperature for in vitro cultivation both, male and female local mating types, was determined as a 30°C. SDA and YGPSA media are suitable for fast growth. For reproductive structures production are the most useful media PDA-BB4, which was newly designed for this purpose, and SDA.

Current trend of microbial insecticides: Challenges and Opportunity in fungal insecticides

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There are many challenges in pest management, such as ecological toxicity of pesticides, pest resistance and strong pesticide regulation. To overcome these issues, global chemical companies have actively merged and acquired biopesticide companies until 2010, but still strategy to develop successful biopesticides is not fully established. Fortunately, many research groups are working on the advanced technology to support the biopesticide R&D. Since 210, collaboration of companies for product distribution is getting more active, for example, Marrone Bio & Isagro or Lidor, STK & BASF or Syngenta. Another trend is to collaborate to develop novel biopesticides, such as Bayer & Forth, Corteva & Evogene, Nu-farm & Marrone Bio, FMC & Chr Hansen, Syngenta & DSM, BASF & Jülich, and Arysta & Koppert. One of the current IP strategy in major companies is to combine chemicals and biological control agents since 2010. From the recent registration of biopesticides in the world, one major interesting trend is to development of fungal insecticides, Bioceres of Anatis Bioprotection (B. bassiana ANT-03), BioAct Prime® of Bayer (P. falciparum), two B. bassiana products of Arysta Life Science, Chongchaesaks® of Farm-Hannong (B. bassiana ERL836), Rizotec® of Rizofora Biotecnologia (P. chlamydospora PC10), and Broadband® & Veilier® of BASF (B. bassiana PPRIS3338). All the fungal insecticides could be combined with chemical spray program in a crop protection calendar, which is probably followed by successful application of fungal biopesticides and production of safe foods. In near the future, biopesticide can be an excellent control agent in smart farming.

Characterization of Pr1 family proteases in Beauveria bassiana

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Most of 11 Pr1 family proteases (Pr1A1/2, Pr1B, Pr1C, Pr1F1/2/3/4, Pr1G and Pr1H1/2) in Beauveria bassiana, an insect mycopathogen, are functionally unknown although they are presumably involved in insect cuticle degradation required for normal infection through cuticular penetration. This study seeks to characterize the functions of these proteases by phenotypic analyses of single-gene deletion and complementary mutants. Six of those proteases, including Pr1A1, Pr1B, Pr1C, Pr1G, Pr1H1 and Pr1H2, are functional and play critical roles in biopesticide function. The Pr1A1, Pr1B, Pr1C, and Pr1G proteases are required for penetration of insect cuticle. The Pr1F1, Pr1F2, and Pr1G proteases are required for germination of conidia and are involved in the biopesticide activity.
and Pr1H2, were found contributing significantly to the fungal virulence through the normal infection due to attenuated virulence in absence of each. Total activity of extracellular (secreted) Pr1 proteases in 12- and 24-h old liquid cultures decreased by 40–60% in Dpr1C, Dpr1H1 and Dpr1H2, 20–30% in Dpr1B and Dpr1G and 5–10% in Dpr1A1. Similar changes in extracellular Pr1 activity were also observed in the deletion mutants grown in a broth based on powder of locust cuticles. However, other Pr1 proteases contributed little to the fungal virulence. These results demonstrate differential roles of the Pr1 family proteases in the infection course of B. bassiana against insect host.

First records of Beauveria bassiana occurrences in the invasive pest Box Tree Moth, Cydalima perspectalis in Georgia


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The Box Tree Moth (BTM), Cydalima perspectalis (Walker, 1859) (Lepidoptera; Crambidae) was introduced in Georgia and began to defoli- ate Buxus spp in large quantities. Today the situation is quite alarming in Western Georgia (WG), in that BTM damages Buxus colchica, which is an endemic species of Caucasian flora and threatened by habitat loss. The larvae feed on leaves and shoots, able to defoliate box trees and cause economic, social and environment problems. During 2017-2018, from different regions and sites (Tsageri, Samegrelo), adults and larvae of C. perspectalis (L3-L5) were collected. In 2017 in mature, Tsageri boxwood forest, adults with developing fungal hyphae on the body were found. We screened living and dead larvae for pathogens. More then 460 larvae were dissected and studied with cultural and morphological methods. Twelve larvae presented fungal growth after death. Nine sporulated fungal isolates from larvae corresponded to Fusarium sp., three to Aspergillus sp. and one to Beauveria bassiana. These isolates showed high susceptibility to ultraviolet radiation, with 33, 49 and 81% of inactivation after irradiation. Optimal conditions for fungal growth were 25°C and pH 6.5. The isolate of B. bassiana showed high potential for the control of C. perspectalis, which caused 81.3% - 75.5% mortality of larvae. This is the first evidence of B. bassiana in this pest and the first record in Georgia. These data contribute to the knowledge of the pathogens of C. perspectalis and constitute the basefor the development of a new biopesticide.

Effectiveness of Beauveria bassiana against Redbanded Stink Bugs (Hemiptera: Pentatomidae), a key pest of soybeans in the southern United States

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The Redbanded stink bugs, Piezodorus guildinii (Westwood), are a neotropical pest of soybean production across the southern United States and the Neotropical Americas. Two strains of Beauveria bassiana (Bals.-Criv.) Vuil. (Hypocreales: Cordycipitaceae) were evaluated for potential control of redbanded stink bug. Four concentrations (7x104, 105, 106, 107) of both a Mississippi Delta native strain (N88) and a commercially available formulation (GHA) were tested against field collected adult redbanded stink bugs in the laboratory and evaluated at four time points (3, 5, 10, and 15 days). Application methods simulated field sprays and were similar to those previously used to screen several other pests and beneficial insects. Both isolates of B. bassiana were tested in pathenogenic to adult redbanded stink bugs. Significant overall differences between strains (regardless of the concentration) was only observed at 3 days after treatment, while differences among concentrations were observed at 5, 10, and 15 days after treatment. The lethal concentration (LC50) and lethal sporation (LS50) were not significantly different between strains at either 10 or 15 days after treatment. However, higher mortality was observed in cohorts sprayed with GHA at lower concentrations that those sprayed with N88. Unlike other control options, B. bassiana has a short re-entry interval and no harvest interval, making it a potentially attractive alternative to conventional synthetic insecticides. Further testing on juvenile life stages and field testing is needed to evaluate potential for in field control.

Entomopathogenic fungi as biocontrol agent against the bulb mite, Rhizoglyphus robinii

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The bulb mite, Rhizoglyphus robinii is an important pest of garlic, onion and ornamentals. The mite is usually found in association with fungal pathogens such as Fusarium spp. Chemical control of the pest is difficult because of its resistance to some acaricides. Due to this reason and concerns about harmfulness of pesticides, alternative methods of plant control are being sought. The aim of this study was to determine the oc-

Bacterial decomposition of insects post Metarhizium infection, possible influence on plant growth.

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Strains of entomopathogenic fungi may have substantial distinction at the final stages of mycoses. Insect cadavers usually overgrow with currence of entomopathogenic fungi (EPF) in onion and garlic fields in Israel and the Czech Republic and test selected EPF strains against R. robinii under laboratory conditions. Strains of Beauveria sp., Isaria sp., Lecanicillum sp., Metarhizium sp., and Purpureocillium sp. were isolated from soil samples in both countries. A total of 20 strains (11 isolates of the genus Metarhizium, 5 isolates of the genus Beauveria, 2 isolates of the genus Isaria and 2 isolates of genus Lecanicillum) of EPF were tested against R. robinii females. A genetic analysis was performed for each tested EPF strain. Isaria fumosorosea strain CCM 8367 was tested as a reference strain and its efficacy was low (38.2 %) compared to some isolated strains. Almost 100% efficacy against R. robinii was found in strains of Metarhizium sp. isolated from Kolence (99.3 %) and Mezltiški (98.6 %) from the Czech Republic and strain isolated from the Gazit in Israel (98.3 %). We can conclude that some EPF, especially genus Metarhizium are promising biocontrol agents against R. robinii.
mycelium after colonization of insect body, but in many cases bacterial decomposition of the colonized hosts is occurred. We studied two \textit{Metarhizium robertsi} isolates: Mak-1 (cadavers overgrow with mycelium), and P-72 (cadavers decay after fungal colonization). We conducted comparative analysis of gut and cadaver microbiota in Colorado potato beetle larvae using 16S rRNA sequencing after infection with these isolates. In addition we estimated content of different forms of nitrogen in cadavers and how the cadavers affect plant growth (\textit{Solanum lycopersicum}) on sand substrate in laboratory conditions. It was shown the infection did not cause significant shift in mid gut bacterial community compared to untreated insects. It is important to note that bacterial communities were similar in both type of cadavers with predomination of enterobacteria. Decomposing cadavers (P-72) were characterized by increased content of nitrate and ammonium and it has more strong growth promoting effect on plants compared to cadavers overgrowing with mycelium and conidia (Mak-1). We also measured colonization and growth promotion of plants after treatment with conidia of both isolates, cultivated on artificial medium. Both cultures successfully colonize plants, but isolates P-72 showed more strong growth promotion relative to Mak-1. We propose that the use of abnormal strains which unable to sporulate on cadavers can lead to only passive (though more rapid) flow of nitrogen from killed insects to plants. The study was supported by Russian Foundation for Basic Research (18-34-20060).

**POSTER SESSION. Wednesday, 16:30 PF-23 STU**

\textbf{Development of a computer-assisted method for the quantification of discharged conidia of an entomopathogenic fungus}

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Quantification of conidia formed by entomopathogenic fungi is one of the most important parameters for the development of fungal formulations designed for biological pest control. The aim of this study was the development of a rapid, simple and objective method for the quantification of discharged conidia of encapsulated Pandora sp. It was assumed that conidia that were spread onto a smooth surface reflect diffuse light and consequently that the emission correlates with the number of discharged conidia. We were able to demonstrate that the correlation between the grey value (as a magnitude of the reflected light by conidia) and the actual number of conidia is applicable for comparable quantification of conidial discharge by the fungus. To separate packages of sticky conidia, the watershed algorithm was applied. Considering conidia counting, automatic and semi-automatic image analysis were tested for their practicability. Automatic counting proved more objective and easier for the experimenters, but semi-automatic counting showed higher correlation with the actual number of conidia. The linear relationship between the number of conidia and the grey value was investigated for calculation within the range of 0.2 and 1.6 x 10\textsuperscript{3} conidia/cm\textsuperscript{2}. In this range, the automatic (R\textsuperscript{2} = 0.946) and semi-automatic (R\textsuperscript{2} = 0.958) method showed no significant difference. The determination of sporulation capacity enables the quality control of fungal formulations. Moreover, the developed method bears the potential to be adapted for the quantification of the sporulation capacity of other fungal biocontrol agents.

**POSTER SESSION. Wednesday, 16:30 PF-24**

\textbf{Determining relative infectivity/virulence of selected entomopathogenic fungi against Asian Citrus Psyllid using spray exposure bioassays}

\textbf{Cisneros, J.} \textsuperscript{1}; \textbf{Wendel, J.} \textsuperscript{1}; \textbf{Jaronski, S. T.} \textsuperscript{1}; \textbf{Vitek, C.} \textsuperscript{2}; \textbf{Flores, D.} \textsuperscript{3}

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The Asian Citrus psyllid (\textit{Diaphorina citri}) vectors "\textit{Candidatus Liberibacter spp.}"; the causative agents of Citrus Greening Disease or Huanglongbing (HLB). Managing the population of psyllids in the Lower Rio Grande Valley (LRGV) is imperative given the continuous increase in detection of HLB-positive trees. A facet of integrated pest management in development is the use of strains of entomopathogenic fungi for the biological control of \textit{D. citri}. In an attempt to find endemic strains of pathogenic fungi that grow favorably under LRGV environmental conditions and naturally infect ACP, psyllid samples were collected from local RV and residential areas, surface sterilized, and plated. Post-mortem fungal samples were isolated and cryostored for later identification. Over 6,000 samples collected from 174 sites throughout the LRGV led to the positive identification of two \textit{Beauveria} spp. isolates which were grown in liquid culture and solid substrate fermentation. Current efforts include the implementation of isolates into applicable bioassays and characterization of the fungus via radial growth plates, UV tolerance, among other techniques. One of the new isolates has shown relative success in spray exposure bioassays and growth kinetics, and may prove to be a good candidate for the control of ACP populations in the LRGV.

**POSTER SESSION. Wednesday, 16:30 PF-26**

\textbf{Heat-exposure of \textit{Metarhizium anisopliae} s.str. conidia in oil suspension and the effects on fungal penetration through the cuticle of the tick \textit{Rhizophalus sanguineus} s.l.}

\textbf{Ferreira, Juliana M.} \textsuperscript{1}; \textbf{Barreto, Lucas P.} \textsuperscript{2}; \textbf{Silva, Carolina S.R.} \textsuperscript{1}; \textbf{Arruda,}
protected suspension previously exposed to heat. We concluded that mineral oil penetration was detected in engorged females treated with aqueous conidial suspension not exposed to heat, and the group treated with aqueous or oil suspensions previously exposed to heat (45 ± 0.2 °C for 4 h). Plates with treated females were then separated into Petri dishes in four groups. Each group was topically treated with aqueous or oil conidial suspension. Two groups were treated with non-heated aqueous or oil suspensions (control groups), and two groups were treated with aqueous or oil suspensions previously exposed to heat (27 ± 1 °C and relative humidity ≥ 98% for 72 or 120 h). We demonstrated, by histological techniques, that the heat exposure delayed the germination of M. anisopliae conidia and the fungal penetration through the cuticle. At 120 h incubation, the groups treated with oil conidial suspension, exposed or not to heat, and the group treated with aqueous conidial suspension not exposed to heat, had the cuticle penetrated by the fungus. However, at 120 h incubation, no fungal penetration was detected in engorged females treated with aqueous suspension previously exposed to heat. We concluded that mineral oil protected M. anisopliae conidia from the effects of heat exposure, and it is suggested as a promising candidate for conidial formulation.

**POSTER SESSION. Wednesday, 16:30 PF-27**

**Distribution pattern of Beauveria fungal entomopathogens in soil habitats: Diversity, natural occurrence and dynamics**

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The density of soil insect pests is regulated by the presence of soil-inhabiting entomopathogenic fungi. A large diversity of these entomopathogenic fungi are considered soil-borne such as *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp. The aim of the present research was to isolate and identify *Beauveria* species in natural soil habitats of Lebanon using different isolation methods and to define the lineage of *Beauveria* strains. Three natural sites from the north western slope of Mount Lebanon were selected based on soil structure and texture, host plants and area. Soils samples were collected and transferred to Laboratory for isolation of entomopathogenic *Beauveria*. Two methods of isolation were used; the first one is the insect bait method where two insect baits were compared the *Galleria mellonella* and *Cephalcia tannourinensis*. The second method of isolation was from the soil where three different media were compared: dodine-based medium, CTAB based medium and low sugar content medium Doc2. Morphological examination was carried on mycelium and conidia where molecular characterisation was based on two protein-coding genes RPB2 and TEF and the intergenic region *Bloc* for cryptic speciation review. A significant difference was recorded between different isolation methods and between the different sites. The highest occurrence of *Beauveria* was recorded with Dodine selective medium (45%, 43.3%, 35%, and 5%). The lowest occurrences were recorded with the insect bait method *Cephalcia tannourinensis* and *Galleria mellonella*, while 0 occurrence was demonstrated for the Doc2 medium. Two *Beauveria* species, *B. bassiana* and *B. pseudobassiana* and one *Isaria fumosorosea* entomopathogens were found.

**POSTER SESSION. Wednesday, 16:30 PF-29**

**Natural occurrence of entomopathogenic fungi in apple orchards in Germany**

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In the project “Biological control as ecosystem service in integrated and organic pome fruit cultivation” we investigated the occurrence of entomopathogenic fungi (EPF) and evaluated their controlling function as natural antagonists. Therefore, soil samples were collected three times a year in spring, summer and autumn from 2016-2018. The sampling took place in three of the main apple growing regions in Germany. These regions are located in the North (Altes Land), the Center (Kraichgau) and the South (Lake Constance) of Germany. The samples were collected in integrated as well as in organic managed orchards and additionally in orchards with very few or without pest management or plant protection. Using the *Galleria* bait method by Zimmermann (1986) and a modified version with *Tenebrio molitor*, the soil was examined for the occurrence of EPF of the genera *Beauveria*, *Isaria* and *Metarhizium*. Furthermore, different tests were conducted with three of the isolated EPF to describe their attributes. We examined the influence of fungicides and one herbicide, which are used in apple orchards on those fungi and tested their virulence against insects, that occur there.

The results show a considerable regional difference in the occurrence of EPF but no major seasonal effect. The influence of fungicides varies not only between those but also between the fungi and the impact on pest insects of apple orchards indicates that there is an ecosystem service of EPF in apple orchards.
Can nematotoxic proteins of *Coptotermes sp.* be used to protect plants against plant-parasitic nematodes? Moradi, A.1, Anagnostid, C.1, El-shetehy, M.1, Wiczorek, K.2, Mauch, F.1 and Künzler, M.2

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Plant-parasitic nematodes have great impact on agricultural production. The plant immune system can recognize the presence of pathogenic nematodes to trigger defensive responses such as the production of nematotoxic proteins. However, plant-parasitic nematodes are able to deliver effectors (virulence factors) into host plants to suppress individual plant immunity responses. To overcome this potential limitation, we used nematode inhibiting proteins (NIPs) from the basidiomycete *Coptotermes sp.* When exposed to fungivorous nematodes, *C. sp.* produces specific defensive proteins to inhibit the attackers. The NIPs are mostly lectins and lipases and functional analysis revealed toxicity towards the model nematode *Caenorhabditis elegans*. We have expressed the NIPs in *E. coli* and show that purified NIPs dramatically impair the development of *C. elegans* larvae by arresting the worms at the L1 larval stage. Overexpression of the NIPs in *Arabidopsis* enhanced the resistance against *Heterodera schachtii*, a plant-parasitic cyst nematode, measured as a lower number of cysts in the roots of the transgenic plants. In conclusion, the NIPs have potential protective activities against plant pathogenic nematodes. The mode of action of these NIPs remains to be demonstrated.

Using Entomopathogenic Fungi to Control the Greenhouse Whitefly (*Trialeurodes vaporariorum*): Developing a Standardised Bioassay

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A standardised bioassay method was created to assess the efficacy of different entomopathogenic fungal isolates against *Greenhouse whitefly* (*Trialeurodes vaporariorum*) (GHWF). A spray tower consisting of a gravity feed dual action airbrush attached to an acrylic cylinder and powered by a mini airbrush compressor was calibrated to apply a uniform coverage of solution to a target area. Egg laying by adult GHWF was restricted to a known area of the lateral side of aubergine leaves and migration during the first instar was observed. The spray tower was used to apply a single concentration of eighteen different entomopathogenic fungi (EPF) onto third instar GHWF. Isolates consisted of both commercially available mycoinsecticides and other isolates obtained from the USDA Agricultural Research Service collection and included *Beauveria*, *Isaria*, *Lecanicillium* and *Metarhizium* species. The sprayer delivered 252±25 fungal spores/mm² equivalent to 2.5x10⁶ spores per hectare. Whitefly mortality ranged from 8% to 89% with half of the isolates resulting in <40% GHWF population mortality. The methods employed in this study could be utilised in the selection of isolates for microbial control of whitefly. Combining the sprayer calibration and bioassay method ensures a reliable and applicable approach to test efficacy of whitefly pesticides.

Insecticidal activity of entomopathogenic fungi against *Gonipterus platensis* under laboratory conditions

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*Gonipterus platensis* (Marelli) (Coleoptera, Curculionidae), commonly known as the *Eucalyptus* snout-beetle, is an important pest of *Eucalyptus globulus* Labill. in Chile and many world regions. This study summarizes twelve months of laboratory strain assessment activities between November 2017 and October 2018 on the biodiversity of pathogenic fungi affecting important weevil and aphid forest pests in the southern Chile. We used different entomopathogenic fungi belonging to the genus *Beauveria*, *Hirsutella*, *Isaria*, *Lecanicillium* and *Metarhizium*, which were isolated from soil and insect samples. In total, nineteen isolates were tested against adults of *G. platensis* in laboratory experiments. Ten adults were submerged for 10 s in a suspension of 10⁴ conidia/ml, then were set in a Petri dish with a disinfected leaf of *E. globulus* and incubated at 25°C in darkness for 10 days. Mortality of adults, and the presence of mycelium or conidia of the tested fungi, and of any saprobie fungi or bacteria on the adults were recorded daily. All tested fungi except *Hirsutella* developed mycelium and conidia on a considerable portion of dead adult within 10 days. Adults treated with the isolates *F. falkense*, 300 or 305 (*Metarhizium* species) at 10⁴ conidia/ml died most rapidly (LT₅₀: 4.3−4.8 days and LT₉₀: 4.9−5.5 days). We found highly virulent *Metarhizium* isolates with potential for the control of *G. platensis* adults. This study was funded by FONDECYT (Fondo Nacional de Desarrollo Científico y Tecnológico, Chile) project 11160555 and 1190390.
Insecticidal effect of entomopathogenic fungus, Beauveria bassiana ANU1, with hydramethylnon to a red imported fire ant, Solenopsis invicta, worker

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Entomopathogenic fungus, Beauveria bassiana ANU1, showed an insecticidal effect in red imported fire ant (RIFA), Solenopsis invicta, and LC₅₀ values were calculated as 1x10⁻⁷ conidia/ml for major worker and 2x10⁻⁷ conidia/ml for minor worker after 120 h application by spraying. Hydramethylnon is generally used as poisoning bait for RIFA control in field and showed 95% of toxicity on major and minor worker at 10 ppm for 96 h. However, dual application of B. bassiana ANU1 (1x10⁻⁷ conidia/ml) by spraying with 1 ppm of hydramethylnon in 10% sugar solution showed 96.7% and 100% of mortality to S. invicta major and minor worker, respectively. These results suggest that B. bassiana ANU1 showed synergism with hydramethylnon. Solenopsis invicta, as an invasive alien pest, could be controlled by combined treatment of entomopathogenic fungus, as biological control agent, and hydramethylnon.

POSTER SESSION. Wednesday, 16:30 PF-35

Influence of the insecticide acetamiprid on the secondary metabolism of Metarhizium sp.

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Entomopathogenic fungi are widespread in nature where they have frequent contact with various anthropogenic pollutants. Although, the mechanisms regulating the pathogenesis of insects are relatively well known, there are only few reports regarding the interaction of toxic contaminants with these microorganisms. In our previous studies we demonstrated that Metarhizium sp. possess the ability to degrade toxic contaminants such as nonylphenol, dibutyltin or ametryn and all of those compounds influence fungal metabolism. The aim of this study was to determine whether the insecticide acetamiprid has deleterious effect on entomopathogenic fungi. Herein, we determined ability of Metarhizium sp. to produce secondary metabolites, the destruxins, which have great importance in the infection process. In culture filtrates we determined by HPLC-MS/MS the presence of 19 destruxins (Dtx) in six Metarhizium strains. Our results showed that acetamiprid in concentrations 5 to 50 mg/L does not have any effect on biomass formation by Metarhizium species but has deleterious effect on destruxin production. This toxic compound decreased significantly Dtx production in all tested strains, even at a concentration of 5 mg/L. The most sensitive strain was M. brunneum which decreased DtxA and DtxB production by 56.4% and 41.9% in comparison to control. These results point to deleterious effect of acetamiprid on the secondary metabolism in Metarhizium sp.

This research was financed by a grant from the National Science Center in Krakow (Poland), contract number UMO-2016/23/B/NZ9/00840.
Virulence of entomopathogenic fungi Beauveria bassiana isolates on the Asia citrus psyllid, Diaphorina citri
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Asian citrus psyllid (ACP), Diaphorina citri, is the most severe threat to the global citrus industry. Its management has mainly depended on the application of chemical insecticides. However, the rapid development of the resistance leads to the difficulty in the control of this insect pest. Entomopathogenic fungi based microbial insecticides are considered as safe alternatives to chemical pesticides. In order to find alternatives for biological control of this vector, the pathogenicity of 16 different isolates of Beauveria bassiana was assessed on adults of D. citri. The psyllids were reared on Myrraya excita in an artificial growth chamber (90%RH, D : L = 12h : 12h). At the spore concentration of 1x10⁶ conidia/ml, the CHL and BbBJ isolates among the 16 isolates gave the highest mortality of 60.20% and 55.70%, respectively. Subsequently, we tested the dose effects of these 2 isolates on D. citri. As the results indicated, the mortality increased with the spore concentration of Beauveria bassiana. Among the serial dilution (1x10⁴, 1x10⁵, 1x10⁶, 1x10⁷ and 1x10⁸ con/ml) of the spores concentrations, the psyllids showed 100% mortality on the 8th days after the infection at the concentration of 1x10⁶ and 1x10⁷ con/ml, respectively. Our data suggested that adult exposure to Beauveria bassiana may be a promising alternative for use in sustainable management programs aimed at microbial control in citrus orchards in China.

POSTER SESSION. Wednesday, 16:30 PMC-3

Multitrophic interactions regulated by an endophytic strain of the entomopathogenic fungus Metarhizium brunneum simultaneously applied with the parasitoid Hyposoter didymator to control Spodoptera littoralis in melon
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Foliar applications of Metarhizium brunneum Petch. EAMa 01/58-Su strain (Ascomycota: Hypocreales) to control Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) in melon plants may have both direct effect via contact with fungal spores and indirect one due to the transient endophytic behaviour of the fungal strain. Meanwhile, the indigenous parasitoid Hyposoter didymator (Thun.) (Hymenoptera: Ichneumonidae) is also considered a good biological control (BC) agent for S. littoralis. Our previous studies showed high compatibility between these two BC agents when the insect larvae were inoculated with the fungus via contact. In the present study, the interaction between M. brunneum and H. didymator to control S. littoralis in endophytically colonized melon plants has been evaluated. The endophytic behaviour of fungal strain was ascertained, with more than 90% of colonization in treated leaves. Interestingly, around 20% of S. littoralis larvae fed with fungal discs of 48h-colonized melon plants were died, whereas there were no signs of fungal outgrowth in the cadavers. The reproductive potential of H. didymator was significantly reduced in S. littoralis larvae fed with colonized plants if compared with larvae fed with non-inoculated plants, with parasitisation percentages of 32.6 and 67.7%, respectively. Nonetheless, total mortality of S. littoralis larvae (86.2%) and mortality with fungal outgrowth (10%) were higher in combined treatment (fungus-parasitoid) than in individual ones.

POSTER SESSION. Wednesday, 16:30 PMC-4

First report of Conidiobolus coronatus in Paraguay as biological control of leaf cutting ants
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Acromyrmex lundorti Fericomis Forel, 1885 (Formicidae: Myrmicinae) is an invasive species with negative impact on forage grasses from Paraguay. Insecticides have proven to have limited effectiveness; thus, the use of biological control agents is an appropriate alternative to improve proper management. The aim of this study is to identify an entomopathogenic fungus, which applied to the soil, can cause epizootics to leaf-cutting ants. In the summer of 2018, Entomophthorales fungal specimens were collected from mummified ant bodies (A. lundorti fericomis) residing in nests, located in Gatton panic paddocks (Megathyrsus maximus, Poaceae), in the Paraguayan Chaco (latitude: 23°17′14.2″S, longitude: 60°45′35.0″O). The collected samples were immediately photographed and brought back to the Plant Protection Laboratory of the National University of Asunción in Paraguay, for spore and mycelium isolation in accordance to standard decontamination procedures. Based on morphological characteristics and molecular phylogenetic analyses, the Entomophthorales fungus was identified as Conidiobolus coronatus. The massive epizootics caused by this fungus in the leaf-cutting ant, was observed to have a 100% rate of mortality, where the entire population (gardiners, workers and soldiers) were expelled from the nest. Through the identification of the entomopathogenic Entomophthorales species, it may be considered a key factor for proper control of the leaf-cutting ant, due to its biological nature and thus reduces the unnecessary use of chemical treatments during growth season. However, the fungus Conidiobolus coronatus has also presented itself to be harmful for human health, due to the peculiar characteristics it has on both humans and animals.

POSTER SESSION. Wednesday, 16:30 PMC-5

Development of a SNP based tool for the identification and discrimination of Melolontha melolontha and M. hippocastani Pedrazzini, C.; Strasser, H.; Holderaggen, R.; Widmer, F.; Enkerli, J.
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The European (Melolontha melolontha L.) and forest (M. hippocastani F.) cockchafer are important pests in grasslands, orchards and forests throughout central Europe. Both species exhibit a three or four year life cycle and occur in temporally shifted populations, which have been monitored and documented for more than 100 years. Nevertheless, the genetic population structure of both species and how they are affected by spatial separation and temporal isolation due to distinct swarming flights have not yet been investigated. Visual identification of these morphologically similar species is challenging and time-consuming when working with large numbers of individuals. As a first step to a detailed population structure analysis, our goal was to develop an efficient molecular genetic tool for the identification and discrimination of M. melolontha and M. hippocastani. We established a collection of both species by sampling 25, 5, and 9 sites in Switzerland, Austria and North Italy, respectively, in 2016, 2017 and 2018. After DNA extraction from legs of individuals, we amplified and sequenced an approximately 1500bp-long fragment of the cytochrome c
oxidase subunit 1 (CO1) mitochondrial gene from 12 M. melolontha and 5 M. hippocastanum beetles. Alignment of the 17 sequences and 40 reference sequences obtained from the NCBI GenBank database and subsequent phylogenetic analysis revealed consistent clustering of the two species. Based on species-specific single nucleotide polymorphisms (SNPs) identified in CO1 alignment we will develop a SNP assay and apply it to the insect collections established in 2016, 2017 and 2018 to verify species identity of collected beetles.

POSTER SESSION. Wednesday, 16:30 PMC-7

Behavioural abilities of EPNs to search the insect host inside sugarcane culm
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The infection process of an entomopathogenic nematode begins with the invasion of infective juveniles (IJs) in the insect. These IJs usually seek subterranean insects but sometimes also insects that attack internally the root of the plant such as the larvae and pupae of Sphenophorus levis (Coleoptera: Curculionidae) that attack the interior of the rhizome of the sugarcane. The objective of this study was to evaluate three species of EPNs (Steinernema sp. IL1, S. rurum PAM25 and Heterorhabditis bacteriophora HB EN01) in respect to their abilities to search the insect host within the sugarcane culms. Galleria mellonella larvae of last instar were held individually in cages and placed individually inside canes culm (7cm long) through a hole of 5 cm diameter accomplished in the base of the culm where it was sectioned. The holes were filled with sugar cane bagasse and the culm were buried vertically in plastic pots fermentation using rice and macerated insects. The biological activity was evaluated in a bioassay under laboratory conditions to determine the lethal concentration (LC) and mean time to death (MTD). For these bioassay, adults of the pest were immersed in aqueous suspensions of M. anisopliae adjusted to five different concentrations (1E+04 to 1E+08 conidia/mL). The mortality of the insects inoculated with P1 ranged between 2 to 10%, and it was not different from the mortality obtained in the control, even for the maximum concentration evaluated. For P2 and P3 the biological activity increased consecutively showing LC50 of 1.2E+08 and 9.4E+06 conidia/mL, respectively. The MTD for P2 and P3 were 10.6 and 6.1 days, respectively. The results help to better understand the impact of in vivo successive passages on the fungal activity which can be used to design a reactivation strategy.

POSTER SESSION. Wednesday, 16:30 PMC-9

Beauveria bassiana against leaf cutter ants: are all strains equally effective to different ant pest species?
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Beauveria bassiana is a well know entomopathogen. Part of its success is due to its great effectiveness against insects from several families. However, recently it has been shown that strains obtained from the pests to control are better in comparison to collection strains. We tested the effect of three different strains of B. bassiana (Bb5, Bb6 and Bb7) against five leaf-cutter pest species (LCP) from Argentina. Although all the strains were pathogenic to the 5 species, there were important differences related to their relative virulence. On one hand, we found a host effect with the species A. ambiguus being more resistant to the three strains than the other LCP. On the other hand, we found a strain effect with strain Bb6 being best for three species and Bb5 for the other two. Finally, we found an important effect of the strain inoculated towards the natural pathogenic load that the ants have in nature. Regarding the latter, we found that Bb6 was better against fungal pathogens whereas Bb5 was more effective towards bacteria pathogens. Therefore, our work shows, again, that not all strains are equally effective against pests even within the same genus. Although all strains will eventually kill the pest is important to use those that will do it fast and with confidence of being the cause of death of the pests.

POSTER SESSION. Wednesday, 16:30 PMC-8

Effect of the successive passes of Metarhizium anisopliae on insecticidal activity to Demotispa neivai
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The entomopathogenic fungi Metarhizium anisopliae (Ascomycetes: Hypocreales) CPMa1502 was isolated by Cienpalma in Barranqueraimeja (Colombia) from a fruit scarper of oil palm Demotispa neivai (Bondar) (Coleoptera: Chrysomelidae). This isolate showed a variability in its biological activity when it is grown consecutively in culture media. The aim of the present study was to investigate the effect of in vivo successive passes on insecticidal activity of M. anisopliae on D. neivai. The initial inoculum of the isolate was reactivated from growing in culture media YM+potato extract, and the successive passes (P1 to P3) were obtained from sporulated insects from the previous pass and were produced in solid fermentation using rice and macerated insects. The biological activity was evaluated in a bioassay under laboratory conditions to determine the lethal concentration (LC) and mean time to death (MTD). For these bioassay, adults of the pest were immersed in aqueous suspensions of M. anisopliae adjusted to five different concentrations (1E+04 to 1E+08 conidia/mL). The mortality of the insects inoculated with P1 ranged between 2 to 10%, and it was not different from the mortality obtained in the control, even for the maximum concentration evaluated. For P2 and P3 the biological activity increased consecutively showing LC50 of 1.2E+08 and 9.4E+06 conidia/mL, respectively. The MTD for P2 and P3 were 10.6 and 6.1 days, respectively. The results help to better understand the impact of in vivo successive passages on the fungal activity which can be used to design a reactivation strategy.

POSTER SESSION. Wednesday, 16:30 PMC-10

Antifungal activity of entomopathogenic Bacillus thuringiensis.
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Bacillus thuringiensis (BT) is known as an insect pathogenic bacteria and is currently used as the most widely sold microbial insecticide in the world. In recent years, it has been found that BT has not only insecticidal activity but also antimicrobial activity against fungi. Many plant diseases are caused by fungi and BT preparations have potential as insects and dual control materials against disease. However, research on insecticidal protein Cry toxin has been increasingly frequent, but research on antimicrobial activity is not so. In this research we will proceed with experiments to search for antimicrobial substances. Those currently sold as BT formulations are BT endosporoses and vegetative spore as the components. However, in this research, it was found that the antimicrobial activity against plant pathogenic fungi (Botrytis cinerea, Fusarium oxysporum and Ver-
ticillum dahliae) was different between endospores and vegetative cell metabolites. Antifungal activity was evaluated on Potato Dextrose Plates using Fusarium oxysporum, the endospores exhibited a high antifungal effect on the mycelium growth but did not in vegetative cell. These results suggest that different mechanism (or metabolism) might occur in endospores and vegetative cells, and antimicrobial active substances of the endospores could be produced at higher expression levels of related genes than vegetative cells. Therefore, by comparing the gene expression levels of endospores and vegetative cells by transcriptome analysis, antimicrobial active substances are identified.

POSTER SESSION. Wednesday, 16:30 PMC-11

Development of Attract-Kill granule for white grubs control

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White grubs are very important underground pest and they always damage plants underground part. Due to the concealment of damage, conventional bio insecticide’s formulations cannot control them effectively; therefore, it is necessary to develop new formulation. “Attract-Kill (AK)” is a good strategy, with the advantages of low dosage and high efficiency, and suitable for underground pest control. To development AK formulation for underground pest’s control, we design to attract the underground pest by the carbon dioxide which released during Bacillus thuringiensis (Bs) growth and metabolism, and kill the underground pests by the multiplicated Bt toxin. Firstly, we screened out the materials that can growth Bt efficiently. The data showed that puffed corn and peanut meal mixture was efficient for Bt growth. Then, we established a process for preparing puffed corn and peanut meal mixture granule (PCPMG). The granule was uniform and the diameter was about 3 mm. The further study shown that the PCPMG not only can growth Bt, but also can growth other white grubs effective fungi, Beauveria bassiana and Metarhizium.

POSTER SESSION. Wednesday, 16:30 PMC-12

Entomopathogenic fungi for the control of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Africa: A first approach towards the development of an alternative small-scale production method

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Spodoptera frugiperda (S. frugiperda) is a destructive pest in maize crops and has currently received tremendous attention due to its introduction to the African continent in 2016. Threatening the livelihoods of millions of small-scale and subsistence farmers, the development of a biological control strategy against this pest is vital. Entomopathogenic fungi possess several advantages in terms of human and environmental safety. A first approach towards the development of an alternative biocontrol strategy using entomopathogenic fungi and aiming at their production on small-scale farms for self-sufficiency was surveyed in laboratory conditions. The M. anisopliae strain JKI-BI-1347 induced 60% mortality on average in a 24 hours post-infection. Gene expression levels in reference and treated insects, whereas the 14B-1 strain of C. sphagni exhibits toxicity against a broad range of important insect pests (including Hemipteran, Coleopteran and Lepidopteran insects), whereas the 14B-1 strain of C. sphagni exhibits toxicity against a more circumscribed subset of taxa. On a pathogen-specific basis, the molecular mode of activity remains poorly understood, and whether and how Lepidopteran host transcriptional profiles might differ when challenged with these two closely related microbial pathogens also remains unknown.

Larvae were orally inoculated, and RNA was extracted and sequenced at 24 hours post-infection. Gene expression levels in reference and treated insects were independently compared at the whole-insect and mid-gut tissue levels to characterize host-specific transcriptional responses to infection. Higher-order comparisons of these expression change ratios were made to identify genes whose differential expression per se varied across midgut and whole-insect tissue types for a particular pathogen, and across pathogenic species in the context of identical tissue types. A subset of genes whose differential expression was suggested by RNA-Seq data was validated using quantitative PCR. These results provide important insights into invertebrate mechanisms for responding to bacterial infections imposed by these two entomopathogenic microbes.

POSTER SESSION. Wednesday, 16:30 PMC-14 STU

Biological Control: Fighting below ground insect pests with Pseudomonas bacteria

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Below ground insect pests are a yet unsolved problem not only in organic, but also in conventional crop production because they are difficult to target and the few effective chemical pesticides are already or will be banned in near future due to raising concerns for environmental and consumer safety. So far, mostly Bacillus thuringiensis was used in biological control of insect pests but resistance against major Bt toxins has been reported. This project aims at developing a new approach for the biological control of soil-dwelling pest insects compatible with organic production. We evaluate the potential of a specific group of plant-beneficial fluorescent Pseudomonas bacteria with entomopathogenic activity (EPP) for insect control. In a first screening, EPP strains were successfully tested against the cabbage root fly Delia radicum, a pest causing increasing losses in the production of brassicaeans crops and for which no satisfactory control measures exist. The most effective strains belonging to the Pseudomonas chlororaphis species are currently further evaluated and combined with entomopathogenic fungi and entomopathogenic nematodes, which are already well-established biocontrol agents used in organic production. Combinations will be investigated for synergistic and additive effects.
This project will give exciting new insights into complex interactions between agriculturally important members of the soil and rhizosphere ecosystem. We hope to provide new methods based on the combined application of beneficial soil organisms for the control of an important insect pest in organic and conventional vegetable production, which may be adapted to other problematic soil pests.

Comparative Gene Expression of Peritrophic Matrix Provides an Insight into its Role in Cry1A.105+Cry2Ab2 Resistance by the Spodoptera frugiperda pest Castellane, T.C.L.1,2; Fernandes, C.C.1; Fatoreto, J.C.1; Pinheiro, D.G.1; Desidério, J. A.2; Lemos, M.V. F.2

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Genetically engineered crops represent one of the most rapidly adopted technologies in the history of agriculture. Some of the environmental and economic benefits of Bacillus thuringiensis (Bt) transgenic crops have been lost because of the rapid evolution of pest resistance. In this study, comparative transcriptomic sequencing and RNA-seq analysis were explored to investigate the role in Cry1A.105+Cry2Ab2 resistance by the fall armyworm (FAW) (Spodoptera frugiperda). The cDNA libraries from Cry1A.105+Cry2Ab2-resistant (RR) and Cry1A.105+Cry2Ab2-susceptible (SS) genotypes of FAW population were successfully synthesized from mRNA enriched fractions and, them were sequenced using the Illumina MiSeq and assembled into a reference transcriptome. The obtained transcriptome provided a list of genes that have potential roles in interactions among insecticidal proteins and FAW. In total, 376 genes and other transcripts (i.e. aminopeptidase, cadherin protein and amino acid ABC transporters) were shown to be differentially expressed between the two comparisons performed. The levels of gene encoding aminopeptidase, cadherin and amino acids-ABC transporters, substrate-binding protein, were highest for RR. In contrast, G-protein coupled receptor mth2-like isoform expression levels remained was down regulated for RR. This receptor when combined with an extracellular signal transmited it across the membrane by activating an associated G-protein. There are evidences of ABC transporter genes associated with resistance because insect ABC transporters often provide protection against xenobiotics. Data generated from this study will be useful in understanding how the insects responds to technologies in the proteomic level, which will provide tools for a better management of insect pest in the field.

Cellulase improves the endophytism of encapsulated Metarhizium brunneum on potato plants

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Biocntrol of insect pests with entomopathogenic fungi is challenging because of the lower efficacy, difficult handling and limited shelf life of these microorganisms compared to synthetic pesticides. However, recent studies have provided evidence that some of these fungi can grow endophytically in plant tissues, paving the way for novel plant protection measures. Furthermore, some of these isolates can also stimulate plant growth. Therefore, the overall aim of our investigations is to combine fine-tuned cultivation with customized formulations that support the delivery of M. brunneum into potato plants for a systemic protection from herbivorous insects and to increase plant growth. Inspired by penetration mechanisms of phytopathicogenic fungi, we hypothesized that an increased expression of plant cell wall-degrading enzymes, such as cellulase will improve plant penetration and colonization. For this purpose, M. brunneum CB 15 was immobilized in a novel bead system with cellulase, cellulolose and inactivated baker’s yeast. We were able to demonstrate that in beads containing only cellulose or in combination with inactivated baker’s yeast there was a significant increase in cellulase activity and improved mycelium growth with 12.6 % and 13.6 % respectively. In addition, a higher enzyme activity, e.g. by co-formulation with cellulase, led to a shift from mycelium to spore formation to a maximum of 2.5 x 10^5 ± 6.1 x 10^4 spores per bead. This correlates with an improved endophytism of 61.2 % compared to the control. Our study thus provided first insights that enzymes as penetration aids can cause an improvement in plant colonization.

Biological control of the Japanese beetle (Popillia japonica) with entomopathogenic fungi

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Agroscope, ecological plant protection in arable crops

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The Japanese beetle (Popillia japonica) is a scarab (Scarabaeidae) that originates from Japan. It was accidentally introduced into the USA, the Azores and recently to northern Italy. Whereas the Japanese beetle is not a serious pest in its natural range, it causes severe damage in the USA. Adults feed on leaves, flowers and fruits of diverse forest trees and many cultivated plants. Larvae mainly subsist on grassroots and damage turf and pastures. The Japanese beetle resembles the garden chafer (Phyllopertha horticola) but has characteristic five white tufts of hair on both sides of the abdomen and two on the pygidium. The main flight occurs between June and July. Larvae are typical grubs and third instars overwinter in deeper soil strata. In the USA, mainly insecticides are used to control the Japanese beetle. However, a range of biological alternatives is currently being investigated, e.g. the use of bacteria, nematodes or entomopathogenic fungi. In 2017 and 2018, Agroscope tested the virulence of different Beauveria and Metarhizium strains against adults and larvae of P. japonica in a quarantine lab. The tested strains are native to Switzerland and were isolated from related scarab beetles. Mortality rates of up to 100% were achieved for adult beetles within a period of 7 dpi only, whereas mortality rates of larvae were between 40 and 100% after 42dpi. While mortality correlated with dosage, sporulation on infected cadavers did not. First field experiments were carried out in summer and autumn 2018 in the infested zone in Northern Italy.

Influence of inundative mass application of Metarhizium brunneum BIPESCO 5 on indigenous Metarhizium strains in maize fields

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The entomopathogenic fungus *Metarhizium brunneum* (BIPESCO 5, production strain of granMet™, Agrifurtur s.r.l.) is actively used or tested against different insect pests in Europe (i.e. *Phylophaera horticola*, *Amphimallon solstitialis*, *Bothynoderes punctiventris*, *Popillia japonica*, *Othorhynchus sulcatus*, Agrotes spp., *Diabrotica v. virens*, *Daktulosphaira vitifoliae*). According to EU Regulation No. 283/2013 (Article 8.6.) the effect of mass application of the biological active agents on non-target organisms, including micro-organisms, has to be investigated. Therefore, the influence of inundative mass application on the indigenous *Metarhizium* population was subject of our investigation. During a three year field study (2016-2018) in Austrian maize fields soil samples were taken 2 (2018) to 3 (2016, 2017) times during planting seasons. From this soil number *Metarhizium* colony forming units were evaluated and 24 randomly selected colonies per site and sampling date were chosen for molecular analysis (2018 only 8 colonies were assessed). After DNA extraction from single cultures genotyping was performed using simple sequence repeat (SSR) marker analysis. Based on these data, the genotype diversity and the influence on the abundance of identified *Metarhizium* genotypes were evaluated. Although the recovery of BIPESCO 5 increased over three years according to the frequency of granMet™ application, the total number of genotypes remained unchanged in all sites. The indigenous *Metarhizium* genotypes were not negatively affected by the application of the production strain. The distribution of genotype recovery was only dependent on the number of samples of each individual sampling.

**POSTER SESSION.** Wednesday, 16:30 PMC-20

It’s necessary new candidate gene for Bt genetically modified crops

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Fall armyworm (FAW) is widely distributed in all American, causing significant damage to maize remain a major crops challenge and involve the interaction of multiple genes and environmental factors. Discovering the relevant genes is difficult although it is known that resistente can originate from the variation of an individual’s genome in contact with insecticide protein. Application of in silico tools can significantly improve the detection of new and differs candidates genes and variation. Data mining tracking of new knowledge facilitate these mapping. Only the vip gene sequences, duly annotated previously, reviewed and stored in the international database UniProt (UNIPROT CONSORTIUM, 2012) were selected for this purpose. Prospecting was carried out exhaustively via BLAST (Basic Local Alignment Search Tool) program to obtain the alignment of the sequences for identification of conserved regions between database ORFs and protein sequences already incorporated in Bt technology. The selected vip3A genes with the following accession numbers: D9IAX4-1, Q7S5G2, Q938Z1, Q938Z1 and I6ROW9, as expected according to the alignment performed by the ClustalW program with Vip3 characterized by MR162 Vip3Aa (ABG204293.1) have similarity in several regions which are conserved between the sequences. There are a great number of Cry and Vip proteins already described; however, the presented results also incite the search for new toxins through Metagenomics allied to Bioinformatics. In conclusion, in silico analysis can differentiate subtle consequences of coding DNA variants and remains the major tool to predict potential new candidate gene transcription.

**POSTER SESSION.** Wednesday, 16:30 PMC-21

Defense mechanism of ixodid ticks to fungal infection: cuticle, the first barrier

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The structure and the chemical composition of tick cuticle act as a barrier to pathogens, such as entomopathogenic fungi. In this study, we investigated the fungal response to the neutral lipids and cuticular hydrocarbons (HC) of four Brazilian ixodid tick species, with significant veterinary and medical importance. Electron micrographs documented a delayed germination of conidia of *B. bassiana* IP 361 and *M. robertsi* IP 146 on cuticle of *Dermacentor nitens* and *Amblyomma scapularis* engorged females 48h and 72h post infection, respectively. On the other hand, fungal penetration through the cuticle of *Rhipicephalus sanguineus* and *Rhipicephalus microplus* was initially evidenced at 24h incubation. A *sculptum* neutral lipid profile was not significantly changed when females were treated with IP361 or IP146, whereas triacyl glycerol profiles were not detected on the cuticle of treated *R. sanguineus*, *R. microplus* or *D. nitens*, and fatty acids profiles were not detected on *R. sanguineus*. Six HCs were exclusively found on the cuticle of *A. sculpmum*. The total lipid extracts from cuticle of *A. sculpmum* or *D. nitens* inhibited the growth of *M. robertsi* in disk diffusion assays; a similar response, however, was not observed with lipids from *R. sanguineus* and *R. microplus*. The growth of *B. bassiana* was not inhibited when exposed to HCs of any tick species investigated, although the extracts from *A. sculpmum* or *D. nitens* caused reduction in the number of germinated conidia to 99.5%. In conclusion, the cuticle of *A. sculpmum* and *D. nitens* has lipid components with anti-fungal activity.

**POSTER SESSION.** Wednesday, 16:30 PMC-22

Blastospores and conidia supplemented or not with secondary metabolite of plants to control flies

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In this study, we evaluated the virulence of five *Metarhizium* spp. and four *Beauveria bassiana* isolates against *Musca domestica* larva. The efficacy of conidia and blastospores of these fungi against fly larvae was tested; one isolate was then selected for efficacy tests of association with the alkaloid matrine. Conidia of *Metarhizium* spp. showed high virulence to *M. domestica* larva compared to *Beauveria* isolates. When comparing both propagules of these fungi, blastospores (IP 146: *Metarhizium robertsi*) had the highest percentages of reduction of adults emergence. The results showed high larval treatment efficacy (LTE) for all matrine concentrations tested when associated to *Metarhizium anisopliae* s.str. conidia (IP 119). When the alkaloid was associated to IP 119 conidia, high virulence was also reported; however, it was not significant different than their individual treatments. Low virulence was obtained for *Beauveria bassiana* conidia, but when associated with matrine in higher concentrations, high and significant (p<0.01) virulence was reported. *Metarhizium* spp. propagules are promising agent to control flies; also, alkaloid matrine

**POSTER SESSION.** Wednesday, 16:30 PMC-23

In the presence of this content in silico analysis can differentiate subtle consequences of coding DNA variants and remains the major tool to predict potential new candidate gene transcription.
showed great toxicity, but it alone can cause high mortality, even in low concentrations, dispensing association with the fungus.

POSTER SESSION. Wednesday, 16:30 PMC-23

Preliminary studies on presence of entomopathogens in outbreak population of great web-spinning pine-sawfly Acantholyda posticalis in urban forest stands

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Sustainable management in urban forests includes effective protection against pests. Research on biological control of forest pests are carried out in the Institute of Biology in cooperation with Latvian State Forest Research Institute “Silava” for many years. In the summer of 2013 the outbreak of great web-spinning pine-sawfly (A. posticalis (Matsunura, 1912)) was observed in forest stands (approximately 100 ha) of the Daugavpils municipality (Latvia). In 2017 mass outbreak with larvae density surpassing 100 larvae per m² covers 336 ha. The aim of the study is to assess affected stands by web spinning sawfly, to acquire preliminary data on mortality factors and identify present entomopathogens. A. posticalis overwintering larvae and nymphs were sampled in 81 sample plots with three samples per plot. Dead, diseased and living specimens were collected from sample plots and checked for the presence of pathogens by light microscopy. Living A. posticalis were reared in the laboratory under optimal conditions (air temperature +20±2°C, RH 75-85 %, photoperiod 16 h), provided with fresh natural food. In 2017 level of A. posticalis mortality caused by parasitoids and pathogens are 20.1%. We observed low level of bacterial and fungal infections. Viral infection was activated by subjecting asymptomatic larvae to physical and chemical stress-factors. Viral infections caused by nucleopolyhedroviruses are observed. In next step – the molecular methods will be used for pathogen identification.

POSTER SESSION. Wednesday, 16:30 PMC-24 STU

Effect of arbuscular mycorrhizal fungi on the susceptibility of Spodoptera exigua to viral and bacterial entomopathogens

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Arbuscular mycorrhizal fungi are known to increase resistance of the host plant to abiotic stressors such drought, salinity or temperature changes. However, their role in improving plant’s ability to cope with biotic stress needs to be studied further. The aim of the present research was to study the influence of the arbuscular mycorrhizal fungus Funneliformis mosseae on the susceptibility of Spodoptera exigua (Hübner) to its natural entomopathogens Bacillus thuringiensis (Bt) and Baculovirus (BV). S. exigua larvae were reared for 48 hours on plant-based artificial diet (Solanum lycopersicum cv. MoneyMaker) prepared with plants derived from different treatments (mycorrhization and/or exposure to herbivory) and infected with sublethal concentrations of Bt and BV. Effects of mycorrhization and herbivory on growth rate and the expression of immune-related genes were also analyzed. When infected with Bt, larvae fed on plants exposed to one or both factors suffered a significant increase in mortality compared to those reared on control plants. Regarding Baculovirus, mycorrhization was the only factor responsible for a significant higher mortality. These results suggest that mycorrhization, and also herbivory, trigger changes in the host plant which affect the insect pest S. exigua and alter their susceptibility to entomopathogens.

POSTER SESSION. Wednesday, 16:30 PMC-25 STU

Management of Colorado potato beetle overwintering adults with entomopathogenic nematodes

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The Colorado potato beetle (CPB) developed resistance to all major classes of chemical insecticides, so other solutions are needed for its control. One possible solution is to reduce adult population during the overwintering. Entomopathogenic nematodes (EPN) seem to be effective, but their efficacy on CPB has not been sufficiently explored in Croatia. This two-year (2018-2019) field study investigated the efficacy of Steinernema feltiae and Steinernema carpocapsae applied in three different doses on overwintering CPB adults. Entomological cages were set up in the treated field from mid April till mid May 2018 to monitor CPB emergence. In 2019, the EPNs were applied in the same doses but on a different field and will be monitored in cages as well. Results from 2018 have shown that in the plots treated with S. feltiae, the emergence of adults occurred from 19 April until 10 May. In the plots treated with S. carpocapsae, the emergence was somewhat earlier (16 April) and lasted ten days shorter (until 30 April) than in plots treated with S. feltiae. On untreated plot, the emergence was recorded from 13 April till 7 May. The results of efficacy were satisfactory for both species: S. feltiae had an efficacy from 79.03% to 100.00%, while S. carpocapsae efficacy was between 77.32% and 96.22%. Both nematodes were most effective at the highest doses. The efficacy at recommended doses was also satisfactory (96.22% for S. feltiae; 77.32% for S. carpocapsae). First results indicate that EPNs are effective tool for overwintering adult control.

POSTER SESSION. Wednesday, 16:30 PMC-26

First report of entomopathogenic nematodes Heterorhabditis bacteriophora from Croatia and its virulence against Lasioperta rubi

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Heterorhabditid nematodes were recovered from soil samples during spring months in 2016, in Croatia, with the overall positive sample rate of 3%. The isolates of entomopathogenic nematodes were identified as three different strains conspecific with Heterorhabditis bacteriophora (Heterorhabditidae). The strains were found from natural sites and vineyard, while no recovery occurred from intensively cultivated agricultural fields. The morphometrical characteristics of infective juveniles and males showed variance between strains and from the original description. Heterorhabditis bacteriophora ISO9 was abundant, and insect cadavers.Showed great toxicity, but it alone can cause high mortality, even in low concentrations, dispensing association with the fungus.
Impact of Moisture on the Viability of Metarhizium Microsclerotia
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Entomopathogenic fungi are a valuable tool used for biological control of a wide range of arthropod pests. Members in the fungal genus Metarhizium have been developed into commercial products. Liquid culture techniques can create an environment for Metarhizium to produce microsclerotia, a natural structure comprised of compact melanized hyphae known to overwinter in the soil, and capable of subsequently producing infective conidia. Microsclerotia have demonstrated potential as an economical, non-traditional fungal structure uniquely suitable for control of soil-inhabiting arthropods and for specialized applications such as to trees trunks when targeting wood borers. It has become apparent that levels of available moisture are paramount for the fungus to maintain viability (measured as conidia production) during storage and after application. Using a prototype clay granule formulation containing M. brunneum strain F52, microsclerotia survived for up to two years when stored at a water activity (aw) < 0.1 and 25 °C. Viability of dried samples decreased when stored under increasing moisture levels, surviving only a few days at 0.5 aw. When considering application environments, microsclerotia require high moisture availability to support conidia production, which is reduced by 99% as water activity falls to 0.94 aw. By contrast, fungal culture (not dried) applied to solid substrate then dried to and stored at 0.5 aw retained viability longer than the same culture dried to <0.2 aw then stored at 0.5 aw. These results highlight the need to fully understand the impact of moisture on the fungus as it relates to varied pest control systems.

Solid State Fermentation at Bayer CropScience Biologics – Ready for the next step
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The solid state fermentation technology developed and applied at Bayer CropScience Biologics GmbH in Wismar, Germany, is among the world’s leading. More than 20 years of experience in this field, a specialized R&D facility, various fermentation platforms and the main production plant at the site enable us not only to produce large quantities of high quality biocontrol products, but also to continuously improve our technology. Still there are many challenges waiting to be tackled: further optimization of substrates and related processes, monitoring of the fermentation process parameters in real-time, fine-tuning of the down-stream procedures - to name a few. We want to present our challenges, discuss potential approaches to their solution and introduce the infrastructure and support we can offer for future collaborations. We want to attract and encourage external collaborators and promote our idea of a solid state fermentation network where Wismar would function as a hub.

Evaluation of the insecticidal activity of Bacillus thuringiensis strains isolated from Algeria: Toxicity of the supernatant and spore-crystal mixtures.
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Bacillus thuringiensis is the most used technology for biological control of insect pathogens worldwide. In order to select new Bt candidates challenging the emergence of insect’s resistance, a mass bioassay and molecular screening was performed on an autochthonous collection isolated from Algeria. Toxicity assays against neonate larvae of three lepidopteran species (Mamestra brassicae, Grapholitha molesta and Spodoptera exigua) were conducted using spore-crystal mixtures and supernatant cultures of 49 Bt isolates harboring at least one lepidopteran specific gene, using as reference strain Btk-HD1. A threshold of 30% of “functional mortality” was used to discriminate between “nontoxic” and “toxic” isolates. The toxicity of many Bt isolates toxicity competed with that of Btk-HD1. However, only three of them (Bt4NA, Bt5NA and Bt15NA) showed high toxicity in both, spore-crystal mixtures and supernatant cultures against the three lepidopteran species. The Bt isolates Bt4NA and Bt5NA express a protein of 130 kDa (with
match with the MW of Cry1 or Cry9 proteins), whereas the Bt isolate B5iNA express a protein of 65-70 kDa (MW of Cry2 proteins). In addition, we evaluate the presence of Vip3 (dot blot assays) and β-exotoxin (LC/MSMS analysis) in the supernatant of the B4iNA, B5iNA and B9iNA. The results indicate that the Bt isolates (B4iNA, B5iNA and B9iNA) do not produce β-exotoxin and overproduce Vip3, as compared with HD1. The results support the need to search for novel Bt isolates that overproduce the Vip3 proteins to be able to combine with the commercial Bt products (Dipel, Xentari).

POSTER SESSION. Wednesday, 16:30 PMC-31 STU

A new method of microsporidia metabarcoding
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The common methods of microsporidia detection base on sequence analysis of specific or group-specific PCR amplicons. Although these methods have proven to be sensitive, they could be unreliable when multiple microsporidian species co-circulate in one host. The aim of our work was to develop a method for the detection of microsporidia based on a short group-specific marker for NGS sequencing. As a model we used mosquitoes (Culicidae) that could be host for more than 100 microsporidia species. In total, 330 mosquitoes and 3 lines of live Encephalitoxozoon sp. spores were screened for microsporidium and host species using V5 18S rRNA and COI sequence data, respectively. DNA was extracted using ammonium hydroxide method. Marker fragments were individually amplified using indexed fusion primers, pooled, and NGS sequenced. Bioinformatic analysis was conducted using a custom workflow in Geneious R11.

The method was successful in detecting 100 spores/ml and let us to detect >10 microsporidian species in mosquitoes, including Microsporidium sp. 1199, first time reported from this host. Moreover, we noticed multiple co-occurrences of different microsporidia species in single host individuals. All mosquitoes were assigned unambiguously to species using COI data. Our method is efficient in rapid and sensitive screening for microsporidian species and their hosts.

In June and July, adults and nymphs were collected directly in the hazelnut orchards, however in May and October overwintering adults were sampled inside of buildings next to the orchards. For the detection of entomopathogens, individuals were dissected, and fresh smears of fat body and midgut tissues were examined microscopically. A naturally occurring microsporidium was detected. Data about the prevalence of the pathogen in various locations and seasons are reported: In Samgegrelo region, from 9 sampling collections only 2 individuals were found infected. From Imreti region none of the individuals were infected in both collection seasons. A higher prevalence (15 %) of the microsporidium was observed in adults and nymphs of Guria region. Highest infection rates occurred in individuals collected in October, in overwintering adults (41.5%), although the prevalence of the microsporidium in adults from Samegrelo region in October was 0.47%.

POSTER SESSION. Wednesday, 16:30 PMC-33 STU

Microsporidian infections in the Gammarus roeselii species complex (Amphipoda) over its geographic range: evidence for both host-parasite co-diversification and recent host-shifts.
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Microsporidia are obligate endoparasites. Both vertical and horizontal transmission routes are known. While the former may promote co-speciation and host-specificity, the latter may promote shifts between host species. Freshwater amphipods are hosts for many microsporidian species. However, no general pattern of host specificity and co-diversification is known. In South-Eastern Europe, Gammarus roeselii comprises of 13 cryptic lineages, but only one lineage has spread throughout North-Western Europe. Our first objective was to explore the Microsporidia diversity in G. roeselii and their phylogenetic relationships with other gammarids. Our second objective was to test if host phylogeographic history might have impacted host-parasite associations. 1904 Gammarus roeselii individuals were collected at 94 sites in 19 countries covering its entire European range. Microsporidia were screened and sequenced for part of the small subunit rRNA. Bayesian phylogenetic reconstruction of microsporidia was performed using sequences product from this study and literature data. Microsporidian diversity was high in G. roeselii with 18 species-level taxa. Ten microsporidia species were rare. Most of them are related to parasites from other crustaceans. Others were widespread genera with high prevalence: Nosema, Cucumispora and Dictyocoela. Microsporidia infecting G. roeselii revealed two scenarios of host-parasite associations: First, vertically-transmitted species Nosema granulosis and Dictyocoela roeselium are infecting G. roeselii over its range and are specific to this host. It suggests a host-specification evolutionary scenario. Second, horizontally-transmitted Dictyocoela muelleri and Cucumispora sp., present only in the region recently colonised. It suggests a host shift from local host species, after the spread of G. roeselii.

POSTER SESSION. Wednesday, 16:30 PMC-34

Development of Anncaliia algerae in Drosophila melanogaster
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Representatives of the genus Anncaliia (Brachiola) are known as natural parasites of dipteran and coleopteran insects, amphibid crustaceans, and humans, primarily with immunodeficiency. A. (Nosema, Brachiola) algerae, the best studied species of the genus, have been reported to infect,
naturally or experimentally, a huge variety of organisms including dipteran, coleopteran and lepidopteran insects, amphipod crustaceans, mice, human, and numerous fish, mammalian and insect cell lines demonstrating the broadest host range among microsporidia limited neither by body temperature nor by other physiological restraints. However, infection of Drosophila melanogaster with A. algerae have never been reported. We present TEM analysis of development of A. algerae in muscles and adipocytes of D. melanogaster 2 weeks after per oral experimental infection (by mixing spore suspension with diet before hatching). We observed typical D. melanogaster A. (Brachiola) vesicularum (spp. features of ultrastructure and cell pathology including spore morphology, characteristic extensions of the plasma membrane, and presence of "ridges" and appendages of vesicular-tubular material at proliferative stages. Tubular-vesicle appendages detached after parasite transformation to sporoplasts and filled in the surrounding cytoplasm. A.algerae development in D.melanogaster was particularly similar to one of A. algerae and A. (Brachiola) vesicularum in humans with acute myositis (Cali et al., 2004). Given D. melanogaster is currently the most established genetic model, with fully sequenced genome, easily available transgenic forms and genomic markers, a novel host-parasite system might provide new genetic tools to investigate exceptional physiological plasticity of A.algerae and other aspects of interactions of microsporidia with their hosts, as well to test anti microsporidia drugs.

POSTER SESSION. Wednesday, 16:30 PMC-35

The Enterocytozoonidae: Emergent Microsporidia in the aquatic-terrestrial food chain
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Enterocytozoon hepatopenaei (Microsporidia: Enterocytozoonidae), is a parasite of two penaeid shrimp (Penaeus monodon and Penaeus vannamei), infecting the hepatopancreatic epithelial cells. It has been associated with (but not found to be the direct cause) of a number of degenerative conditions (monodon slob growth syndrome, white faeces syndrome and acute hepatopancreatic necrosis disease). It was first described in Thailand but has been found to be very prevalent in other South East Asian and Central American countries. Initial indications and use of SSU suggested that the widespread nature of E. hepatopenaei in shrimp farms was a recent expansion, the result of anthropogenic factors. Some recent studies have however suggested that it likely was naturally widespread prior to it’s initial description. Suggesting a change in farming policies, like biosecurity, is the source of the rise in the prevalence of E. hepatopenaei. The aim of my project is to address the two posed hypotheses by assessing connectivity between geographic isolates (shrimp farms), using wgs. Samples were collected from Thailand and China, two of the first countries to describe E. hepatopenaei. The results suggest a change in the way that P. vannamei is currently being farmed. It also has implications for the threat that other widespread enterocytozoonoids have on aquaculture-based farming.

POSTER SESSION. Wednesday, 16:30 PMC-36

Do-it-yourself: On-site detection method for Nosema ceranae infecting honeybees
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Nosema ceranae is currently the dominant microsporidian parasite in honeybee colonies worldwide and large-scale colony losses (colony collapse disorder, CCD) symptoms have been linked to N. ceranae infections. Currently, microscopic examination of Giemsa-stained thin smear is the main diagnosis method. Here, we report the N. ceranae recombinase polymerase amplification-lateral flow (LF-RPA) dipstick detection method targeting the RNA polymerase II largest subunit gene. The reaction takes only 20-30 min under isothermal temperatures between 30 and 40 °C thereby indicating that the reaction mixture can be incubated with simple heating equipment, ambient temperature, or even human body heat. Specificity was evaluated using DNA from closely related microsporidia and other diseases in bees, while the biological sensitivity was validated based on N. ceranae DNA isolated from various geographical locations. Results indicated that the N. ceranae LF-RPA method is 100 times more analytically sensitive than conventional PCR. The in-field applicability of the RPA method was further evaluated using hive associated samples collected from various apiaries in Canada. Overall, the novel N. ceranae LF-RPA assay is effective for the detection of N. ceranae and has conceivable advantages over the conventional PCR in sensitivity, specificity, simplicity in operation, less time consumption and visual detection. The described N. ceranae LF-RPA method may facilitate the surveillance and early accurate detection of N. ceranae infection in hives.

POSTER SESSION. Wednesday, 16:30 PMC-37

Determining eastern spruce budworm population health: Operational utility of recombinase polymerase amplification for detection of Nosema fumiferanae
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The microsporidian, Nosema fumiferanae, is the ubiquitous natural enemy found in field populations of the eastern spruce budworm, Choristoneura fumiferanae. To date, Giemsa-stained thin smears followed by microscopic examination is the main diagnosis method to probe field-collected samples of eastern spruce budworm for N. fumiferanae infection. With the existing detection method, determining the health of field populations for onward forest protection decisions is not feasible on an operational basis. In this study, we developed and optimized rapid and accurate isothermal diagnostic tools, based on recombinase polymerase amplification (RPA), targeting the RNA polymerase II largest subunit gene for detection of N. fumiferanae. The lower detection limit of the assay were similar to those of established diagnostic PCR tests. A simple sample preparation method was optimized to eliminate the need for total DNA extraction using commercial kits. The RPA assay was validated using field collected samples from various Provinces in Canada. The estimated time to completion of the RPA assay from receiving the sample to having a result is 40 min, compared to 7 hr for PCR tests thereby suggesting that the assay was rapid for N. fumiferanae detection. The developed RPA assay is faster and feasible for large-scale assessment of budworm health prior to planning in-depth integrated budworm management approaches.

POSTER SESSION SLEGS AND SNAILS Wednesday, 16:30-18:00

Characterization of two Phasmarhabditis (Nematoda) earthworm isolates and susceptibility of earthworms and invasive slugs to EM434
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Phasmarhabditis hermaphrodita is a slug parasitic nematode commercially available for over 20 years as a slug biopesticide (Nemaslug®) in Europe.
This species, and two others, were recently discovered in California and Oregon. Two isolates of *Phasmarhabditis*, EM434 and DF5056, were recovered separately from undetermined earthworm species in New York, however the nature of their association remains unknown. This study aimed to characterize these two isolates, and determine their infectivity on three earthworm species and two invasive slug species. Both were characterized morphologically and by sequencing the D2-D3 expansion segments of the large sub-unit rDNA and mitochondrial cytochrome c oxidase 1 (COI) genes. Both genes were found identical for both isolates. EM434 and DF5056, at the Nemaglug® recommended dosage of 30 L1/cm², and a higher dosage of 150L1/cm², caused mortality to *Dero cericas reticulatum* and *Lehmannia valentiana*, but not on the three earthworm species *Eisenia fetida*, *E. fetida* and *Lumbricus terrestris* tested. DF5056 is lethal to *E. fetida* and *E. hortensis*, but not *L. terrestris*.

**POSTER SESSION**. Wednesday, 16:30 PSS-2

**LIMACAPT**: a self-powered connected sensor for monitoring slugs

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LIMACAPT is a sensor that can automatically count slugs in fields. Images are acquired during the night during slug activity, using a camera and infrared lighting. The computer embedded into the device runs an algorithm which processes several hundred images taken each night. The user receives the results of this data analysis sent by means of a low speed or GSM chip. This solution offers the chance to choose the best network option suited to rural regions. The whole electronic system, which is self-powered due to its battery and solar panel, is assembled on a fixed network option suited to rural regions. The whole electronic system, which is self-powered due to its battery and solar panel, is assembled on a fixed device, making it easy to deploy in the fields to be monitored. The innovation of LIMACAPT mainly lies in its continuous image capture detecting all active slugs, and on embedded image processing algorithm. This system works with a low error rate, approximately 9%, without any need for user intervention and enables the recognition and identification in the fields of objects appearing and disappearing from the frame without counting the same slug several times. LIMACAPT is a tool in precision farming which detects the present slug populations early and daily, to enable farmers to effectively deal with this pest as soon as the risk becomes apparent, for reasoned interventions. LIMACAPT opens up new scientific perspectives in terms of modelling for better insight into the pest.

**POSTER SESSION**. Wednesday, 16:30 Foyer

**NEMATODES**

**POSTER SESSION**. Wednesday, 16:30 PN-1

New isolates of the nematophagous fungus *Arthrobotrys oligospora* for biocontrol of garlic nematodes

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Phytonematodes pose a very serious threat for plant growing in Kyrgyzstan. Especially garlic crops in many regions, in all climatic conditions suffer from this dangerous pest.

Among microorganisms regulating nematodes population in soil, the fungi play a vital role due to their parasitic, antagonistic or predatory behavior. Nematophagous fungi are carnivorous fungi specialized in trapping and digesting nematodes. They can capture, parasites or paralyze nematodes at all stages of their life cycle and use them as a rich source of nitrogen. The objective of this study was to select native isolates of *Arthrobotrys oligospora* (Orbiliomycetes), a nematophagous fungus with high nema
tofage potential for use in biocontrol of garlic nematodes.

Native isolates of *A. oligospora* were characterized by using light micros-

**POSTER SESSION**. Wednesday, 16:30 PN-2

**Effects of pre-maize cultivation on soil conditions in apple replanting habitat with alleviating soil deterioration indicated by soil nematode community**

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Crop replanting changes soil physicochemical parameters, enzymes and microorganism communities, causing “replant problems” in crop cultivation. Pre-crop cultivation alleviates the problems. Soil nematode community would reflect the changes in soil conditions caused by replanting crops, in ways that are consistent and predictable.

We studied that the soil nematode communities reflected the alleviation made by pre-maize cultivation on soil physiochemical parameters in apple replant habitat during flowering, fruitlet, expanding and maturity stages. The results showed that soil parameters, including the pH value, the soil organic matter, ammonium nitrogen, and available P and K contents, were lower in replanting plots. Apparently, the soil deterioration is one of main contributors to apple replant problems. The values of the soil parameters were raised in 2-year maize pre-planting plots up to the level that in no replanting plots during the 4 apple growth stages, these changes can be indicated by soil nematode community. Soil pH (6.9) significantly decreased in replanting apple habitat, which highly decreased as well to bacterivorous nematode genera of *Plectus*, *Monhystera* and *Phasmatoaulus*. Two-year pre-maize cultivation raised pH value up to 7.6 and *Plectus* spp. became eu-dominant genus which reflected the available plant nutrition in the soil. The herbivorous nematodes, however the population of *Tylenchus*, *Paratylenchus* and *Pratylenchus* genera were significantly decreased in 2-year maize pre-cultivated soils.

Our results suggest that maize pre-planting could improve soil conditions and alleviate soil deterioration, which may mainly attribute to hydroxamic acid, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-2-benzoxazolinone (MBOA) indirectly in maize roots.

**POSTER SESSION**. Wednesday, 16:30 PN-3 STU

**SNP analysis for generation of trait-related molecular markers in the entomopathogenic nematode Heterorhabditis bacteriophora**

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The entomopathogenic nematode *Heterorhabditis bacteriophora* is a highly efficient biocontrol agent currently taking the step from niche markets into arable crops. Modern breeding technology targets at optimization of biocontrol traits, like dauer juveniles (DJ) longevity. This trait correlates with tolerance to oxidative stress and has a stronger genetic component (higher heritability) than DJ-longevity. To exploit this genetic component for breeding purposes, easy-to-use PCR markers predicting DJ-longevity were produced. Until recently, no more than 100 site-specific polymorphic markers had been reported for *H. bacteriophora*. To fill the genetic tool box, genotyping by sequencing (GBS) was carried out with *H. bacteriophora* strains and inbred lines of different origin and recombinant
inbred lines (RLIs) derived from crosses. More than 100,000 sequence clusters were obtained and yielding more than 1,000 single nucleotide polymorphisms (SNPs). Thereafter, the genotype information was combined with oxidative and desiccation stress tolerance phenotypic data by QTL analysis to identify genomic regions associated to survival traits. In parallel, we transferred the GBS information into Competitive Allele Specific PCR (KASP) markers and used them to genotype more than 40 WT strains. We determined that QTL-flanking SNPs can accurately predict DJ-longevity in wild type (WT) materials. We established a platform containing the genotype information from more than 700 robust SNPs in a collection of 48 WT inbred lines. Using this information other traits are studied by association analysis such as virulence at low temperature and nematode attraction towards plant and insect volatiles.

**POSTER SESSION. Wednesday, 16:30 PN-4**

**Putative receptor of a Bacillus thuringiensis toxin in Caenorhabditis elegans**

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The study of Bacillus thuringiensis toxins on plant-parasitic nematodes is complex, as these organisms require puncturing the host plant cells with their stylet to suck their content. For this reason, the model nematode Caenorhabditis elegans, naturally bacteriophagous, is used for this purpose, and several nematicidal B. thuringiensis strains have been found in this way. These strains may show potential activity against plant-parasitic nematodes. The interaction between Cry toxins and nematode intestinal receptors is poorly known. Therefore, we followed the RNAi silencing strategy to predict putative receptors, by using a B. thuringiensis strain with nematicidal activity, previously selected in our laboratory. Several genes encoding C. elegans intestinal membrane proteins were selected (abt-4, bre-1, bre-2, bre-3, B0024, abl-1, AC3.5) as potential Cry protein targets, some already known for having this attribute. Once sequences from 800 to 2,500 bp from each gene were cloned in the pL4440 vector and transferred to the E. coli HT115 strain to obtain dsRNAs, nematodes with silenced genes were tested in bioassays with the LBIT-107 strain, which contains Cry14 and Cry21 toxins. All tested genes were silenced, as no mRNAs were detected. Interestingly, the nematodes with the silenced abt-4 gene (a member of the ABC ATP-dependent transporters family) were partially but significantly more resistant to the LBIT-107 strain, as compared with the susceptible nematode strain. Therefore, the abt-4 gene product is a strong candidate for a toxin receptor.

**POSTER SESSION. Wednesday, 16:30 PN-5 STU**

**Steinernema feltiae scavenging behavior: offspring fitness is modulated by various cadaver scenarios**

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The entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are well-known biocontrol agents of soil-dwelling insect pests. EPNs are widely considered obligated parasites, despite their capacity to colonize and reproduce in insect cadavers, even in the presence of saprophagous organisms of distinct nature. We aimed to determine the possible impact of the scavenger behavior of Steinernema feltiae on subsequent offspring fitness. We investigated the pathogenicity and reproductive success of the infective juveniles (IJ$s) resulting of various environments inside Galleria mellonella cadavers: (i) emerged from freeze-killed or initially alive larvae, and (ii) exposed or not to scavenger nematodes (Oscheius onirici or Pristionchus maupasi) or opportunist fungus (Aspergillus flavidus). We speculated that the IJs emerging after scavenger activity will display a reduction of their pathogenicity and reproductive success, and this decline in fitness will be amplified when exposed to other saprophages. We observed that the IJs emerged from freeze-killed larvae recorded subsequent lower pathogenicity rates, but also a lower reproductive success when support high competitive pressure by scavenger nematodes (O. onirici in particular). Our results suggest that scavenging can be a plausible but suboptimal solution as an alternative pathway for EPNs to reproduce.

**POSTER SESSION. Wednesday, 16:30 PN-6**

**Mortality of Phyllophaga vetula larvae by the separate and combined application of Metarhizium anisopliae, Steinernema carpocapsae and Steinernema glaseri**

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Phyllophaga vetula is an edaphic pest that is present in the corn crop (Zea mays), which is usually uncontrolled with broad-spectrum chemical insecticides. However, the synergistic interaction between fungi and entomopathogenic nematodes could improve the biological control of this insect. This study investigates the mortality of larvae of Phyllophaga vetula (Pv) by the effect of the separate or combined application of the fungus Metarhizium anisopliae strain M101 (Ma) and the nematodes Steinernema carpocapsae All strain (Sc) or Steinernema glaseri strain NJ-43 (Sg). In laboratory, dosages of 18 × 10^7 or 9 × 10^7 spores and 250 infective juveniles were applied on Pv larvae of medium or large size contained in vials with sterilized agricultural soil as the assay arena. The separate application of Ma did not kill any larvae, but Sg and Sc killed 40 % and 80 % of the larvae, respectively. Though, the Ma and Sc combination had an important antagonistic interaction that decreased the mortality to 40 %, but the combination Ma and Sg had a slight additive interaction that increased the mortality to 47 %. The most determinant factor in larvae mortality was the nematode used, with Sg as the species of best performance in 6 of the 12 treatments evaluated and with a maximum effectivity of 80 % on medium size larvae. The results are discussed in light of available information on the individual and combined effective of the entomopathogens used against Phyllophaga spp.
of EPN were isolated from different sites and habitats across Israel. Phylogenetic analysis revealed these isolates belong to the species *Heterorhabditis bacteriophora* (5 strains) and *H. indica* (5 strains). All strains were characterized for heat and desiccation tolerance. Results indicated differences between the species and within the strains belonging to the same species regardless of the habitat characteristics. In general, strains of the species *H. indica* were more tolerant than strains of the species *H. bacteriophora* to each of the stress conditions examined. For studying the genetic basis for differences in tolerance to abiotic stress, from each nematode species strains with phenotype of high or low tolerance were chosen for transcriptome analysis. The transcriptomic comparison between strains in each of the nematode species with varied degree of tolerance to abiotic stress allow the identification of crucial metabolic pathways and genes involved in response to each stress. These sets of genes are examined as possible molecular markers for breeding lines and strains with improved tolerance to abiotic stress.

**POSTER SESSION. Wednesday, 16:30 PN-8 STU**

**Behavioural response in model insect under biological and chemical stress condition**

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**Drosophila melanogaster** and *Galleria mellonella* are among the most widespread model organisms used in various fields of research. For behaviour research a new method called FIMTrack was developed to simplify the collection and processing of locomotor data. It is based on frustrated total internal reflection and allows us to observe groups of larvae crawling on the translucent agar gel. FIMTrack was used to evaluate the behaviour of *Drosophila* larvae during infection with entomopathogenic nematodes (EPN). We observed some differences in velocity, bending, rolling and twisting in attempt to avoid the contact with nematodes. When enabled to choose, the larvae preferred the safer route without EPN influence. We observed that nematodes evade the encapsulation by hemocytes, we examined the effect of *C. elegans* on hemocytes in the hemocoel of *G. mellonella* larvae. Injection of nematodes resulted in the decrease in hemocyte density while hemocyte mortality, spreading ability of plasmatocyte and hemocyte production by the insect hematopoietic organ were not affected. In vitro co-incubation of hemocytes and nematodes resulted in the decrease of the hemocyte number and we could observe nematodes feeding on hemocytes. Injection of *C. elegans* feeding-delay mutants into insects did not cause the decrease of hemocyte density comparing to the injection of wild type nematodes. Our results suggest that the decrease of hemocyte density in insects after injection of *C. elegans* is due to the nematode’s predation on hemocytes. Furthermore, an entomopathogenic nematode as well as other bacterial feeding nematodes were also observed to feed on hemocytes. These results suggest that feeding of hemocytes may have played an important role in the evolution of nematode parasitism.

**POSTER SESSION. Wednesday, 16:30 PN-10**

**Potential four entomopathogenic nematodes for the control of Brown marmorated stink bug - Halyomorpha halys**

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (BMSB) is an exotic invasive insect for Italy and Georgia. At present BMSB is very active and makes alarming situation. Pest characterized by the massive increase and formation the large tracts of agroecosystems and urban area (inducing foliage, coniferous, ornamental plants as well). At present, BMSB has become a key pest in many crop plants and makes grade economical losses. Nowadays, due to lack of specific natural enemies, population density of this insect is not controlled by them. The aim of our study was to established potential entomopathogenic nematodes (EPNs) and determine their effectiveness on *H. halys* for its control. In experimental trial four entomopathogenic nematodes were used: two of Georgian species *Heterorhabditis bacteriophora* (HRB, GEO) and *Steinernema borjomiensis*; two Italian species *Heterorhabditis bacteriophora* (HRB, IT) and *Steinernema apuliae*. In laboratory assay (22°C and 80% RH), the mentioned nematodes were used in the following concentrations: 1:1000, 1:500, 1:200 infective juveniles (IJ) per adult of *H. halys*. The mortality of tested insects was estimated from the third day after treatment. Significant differences were observed between Georgia and Italian species. HRB (GEO) and *S. borjomiensis* at the high concentration -1:1000, mortality 46.6% -33.3%, in case 1:500 – 33.3%-32% and in 1:200 – 33.3%-13.3% were reached accordingly. More pathogenic were Italian species. HRB(II) and *S. apuliae* in high concentration –1:1000, mortality achieved 93.3%-53.3% in case 1:500 – 93.3%-40%, and in 1:200 – 73.3%-33.2% were observed respectively. The emerging IJs were harvested and counted throughout the interval of 11-15 days.

**POSTER SESSION. Wednesday, 16:30 PN-11**

**Susceptibility of olive fruit fly, Bactrocera oleae (diptera: tephritidae) larvae and pupae to entomopathogenic nematodes**

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Hemocytes play important roles for recognition and attack against larger invading organisms such as nematodes. However, very limited information on nematode-hemocyte interaction is currently available. In our previous study, we demonstrated that non-insect parasitic nematodes such as *Caenorhabditis elegans* are not encapsulated in the larvae of the greater wax moth *Galleria mellonella*. To understand a mechanism in which nematodes evade the encapsulation by hemocytes, we examined the effect of *C. elegans* on hemocytes in the hemocoel of *G. mellonella* larvae. Injection of nematodes resulted in the decrease in hemocyte density while hemocyte mortality, spreading ability of plasmatocyte and hemocyte production by the insect hematopoietic organ were not affected. In vitro co-incubation of hemocytes and nematodes resulted in the decrease of the hemocyte number and we could observe nematodes feeding on hemocytes. Injection of *C. elegans* feeding-delay mutants into insects did not cause the decrease of hemocyte density comparing to the injection of wild type nematodes. Our results suggest that the decrease of hemocyte density in insects after injection of *C. elegans* is due to the nematode’s predation on hemocytes. Furthermore, an entomopathogenic nematode as well as other bacterial feeding nematodes were also observed to feed on hemocytes. These results suggest that feeding of hemocytes may have played an important role in the evolution of nematode parasitism.
Assessing the immunocompetence of Rhynchophorus ferrugineus in response to bacterial challenge and entomopathogenic nematode infection

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The most susceptible stage or sex and the most virulent pathogen is particularly important to develop effective control strategies against insect pests. In this study, we carried out laboratory tests to identify the most susceptible target of the Red Palm Weevil Rhynchophorus ferrugineus by infecting different stages and sexes (larvae, pupae, male and female adults) with both a generic pathogen, Gram-negative bacteria Steinernema feltiae and Heterorhabditis bacteriophora and two indigenous Italian strains of H. bacteriophora and Steinernema carpopapsae. The susceptibility assays with B. oleae were performed in 24-well plates, filled with about 2 g of sterile soil. A single pupa or larva was inserted into the bottom of each well and 100 IJs/0.5 ml of distilled water were inoculated onto the soil surface (n = 30 for each EPN strain and insect stage). In the control (n = 30), only sterile water was added to each well. Adult emergence and mortality were recorded after 15 days. Dead pupae and adults were dissected to assess nematode infection. The most noteworthy result was obtained with S. feltiae which was able to infect more than 80% of larvae. Since this tephritid fly spent several months in the soil, the use of EPNs could be a promising method to control this pest, but further studies are necessary to evaluate this topic.

Recombinant Bacillus thuringiensis for intestinal nematodes

Flanagan, KA1; Abraham, A.; Gazzola, D.; Li, H.; Kellog, T.; Kim, Y.; Rus, F.; Ostroff, G.; and Aroian, RV

POSTER SESSION. Wednesday, 16:30 PN-13

Gastrointestinal nematode (GIN) parasites are a leading threat to mammalian livestock agriculture and ~1.5 billion people worldwide. The rise of drug resistance in parasitic nematodes towards the available small molecule drugs currently used to treat GIN infections ushers in an urgent need for new and inexpensive cures. Cry5B, a member of the crystal protein family found in Bacillus thuringiensis (Bt), is an effective nematocidal protein and a pioneer for the use of therapeutic crystal (Cry) proteins against GINs that infect mammals. Cry proteins have already been generally accepted by federal agencies as non-toxic to nontarget mammals. The Bt Cry protein family is an extensive and potentially rich resource of other nematocidal Bt Cry genes and variants. Here, we will present a wide range of parasites of humans and livestock animals that we have tested Cry5B against. In addition, we have begun to delve wider into how nematocidal Cry proteins and Cry protein variants themselves might be useful against GINs alone and in combination. Recombinant Bt strains expressing various Cry protein and Cry protein variants were harvested, processed, and bioassayed for lethal toxicity against GINs in vitro and in vivo. The long-term goal of this study is to engineer Bt with therapeutically relevant proteins for safe, cheap, and community-wide clearance of gastrointestinal parasites. These experiments are important steps towards generating a recombinant Bt for mass production to cure mammals of pathogenic gastrointestinal nematodes.

Pheromones as drivers of entomopathogenic nematodes movement and infectivity

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Inconsistencies in entomopathogenic nematode (EPN) efficacy is still one of the biggest challenges for the wider adoption of EPNs as biocontrol agents. Previous studies demonstrated that extracts from EPN infected hosts enhance dispersal and efficacy, two key factors in EPN’s success. Some active components in the insect host cadavers responsible for dispersal were identified as nematode pheromones, ascarosides. We hypothesized that pheromones increase EPN infective juveniles (IJ) dispersal leading to increased efficacy. First, we determined whether pheromone pretreatment induced higher numbers of Steinernema carpocapsae and Steinernema feltiae IJs to move towards T. molitor larvae in the bottom of the column. This was in comparison to IJs treated with infected cadaver macerate and water, positive and negative controls, respectively. Consistent with the soil column tests, both S. carpocapsae and S. feltiae IJs treated with pheromones performed better in killing two important insect larvae (pecan weevil, Curculio caryae, and black soldier fly, Hermetia illucens) in greenhouse tests when compared to the IJs treated with water. Moreover, to further elucidate pheromone modes of action, eppendorf tube tests with T. molitor showed that infectivity, measured as invasion rate, of S. carpocapsae and S. feltiae was also greater in pheromone treatments in comparison to control treatments. This novel project demonstrates the use of pheromones to enhance pest control potential via increased dispersal and therefore more contact with hosts, but also via increased infectivity.
control of box tree moth – Cydalima perspectalis (Walker, 1859) in laboratory conditions
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Cydalima perspectalis or the box tree moth is a pest of Buxus trees. It originates from South East Asia and recently has been introduced in Europe, and in 2014 in Bosnia and Herzegovina. It has quickly spread over the country and started to make devastating damage to this ornamental plant. Buxus ornamentals are usually grown in private gardens or in public parks. Its damaging potential requires implementation of control measures at regular basis. In this study four species of entomopathogenic nematode Steineremna feltiae, S. carpocapsae, S. kraussei and Heterorhabditis bacteriophora local strains were used. Caterpillars of the box tree moths were collected from naturally infested trees during April and May and treated with nemathodes in laboratory assay. Four concentrations of nematodes 250, 500, 1000, and 2000 IJ were applied against 10 larvae in Petri ditches (diameter 5.5 cm) at room temperature. Mortality was assessed 24 hours and 5 days after the nematode application. At the highest concentration of IJ S. kraussei showed 100% mortality after 24 h, while other nematodes this efficacy expressed in observation 5 days after the application.

POSTER SESSION. Wednesday, 16:30 PN-16
Management of black vine weevil (Otiorhynchus sulcatus) by entomopathogenic nematodes in Georgia
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The aim of this study was to determine the biological control effect of entomopathogenic nematodes species, Steineremna carpocapsae and Steineremna feltiae against of Otiorhynchus sulcatus. Adult weevils can be controlled by using nematodes as biocontrol. Also grubs can be controlled using the fungus Beauveria bassiana. Experiments was evaluated under laboratory conditions. Various laboratory bioassays were conducted to determine the effectiveness of entomopathogenic nematodes to control Otiorhynchus sulcatus. Adults of O. sulcatus were screened for susceptibility to two introduced from Israel nematode species. Otiorhynchus sulcatus was found to be most susceptible to S. carpocapsae and S. feltiae, causing mortality 34, 52, 83% and 27, 34, 69% on the temperature 25°C and 1000 IJs/ml cm² concentration, respectively. Larvae of O. sulcatus was controlled using the fungus B. bassiana. Further bioassays illustrated a linear relationship between black vine weevil, mortality and the concentration of nematodes applied, with the highest level of control using a concentration of 1000, 1500 infective juveniles (IJs)/insect. Steineremna carpocapsae proved able to locate and infect black vine weevil, quicker than S. feltiae. For all nematode species, the highest virulence was observed 48,56,88% and 39,45, 78% on the temperature 25°C and 1500 IJs/ml cm² concentration for S. carpocapsae, and S. feltiae, respectively. Fungal isolate B. bassiana imposed more than 50% larval mortality of O. sulcatus. In conclusion, it was determined that O. sulcatus can be controlled by S. carpocapsae, S. feltiae and fungus B. bassiana, but further studies should be conducted at field conditions.

POSTER SESSION. Wednesday, 16:30 PN-17
Molecular and phenotypic characterization two strains of Photorhabdus luminescens associated with Iraqi Heterorhabditis bacteriophora
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The bacterial symbiont IRQ.1 and IRQ.2 were isolated from populations of the insect pathogenic nematode Heterorhabditis bacteriophora collected from Iraq. These bacteria were symbiotically associated with entomopathogenic nematodes of genus Heterorhabditis, contributing actively to the biological cycle of their host. The Heterorhabditidae family of nematodes involves of obligate insect pathogens. Both the nematode and bacteria work together to overcome the immune response of target insect. The bacteria were isolated from crushed number of infective juveniles. On the indicator NBTA plates, characteristic blue colonies of Photorhabdus were developed slowly, then, the colony was picked from isolation plates only after 48 h. This study was based on phylogenetic analysis of sequence data of two genes: 16S rRNA and gyrB. The bacteria were also characterized phenotypically by biochemical and physiological tests. Our results have shown that the Photorhabdus strains isolated from H. bacteriophora belong to Photorhabdus luminescens subsp. akhurstii. This is indeed the case for the strain examined in this study which has been isolated from the recently described H. bacteriophora from Iraqi soil.

POSTER SESSION. Wednesday, 16:30 PN-18
Comparison of entomopathogenic nematode and insecticide management of western corn rootworm larvae
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The Western Corn Rootworm (WCR), Diabrotica virgifera virgifera LeConte, 1868, [Coleoptera, Chrysomelidae], whose larvae cause damage to maize roots, is an important economic insect pest in America and Europe. Its larvae are usually controlled by granular soil insecticides or insecticide-treated seeds. Biological control options, such as entomopathogenic nematodes (EPN), may provide an alternative management option. In a three year field experiment we compared the effectiveness of inundative biological control on the basis of EPN Heterorhabditis bacteriophora Poinar, 1976 (Rhabditida: Heterorhabditidae; product Dianem) and the chemical insecticides Force 1.5 g (active substance tefluthrin, pyrethroid) and Sonido (a.s. thiacioprid, neonicotinoïd). Additionally, a soil conditioner (a.s. alcohol ethoxylate, product Transformer) was used with the EPN, to check for potential increase of EPN effectiveness. Treatment efficacy was evaluated by counting the emerged beetles in the experimental plots using field cages. Two experiments were performed, one in eastern (Priekļķi) and the other in northern (Gorenjka) Slovenia. The efficacy of the treatments was very similar at both locations, despite the approximately 5-fold lower WCR population in Gorenjka compared to Priekļķi, as well as consistent over time. The highest number of WCR beetles was caught in the negative control, followed by the treatment Sonido (insignificant decrease). Treatments Force, Dianem with and Dianem without Transformer significantly decreased the number of emerged beetles and were statistically indistinguishable. WCR larvae control in maize using entomopathogenic nematode Heterorhabditis bacteriophora was comparable to conventionally used chemical control and could thus provide a sustainable WCR biological control management option.

POSTER SESSION. Wednesday, 16:30 PN-19 STU
The earthworm mucus and their feeding activity can decrease the biological control action by entomopathogenic nematodes and entomopathogenic fungi
Chelkha, M.1,2; Blanco-Pérez, R.2,3; Bueno-Pallero, F. Á.2; El Harti, A.1; Amghar, S.1; Campos-Herrera, R.2,3
1 Research Team »Lombricidae, Improving Soil Productivity and Envi-

1868, [Coleoptera, Chrysomelidae], whose larvae cause damage to maize roots, is an important economic insect pest in America and Europe. Its larvae are usually controlled by granular soil insecticides or insecticide-treated seeds. Biological control options, such as entomopathogenic nematodes (EPN), may provide an alternative management option. In a three year field experiment we compared the effectiveness of inundative biological control on the basis of EPN Heterorhabditis bacteriophora Poinar, 1976 (Rhabditida: Heterorhabditidae; product Dianem) and the chemical insecticides Force 1.5 g (active substance tefluthrin, pyrethroid) and Sonido (a.s. thiacioprid, neonicotinoïd). Additionally, a soil conditioner (a.s. alcohol ethoxylate, product Transformer) was used with the EPN, to check for potential increase of EPN effectiveness. Treatment efficacy was evaluated by counting the emerged beetles in the experimental plots using field cages. Two experiments were performed, one in eastern (Priekļķi) and the other in northern (Gorenjka) Slovenia. The efficacy of the treatments was very similar at both locations, despite the approximately 5-fold lower WCR population in Gorenjka compared to Priekļķi, as well as consistent over time. The highest number of WCR beetles was caught in the negative control, followed by the treatment Sonido (insignificant decrease). Treatments Force, Dianem with and Dianem without Transformer significantly decreased the number of emerged beetles and were statistically indistinguishable. WCR larvae control in maize using entomopathogenic nematode Heterorhabditis bacteriophora was comparable to conventionally used chemical control and could thus provide a sustainable WCR biological control management option.
Entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPF) are excellent biological control agents of insects. However, many extrinsic factors, such as the presence of other soil inhabitants, modulate their efficiency. Earthworms are considered soil eco-engineers because their activities enhance soil properties. Earthworms’ movement promote EPN and EPF biocontrol activity by helping their dissemination in the soil profile; however, little is known about the impact of other actions such as feeding activity or mucus secretion. We hypothesize that earthworms will decrease the EPN/EPF biocontrol activity by feeding action, but mucus secretion will not be a limiting factor. In laboratory experiments, we combined earthworms (Eisenia fetida) or its mucus with six EPN species (Steinernema carpocapsae, S. feltiae, S. glaseri, S. khuongi, Heterorhabditis bacteriophora, and H. zealandica) or one EPF species (Beauveria bassiana). In soil mesocosm, mucus altered biocontrol potential of certain EPN and EPF species by reducing the Galleria mellonella larval mortality compared to control treatments. A subsequent experiment showed that when low concentration of EPNs were exposed to mucus, the biocontrol activity was reduced in a species-specific manner, increasing EPN emergency times and reducing the number of emergencies in certain species, highlighting a possible trade-off of the presence of EPN/EPF and earthworms.

**POSTER SESSION. Wednesday, 16:30 PN-20**

**Fighting parasitic nematodes with natural products and microbial crystals**


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The discovery of new broad-spectrum anthelmintics to target parasitic worms, which affect 24% of humans, crops, and livestock, remains a challenge. We are using small molecule and natural products to identify novel compounds that affect nematodes and study their modes of action. We established a high-throughput automated platform for chemical and functional genomic screening that accommodates both cell-based and whole-organism assays. We are using the free-living nematode model *C. elegans* and the distantly related *P. pacificus* as model organisms. Given the short life cycle of the worm, our platform enables one person to screen 20,000 chemicals per week and perform one genome-wide RNAi screen every three weeks. We validated our approach in a pilot screen of an FDA-approved drug library, which confirmed the effects of known anthelmintics and revealed novel anthelmintic compounds. We screened a library of 32,000 small molecules, selected using a computational approach to predict bioavailability in nematodes and identified numerous candidate molecules that will be assayed for toxicity in mammalian cells. We have also screened a *Bacillus thuringiensis* library of 300 uncharacterized strains to identify Cry proteins showing toxicity against nematodes. We found 95 strains that hinder the development of worms, and among them 50 strains that act through a Cry5-independent mechanism. Tests in the plant root-knot parasite Meloidogyne and the veterinary parasite *Haemonchus contortus* revealed 20 strains with variable severity effects. Virulence factors of these strains are being characterized by DNA sequencing combined with proteomics and functional genomic assays to elucidate their mechanisms of action.

**POSTER SESSION. Wednesday, 16:30 PV-1**

**Phylogenetic analysis of six strains of baculovirus with activity towards Spodoptera frugiperda**

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In order to know the phylogenetic relationship of six strains of baculovirus (SINPV-An2, SINPV-Arg, SINPV-Fx, SINPV-Ho, SINPV-Sin and SIGV-RV) the genes of polyhedrin (polh) were partially amplified, late expression factor 8 (lef-8) and late expression factor 9 (lef-9) and were sequenced. The nucleotide sequences were aligned by ClustalW and the sequence obtained from the NCBI GenBank of the *Spodoptera frugiperda* nucleopolyhedrovirus (SINV) was used for the five NPV studied and the *S. frugiperda* granulovirus (SIGV) for GV. In the alignment of the NPV, nucleotide changes and identities were observed between amino acids higher than 97% (lef-9) and 98% (lef-8), however polh did not present any nucleotide change. For the GV alignment there were also nucleotide changes in the three amplified genes and identities higher than 98% in lef-8, lef-9 and granulin. For the phylogenetic reconstruction, the amino acid sequences of 47 *Alphabaculovirus*, 23 *Betabaculovirus* and one *Gammabaculovirus* (external group) obtained from the NCBI GenBank, as well as the sequences of the six baculoviruses were used. The five nucleopolyhedroviruses studied were grouped in group II of *Alphabaculovirus* and were included in the same clade in which the SINPV is used as reference, but with phylogenetically distinct identities, which is consistent with the geographical distribution of the isolates (México, United States, Honduras and Argentina); for the case of the granulovirus it was grouped together with the *Betabaculoviruses* in the same clade of the SIGV used as a reference. The SINPV and SIGV-RV of the present study show a common ancestor.

**POSTER SESSION. Wednesday, 16:30 PV-2 STU**

**Functional and genomic approaches to study the evolution of recently domesticated virusts in Campopleginae parasitoid wasps**


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**Nudiviruses** are known to be endohenedized in hundreds of wasp species. In braconid wasps, which have integrated a virus belonging to the Betanudivirus genus, the wasp makes use of this viral integration to successfully accomplish their parasitic lifecycle in lepidopteran hosts by deregulating the host immune system with viral particles containing DNA circles inject- ed during oviposition. *Venturia canescens*, an ichneumonid wasp belonging to the *Campopleginae* subfamily, has endogenized nudivirus genes belonging to the *Alphanudivirus* genus. Contrary to braconid wasps, *Venturia canescens* wasps produce Virus-Like-Particles (VLPs) which do not package DNA circles but instead package proteic virulence factors. More recently, nudivirus genes coding for VLPs have been found in another species of *Campopleginae* wasps: *Campoplex capitator*, phylogenetically close to *Venturia canescens*, offering the opportunity to perform comparative functional and genomic approaches to better understand early mechanisms involved in viral domestications and to determine if the same evolutionary paths were taken for the production of VLPs. The work pre-
sented here will present the functional (kinetic expression analyses and silencing and interfere with gene expression of the host.

In recent years, several cricket species are mass reared as an alternative biocontrol agents of some specific plant diseases spread in Europe. A new iflavivirus was occasionally found in some Italian and French insect. The USA samples were richer in viral biodiversity, as members of the families Bunyaviridae, Nodaviridae, Partitiviridae, Reoviridae and Permutotetroviridae were identified. Samples of F. occidentalis and T. tabaci were collected from seven Italian regions and from two laboratory populations from the USA. Overall, full-length genomes of more than 40 new viral species were assembled. Among them, a densovirus and a mesonivirus were highly prevalent in F. occidentalis, while a virga-like and a dimarhabdovirus were present in all the T. tabaci samples. Some of the identified viruses will be selected to measure their effects on insect fitness parameters and transmission efficiency. The most promising one for each pathosystem will be used to promote virus-induced gene silencing and interfere with gene expression of the host.

Unraveling the multiple nudiurnal integration traces within insect genomes

Bézier, A.; Leibold, M.; Gayral, P.; Drezen, J-M.; Herniou E.A.

Nudiviruses are dsDNA viruses that infect a wide range of arthropods, from crustaceans to insects. Recently, new genomes have been published raising their number to twelve available complete exogenous nudi-

nudivirus genomes at the end of 2018. Moreover, nudiviruses are occasionally susceptible to integrate their genome within their host's genomes. Some endogenous nudiviruses have been further the source of biological innova-

tion for their hosts as previously shown in braconid and ichneumonid parasitoid wasps, in which they respectively produce bracoviruses and virus-like particles. The advent of affordable Next Generation Sequencing led to a tremendous increase of complete genome and transcriptome data of complex non-model organisms such as insects that may carry dsDNA
viruses. A dedicated bioinformatic pipeline was used for data-mining billions of raw sequence data to discover new exogenous or endogenous nudiviruses. A thorough search based on databases screening using first a set of conserved nudiviral sequences allowed the identification of new insect species potentially harbouring nudiviruses. Their transcriptomic or genomic data were then investigated in depth and similarities with nudiviral genes were confirmed by BLAST searches against NCBI and against a manually curated nudiviral database. Ultimately over 250 new integrat-ed nudiviral sequences were identified from 13 species belonging to five insect orders. These results highlight the close relationships nudiviruses have had with many insect species since the Paleozoic.

POSTER SESSION. Wednesday, 16:30 PV-7

**Construction of a novel baculovirus expression system with increased foreign protein production and expression time**


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The baculovirus expression system (BES) is widely used for the expression of vertebrate proteins or production of recombinant subunit vaccines in insect cells. The BES is inexpensive than mammalian cell expression systems, has a short production time, and has a convenient protein expression method. The most useful feature of the BES is that it can produce the post-translationally modified and correctly folded proteins at a similar level to mammalian expression systems. The BES uses a polyhedrin promoter or p10 promoter with strong transcriptional activity. However, the production efficiency of a foreign protein using these polyhedrin promoter or p10 promoter are not as high as that of native polyhedrin or p10 proteins. Therefore, a variety of efforts have been made to increase the mass production of foreign proteins, such as the modification of culture media or fusion with a carrier protein as a “fusion partner”. Previous attempts, however, have shown limited efficacy on some proteins and have not become popular. Therefore, in this study, overexpression vectors and productivity-enhanced Bacmid were constructed to fundamentally improve the low efficiency of protein production.

**POSTER SESSION. Wednesday, 16:30 PV-8 STU

**Construction of novel baculovirus inducible vectors for rapid production of foreign proteins**

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The baculovirus expression system is a very useful tool widely used for expression of foreign proteins. In order to utilize baculovirus expression system, it is necessary to prepare the recombinant baculovirus. The development of the Bac to Bac system has reduced the time and effort required to generate recombinant baculovirus. However, it still takes at least two weeks and more than that for unskilled persons. In addition, more time is required to measure the titer of the recombinant baculovirus. In order to overcome this problem, a virus inducible expression system is being studied recently. Although baculovirus is able to rapidly express foreign proteins, it still has a low expression level. Therefore, in this study, we aimed to construct a novel baculovirus inducible expression vector that not only shortens the production time of protein but also can express at a high level. The novel baculovirus inducible expression vector has been evaluated using EGFP and is expected to be a very useful tool for production of various proteins.

**POSTER SESSION. Wednesday, 16:30 PV-9 STU

**Chrysoperla carnea’s performance when fed with two nucleopolyhedroviruses (SeMNPV and AcMNPV) Spodoptera exigua infected larvae**

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To determine the compatibility of use of the nucleopolyhedroviruses (NPVs) of Spodoptera exigua (SeMNPV) and Autographa californica (AcMNPV) with the neuropeptide predator Chrysoperla carnea, laboratory assays with last instar larvae were developed. Four diets composed the treatments: (1) *Ephestia kuehniella* + *Artemia* spp. eggs (control 1), (2) healthy L2 *S. exigua* larvae (control 2), (3) SeMNPV infected L2 *S. exigua* larvae, (4) AcMNPV infected L2 *S. exigua* larvae. *Spodoptera exigua* newly molted L2 larvae were infected via droplets ingestion with the LC50 of SeMNPV (specific NPV of *S. exigua*) and the LC50 of AcMNPV (wide range NPV of *Noctuidae*) respectively, and offered *ad libitum* 3 days post inoculation to L3 *C. carnea* larvae until pupation. The parameters evaluated were: pupation, weight of pupae, adult emergence, sex ratio, fecundity, egg hatching, adult lifespan, viability of the offspring and presence of occlusion bodies (OBs) in the meconium. LC50 of both NPVs for L2 *S. exigua* larvae were previously calculated with a PROBIT analysis (SeMNPV: 1.9x10^5 OBs/ml; AcMNPV: 1.6x10^6 OBs/ml). There was a delay in the pupation of *C. carnea* larvae treated with both virus as well as a slight reduction of the pupal weight. No differences were found on the adult emergence. When reproduction parameters were assessed, some differences between treatments were detected. Our findings are relevant for the combined use of virus-based insecticides and natural enemies of lepidopteran pests.

**POSTER SESSION. Wednesday, 16:30 PV-10

**Identification and Genomic Analysis of a Second Species of Nucleopolyhedrovirus Isolated from Spodoptera exigua (Lepidoptera, Noctuidae)**

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SeMNPV-QD is a nucleopolyhedrovirus isolated from naturally infected *Spodoptera exigua* (Lepidoptera, Noctuidae) larvae in the field in Qingdao, Shandong, China. The virus has a polyhedron size of 1.39 ± 0.28 μm, and typical virions contain one to seven nucleocapsids per envelope. SeMNPV-QD only infects the larvae of *S. exigua*, LC50 and LT50 for the 4th instar larvae of *S. exigua* were 3.81x10^5 PIB/mL and 5.3 d, respectively, which were not significantly different from the SeMNPV American isolate (SeMNPV-US1). SeMNPV-QD has a circular double-stranded DNA genome of 128,525 bp with a GC content of 37.41% and 127 putative open reading frames (ORF), each of which encodes more than 50 amino acids. These were identified and annotated in the SeMNPV-QD genome, accounting for 87.53% of the whole genome. The SeMNPV-QD genome is 7,086 bp smaller than SeMNPV-US1, SeMNPV-QD has 123 ORFs similar to those of SeMNPV-US1, and the genomic similarity with SeMNPV-US1 was only 45.8%. Although gene arrangement is virtually identical, there are 4 ORFs unique to each locus, including 4 ORFs unique to SeMNPV-US1 and other baculoviruses isolates were all above 0.05 substitutions/site for each locus, indicating that SeMNPV-QD is a new species, previously unrecognized species and not variants of members of currently existing
Arboviruses associated with *R. microplus* ticks in Yunnan China

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Ticks are well known as vectors of many arboviruses which usually do great harm to human and animal health. Yunnan Province in China widely covered by flourishing vegetation and mainly relying on farming husbandry is abundant with *Rhipicephalus microplus* ticks. Therefore, it is of great significance to characterize the viral profile present in *R. microplus* parasitizing on cattle in Yunnan China. A total of 7387 ticks (*R. microplus*) fed on cattle and 100 bovine serum samples and healthy/febrile human serum samples were collected in Yunnan during 2015 to 2017. There are nine arboviruses characterized with high diversity concerning chuvrhabdo-, phlebo-, flavi- and parvo- viruses are identified via metagenomic sequencing. PCR-based and IFA-based investigations indicate five of the nine viruses may broadly distributed in Yunnan and may be infectious to cattle. Our study revealed the presence of diverse viruses which expanding the biogeography of these identified viruses. We described the genomic and phylogenetic characterization of these viruses. Furthermore, continuous survey among ticks reveals the broadly prevalence of three viruses including a flavivirus-like segmented virus (Jingmen tick virus). Serological investigation among cattle indicates that these identified viruses may be infectious to cattle and can elicit corresponding antibody. Further analysis is needed to better elucidate the natural circulation of these virus among ticks and cattle. In the future, the influence of these novel arboviruses to cattle or other animals, even plants and humans should be taken into consideration.

Comparison of vertical transmission of high and low virulent nucleopolyhedrovirus strains after *Lymantria dispar* L. infection

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It is known that baculoviruses are able to persist in the organism of their hosts after infection and may be vertically transmitted to the next generation. Obviously, that viruses with low virulence have higher probability for this way of transmission. The aim of our study was to compare the efficiency of vertical transmission and following reactivation of multiple nucleopolyhedrovirus (LdMNPV) in the offspring of *Lymantria dispar* L. after infection of parental generation. As the result of parents infection, fecundity of survived females, pupae weight, fertility (egg hatch) were significant affected. The offspring of infected parents were grown in the laboratory conditions. When larvae reached IV instar, they were starved to activate the vertically transmitted virus. Frequency of survived females, pupae weight, fertility (egg hatch) were significant affected by virus to compare with control group. However, differences in these parameters between the strains were not found. The prevalence of high and low virulent strains in filial generation measured as target DNA presence by qPCR was not differed. The offspring of infected parents could be considered as the main effector of observed variation in virulence. This fact indicated a complex genetic nature of virulence variation could be considered as the main effector of observed variation in virulence. Phylogenetic analyses indicated recent divergence of LdMNPV-studied isolates from a common ancestor. We found a number of nonsynonymous nucleotide substitutions and indels in many genes. However, we could not find a locus that could be considered as the main effector of observed variation in virulence. This fact indicated a complex genetic nature of virulence variation among closely related virus isolates. Variation in virulence among related LdMNPV isolates can be explained by the complex effect of different loci. The study was supported by Russian science foundation (grant # 17-46-07002).

The role of parasitic larvae and their symbiotic viruses as hidden players in plant-insect interactions

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Insect herbivores are often associated with other organisms (e.g. microbiota, parasitic invertebrates) that may play an important role in the expression of the herbivore’s phenotype and consequently on interactions of the herbivore with its host plant. Here we focused on the role played by parasitic wasp larvae and their associated polydnnaviruses (PDVs) as hidden players in plant-insect interactions. Using a dual-choice olfactometer set-up, we have shown that PDVs injected in the caterpillar affect the attraction towards the plant of other parasitoids and herbivores. To explore the molecular mechanisms underlying the ecology of plant-herbivore interactions induced by PDVs, a proteomic analysis of caterpillar’s oral secretions was performed. Indeed, oral secretions often contain elicitors that the plant can use to mount a defense response against herbivores. Results showed that PDVs affect plant-insect interactions by alter-
ing key proteins in oral secretion of infected caterpillars feeding on plants. As a consequence of alterations in oral secretions, plants display phenotypic changes which in turn affect the interaction with other insect community members.

**POSTER SESSION. Wednesday, 16:30 PV-15 STU**

**Reduced AcMNPV budded virus production facilitates generation of persistent infections in vitro**


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**Autographa californica nucleopolyhedrovirus** (AcMNPV) normally causes lethal infection in insects. However, many species harbour persistent, non-lethal virus infections throughout their life stages. This state in vivo is rare but in 2011 we generated a persistent infection in *Trichoplasia ri* (Hi5) cells with an AcMNPV n10-deletion mutant (AcULW1.lacZ) that has been in continuous culture ever since. Similar persistent infections in Hi5 cells with other AcMNPV recombinants have also been established, but the process is inconsistent, with highly variable success rates. If it could be made reproducible, persistent infections might be established with baculovirus expression vectors for the continuous synthesis of recombinant proteins. We tested the hypothesis that reducing the budded virus (BV) yield in Hi5 cells challenged with recombinant baculoviruses might render it easier to generate persistent infections by improving cell survival in the early phases. We used viruses with mutations in lef2 having impaired BV production to inoculate Hi5 cells. These also expressed dsRed or urokinase nase under late and very late promoters. All viruses initiated persistent infections within the first or second attempts. Fluorescent and flow cytometry studies suggested DsRed was produced over several passages although assays for urokinase activity proved negative. This study suggests that modulating BV output in the early stages of infection increases the opportunity for the cells to adapt to the virus. However, we also noted a 200bp insertion in cp25k within cell passages 1-3. Such mutations are often associated with increases in BV production, which raises questions about how the persistent virus infection becomes established.

**POSTER SESSION. Wednesday, 16:30 PV-16 STU**

**Constructing a model of the larval Spodoptera exigua brain to study baculoviral entry and localization**

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Alteration of host behaviour induced by parasitic infections can be caused by a broad range of organisms. Only few of these parasites manifest and alter these behavioural-changes from the central nervous system (CNS) itself, and little is known about the mechanisms behind these alterations. Neuroparasitology intertwines the existing fields of neurology, biology and parasitology – covering the cases of parasitic manipulation of the CNS. After infection by baculoviruses, infected caterpillars express hyperactivity, and/or climb the vegetation in “tree-top”-disease. These infections result in liquefaction of the caterpillars and release of virus progeny. Both behavioural alterations are thought to increase the transmission to susceptible hosts. Caterpillars are most susceptible to baculovirus infections in their earlier stages (1st – 3rd). Nevertheless, as previous studies modelling lepidopteran brains have focused on 5th instars, pupae or even adults, there is a need for developing a reliable model for the brains at earlier stages. In this project we will make a 3-dimensional model of the brain-complex of uninfected 3rd instar *Spodoptera exigua*, by immunolabeling for different rate-limiting enzymes in the biosynthesis of the major biogenic amines in the signalling of the invertebrate CNS; serotonin, dopamine and octopamine. This 3D brain-model is an essential step, as we furthermore will study the localization of Autothoga californica multiple nucleopolyhedrovirus (AcMNPV) in the brain-complex of *S. exigua* using a fluorescently tagged virus.

**POSTER SESSION. Wednesday, 16:30 PV-17**

**Rhabdovirus-free clones from a Spodoptera frugiperda (Sf21) cell population?**

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**Spodoptera frugiperda** cells have been a vital component of the baculovirus expression system (BES) based on the use of *Autographa californica* nucleopolyhedrovirus. They are used widely to isolate virus expression vectors and to produce recombinant proteins. However, recent studies have shown that both the original *S. frugiperda* (Sf21) cell line and its clonal derivative (Sf9) harbour a persistent rhabdovirus. Whether or not this virus can produce fully formed particles is unclear. Efforts in some laboratories have succeeded in curing Sf9 cells of this virus contaminant. We were interested to determine if all cells in the original Sf21 cell line supported replication of the rhabdovirus. Using a cell stock stored cryogenically since 1981 we conducted two rounds of dilution cloning in 96-well plates and isolated a number of clones using an animal product-free culture medium. These were amplified and tested subsequently for the presence of rhabdovirus sequences using reverse transcriptase-polymerase chain reaction (RT-PCR). Most of the clones were positive for rhabdovirus sequences, but two appeared to be negative with only very faint products visible in agarose gels. Further analysis of material that was pelletted from cell culture medium also failed to produce a positive result in RT-PCR. We conclude that these two cell lines may be negative for rhabdovirus sequences or contain a virus that is genetically distinct from the isolate present in Sf9 cells or other clones derived from Sf21 cells. Both cell lines support the BES and may be useful in applications involving therapeutic protein or vaccine production.

**POSTER SESSION. Wednesday, 16:30 PV-18 STU**

**Usage of highly specific indel mutations for distinguishing Cydia pomonella granulovirus isolates**

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Since the late 1980s, various isolates of the Cydia pomonella granulovirus (CpGV) have been worldwide used as insect pest control agents against codling moth (CM, *Cydia pomonella* L), a main pest in apple orchards. Commercial formulations are based on *in vivo* produced CpGV occlusion bodies (OBs) that are sprayed in aqueous suspensions on leaves and fruits, where they are ingested by CM first larval instar initiating a fast developing and fatal viral infection. Since the genetic diversity of CpGV is more and more exploited and virus isolates from different phylogenetic lineages, e.g. genome groups A, B and E, are registered, a fast and reliable detection method of different isolates is of eminent scientific and economic value. Furthermore, populations of CM are known to host potentially latent CpGV infections, challenging the propagation process of a given isolate. Recently, the isolates CpGV-M, -S and -E2 were NGS deep sequenced using reverse transcriptase-polymerase chain reaction (RT-PCR). Most of the clones were positive for rhabdovirus sequences, but two appeared to be negative with only very faint products visible in agarose gels. Further analysis of material that was pelletted from cell culture medium also failed to produce a positive result in RT-PCR. We conclude that these two cell lines may be negative for rhabdovirus sequences or contain a virus that is genetically distinct from the isolate present in Sf9 cells or other clones derived from Sf21 cells. Both cell lines support the BES and may be useful in applications involving therapeutic protein or vaccine production.
late specific and allow the rapid detection and identification of these three CpGV isolates by PCR techniques. Two different open reading frames, namely pe38 and orf47, as well as an intergenic region with specific indels mutations were chosen for this approach. Indels were not smaller or larger than 19 to 25 bp, respectively. The PCR approach was used for the identification of CpGV-M, -S and -E2 in infection treatments as well as uninfected control larvae of susceptible and resistant CM populations to detect and characterize CpGV latency and to offer a rapid tool in quality control for CpGV OB production.

confirmed the presence of a new cypovirus isolate and a betabaculovirus (Erinnys ello granulovirus, ErelGV). Phylogenetic analysis of the RdRp showed that the new cypovirus was closely related to another lepidopteron-infective cypovirus, Inachis io cypovirus 2 (ICPV-2) and according to these results we proposed the name Erinnys ello Cypovirus 2 (ErelCPV 2) for this new virus isolate. Moreover, BLASTX search using the segment 8 (S8) amino acid sequence against the NCBI non-redundant protein database revealed amino acid identity (35.82%) to a hypothetical protein of betabaculovirus, suggesting a horizontal gene transfer.

Quantification of Erinnys ello granulovirus in a biopesticide formulation.

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The hornworm, Erinnys ello Linnaeus 1758 (Lepidoptera: Sphingidae) is a widely distributed, polyphagous pest in the Americas. In Colombia it is considered a pest of rubber trees and cassava. In a previous work, the insecticidal activity of an Erinnys ello Granulovirus (ErelGV) isolate VG010 against the insect was demonstrated and this isolate was used for developing a biopesticide as a wettable powder (WP). For the manufacture and quality control of the biopesticide is necessary to quantify the active ingredient; however, the small size of the ErelGV occlusion bodies (OBs) and excipients in the formulation, do not allow to quantify virus particles by conventional methods. In this work, a sensitive tool for detection and quantitation of ErelGV in a biopesticide formulation was developed. Specific oligonucleotides and a Taqman probe were designed using the isolate VG010 granulin gene sequence. To validate the methodology, a purified viral suspension was quantified by spectrophotometry using a calibration curve, as a control for quantitative PCR. The WP was suspended in sterile water (1:50 w/v) and an aliquot of 1 mL of the sample was heat-treated and then centrifuged. The supernatant was used as template to PCR reactions. The results showed that the OBs concentration of the control viral suspension was similar that the calculated by spectrophotometry, the viral concentration in the WP (1.78 x 10^6 OBs/g) was very close to that expected in the manufacturing procedure (1.0 x 10^6 OBs/g). This methodology could be used as a tool for quality control of the biopesticide manufacturing process.

A draft of the encapsidated genome of the Cotesia flavipes Bracovirus

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The parasitoid braconidae wasp Cotesia flavipes has been introduced from Southeast Asia and is currently used extensively in sugar cane fields of Brazil to control the stemborer pest Diatraea saccharalis, a significant disruptor of sugar production. Similar to other parasitoid wasps of Lepidoptera, there is an integrated Polydnavirus within the genome of C. flavipes that allows ovary cells to produce abundant virus particles carrying the segmented bracovirus genome that will be injected with the wasps’ eggs into the hemolymph of the larval host, wherein the wasps’ larvae will grow. The encoded genes are capable of inducing immunosuppression in the hosts cells thus granting a higher survival chance of the wasps’ offspring within the hemolymph. We have sequenced the encapsidated genome of the C. flavipes Bracovirus (CfBV) using a combination of techniques that yielded long and short reads derived from rolling circle amplification and total DNA of ovary samples. The current draft genome of 31 viral segments with lengths ranging from 734 to 37388 bp yielded more than 400000 bp in total size and 290 ORFs. These sequences showed a very high synteny and identity of CfBV with the C. sesamiae kitale BV genome segments available (> 90%) and the C. vestalis BV genome (74 to 90%). An estimation based on mapped reads indicated a higher abundance of segments encoding PTP and EP genes. The CfBV genome presents a new contribution to the comparative genomics and the understanding of the complex interactions between the parasitoid wasp and its host.

Baculovirus hyper expression system for virus like particles and vaccines production in insect cells

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Baculovirus expression vector system (BEVS) is an effective and widely used method for the production of recombinant protein by referring to the high expression level of polyhedrin protein. However, the problem of producing a recombinant protein exhibits a lower expression rate than the native polyhedrin protein is considered to be a problem of the baculovirus expression system. To solve this problem, many researchers have studied the polyhedrin promoter in order to improve the efficiency of the expression system. In the present study, we used a new expression vectors were constructed by combining the transcriptional enhanced factor and promoter that improve the expression level of polyhedrin promoter. To confirm the usefulness of hyper expression vector system, the recombinant virus expressing virus like particle (VLP) was generated using them. Human papilloma virus (HPV) type 16 L1 protein have been expressed by baculovirus hyper expression system. We investigated production and immunogenic efficacy of HPV type 16 VLPs. VLPs were
produced by self-assembly of L1 proteins, self-assembled VLP was confirmed using transmission electron microscope. VLPs were purified using a Capto™ Core 700 chromatography approach. Baculovirus hyper expression system production efficiency was influenced by the VLP production. HPV type 16 VLPs vaccination to Balb/c mice induced the generation of antibody confirmed by ELISA. In addition, it was confirmed that the neutralizing antibody was formed in mouse serum treated with VLP. This study could provide improvements on the vaccine production for the development of VLP vaccines and high expression of useful foreign recombinant proteins using BEVS.

POSTER SESSION. Wednesday, 16:30 PV-23

A Study about Chimeric OBs Based on d-POLH

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The Autographa californica multiple nucleopolyhedrovirus (AcMNPV) p48 (ac103) gene has been shown to be essential for the nuclear egress of nucleocapsids and the formation of occlusion-derived virions (ODVs). However, the exact role of p48 in the morphogenesis of ODVs remains unknown. In this study, we present evidence that p48 is required for the efficient scission of inner nuclear membrane (INM) invagination structures and the subsequent formation of intranuclear microvesicles. P48 protein is associated with both the nucleocapsid and envelope fractions of budded virions (BVs) and ODVs. In virus-infected cells, P48 is predominantly localized to the nucleocapsid in the virogenic stroma and the nucleocapsids enveloped in ODVs, and this protein is also detected in the plasma membrane, nuclear membrane, intranuclear microvesicles, and ODV envelope. P48 associates with Ac93, another protein that is involved in intranuclear microvesicle formation, independent of other viral proteins. Residues N318, V319, C320, R321, and I323 of P48 not only play critical roles in the association of P48 with Ac93 but also participate in the efficient scission of INM invagination structures and the subsequent formation of intranuclear microvesicles. Thus, we hypothesized that the association between P48 and Ac93 might be involved in the scission of INM invagination structures. These data may help to reveal insights into the mechanism of baculovirus ODV morphogenesis.

POSTER SESSION. Wednesday, 16:30 PV-24

Evaluation of CRISPR/Cas9 based procedures for the edition of baculoviral genomes

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Baculoviruses are invertebrate viruses that efficiently transduce mammalian cells, remaining as non-replicative episomes. In this context, we set out to develop and evaluate recombinant AcMNPV, both in vitro and in animal models. Thus, 3 viruses were generated that express a fluorescent protein (eGFP, mCherry or BFP) under the CMV viral promoter. Through their use, transduction efficiency was optimized and measured on mammalian primary cultures, which were analysed by fluorescence microscopy and flow cytometry. Using such results, in vivo tests were then performed on rabbits with peripheral artery ischemia (PAD). Animals were injected in the ischemic muscle, measuring the expression of reporter gene by RT-qPCR and the viral DNA by qPCR, at different days post-treatment. These tests revealed an adequate performance in short times (less than 7 days). Besides, another virus was generated that expresses a mutant version of HIF gene (Hypoxia inducible factor), with the purpose of proving its usefulness in a therapy for PAD. In parallel, to lengthen the expression of transgenes in vivo, a virus containing a gene circuit that allows the release of a minicircle by Cre/LoxP technology in an inducible manner (TetON/TetOFF system), is being developed. This tool will allow a controllable and lasting expression of the gene of interest. So far, we have preliminary in vitro results, which have shown a correct functioning. In summary, evaluations carried out in vitro and in vivo using recombinant baculoviruses showed satisfactory results, which are necessary to proceed with the preclinical evaluations of gene therapy active ingredients.

POSTER SESSION. Wednesday, 16:30 PV-25

BacMam technologies in cells and animals; Advances towards the transport of DNA in mammals


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Baculoviruses are insect viruses that efficiently transduce mammalian cells, remaining as non-replicative episomes. In this context, we set out to develop and evaluate recombinant AcMNPV, both in vitro and in animal models. Thus, 3 viruses were generated that express a fluorescent protein (eGFP, mCherry or BFP) under the CMV viral promoter. Through their use, transduction efficiency was optimized and measured on mammalian primary cultures, which were analysed by fluorescence microscopy and flow cytometry. Using such results, in vivo tests were then performed on rabbits with peripheral artery ischemia (PAD). Animals were injected in the ischemic muscle, measuring the expression of reporter gene by RT-qPCR and the viral DNA by qPCR, at different days post-treatment. These tests revealed an adequate performance in short times (less than 7 days). Besides, another virus was generated that expresses a mutant version of HIF gene (Hypoxia inducible factor), with the purpose of proving its usefulness in a therapy for PAD. In parallel, to lengthen the expression of transgenes in vivo, a virus containing a gene circuit that allows the release of a minicircle by Cre/LoxP technology in an inducible manner (TetON/TetOFF system), is being developed. This tool will allow a controllable and lasting expression of the gene of interest. So far, we have preliminary in vitro results, which have shown a correct functioning. In summary, evaluations carried out in vitro and in vivo using recombinant baculoviruses showed satisfactory results, which are necessary to proceed with the preclinical evaluations of gene therapy active ingredients.
non-homologous recombination processes made it possible to facilitate genome mutagenesis. In this sense, we evaluate a combination of in vitro and in vivo procedures, based on CRISPR/Cas technology and homologous recombination to edit baculoviral genomes: firstly, to convert them into modifiable bacmids that multiply in Escherichia coli; and secondly, to remove or add genetic information from them. The methodology fine tuning and the conceptual tests were carried out on genetic constructions made for that purpose and on the genomes of AcMNPV and AgMNPV. Our results showed that the CRISPR/Cas technology would be applicable made for that purpose and on the genomes of AcMNPV and AgMNPV.

The persistent infection of PnV (Perina nuda illavirus) to its heterologous cell line, NTU-LY cell line (Lymantria xylina cell line)  
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PnV (Perina nuda illavirus) was first found to infect persistently to its heterologous cell line, NTU-LY-16 cell line, which was a subclone of Lymantria xylina cell line (NTU-LY-1 cell line). The cytopathic effect of PnV to LY-16 cells was different from that of PnV to its homologous NTU-PN-HF cells. The membrane-bound inclusion bodies (IBs) were found in newly seedling LY-16 cells. The lysed LY 16 cells formed many microsomes after 15 days post-passage, three different inclusion bodies (IBs) could be distinguished among the microsomes, except the membrane-bound, two added IBs, fibril-bound and sheath-bound IBs. After 15 days post-passage, the cytoplasm of un-lysed LY-16 cells contained zipper-like array of unmembrane-bound virogenic stroma, the parallel rows of viral particles were restricted by two membranes. These viral particles were purified and showed a typical PnV morphology by negative stain. Further examined by using RT-PCR with a primer set designed from the sequence of a putative PnV helicase gene and Western blot, Indirect Immunofluorescent assay IFA with a specific anti-P. nuda picorna-like virus (PnV) antiserum confirmed that PnV extends its in vitro host range to NTU-LY cell line. These results suggest that PnV could extends its in vitro host range to LY cell line and this persistent infection of PnV is a model for the membrane-associated replication and translation for positive sense ssRNA viruses.

Improved generation of baculovirus for biopesticide by CRISPR/Cas9 editing  
Pazmiño-Ibarra, V.1; Mengual, A.1,2,3; Targovnik, A.1,2,3; Herrero, S.1  
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The CRISPR (Clustered Regularly Interspaced Short Palindromic repeats) system associated Cas9 endonuclease is a molecular tool that enables specific sequence edition with high efficiency. The edition using CRISPR/Cas9 system has been successfully reported in small and large viral genomes. In this study, we have explored the use of CRISPR/Cas9 system for the edition of the baculovirus genome. Guide RNAs (sgRNAs) were designed to direct a cleavage of Cas9 to different loci of the AcMNPV genome. We showed that the delivering of Cas9-sgRNA ribonucleo-protein (RNP) complex into Spodoptera exigua cells through lipofection might be efficient to generate Indel mutations. To evaluate potential application of our CRISPR/Cas9 method to improve baculovirus as protein expression vector and as biopesticide, we attempted to knock-out several genes from a recombinant AcMNPV form used in the baculovirus expression system as well as in a natural occurring viral isolate from the same virus. Sequencing analysis revealed that the edition efficiency and the type of changes was variable. Depending on the targeted gene, the rate of edition ranged from 10% to 40%. We analyzed the effect of the mutations in vivo and showed, for certain mutants, improvement of baculovirus as protein expression vector and as biopesticide. This study established the first evidences about the potential of CRISPR/Cas9 edition of baculovirus that could contribute to the generation of mutants with biotechnological applications.

Identification of Dendrolimus punctatus cypovirus (DpCPV) viral attachment proteins and its ligands in the host midguts  
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Dendrolimus punctatus cypovirus (DpCPV) is an important viral pathogen of Dendrolimus punctatus walker, which is considered as the most destructive pest in pine forest worldwide. In China, this virus species has been exploited as a commercial insecticide to control the pine caterpillar since 2010, not only because DpCPV has a relative wide host range infecting 35 insect species spanning 10 Lepidoptera families but also that DpCPV infection is mostly restricted to the midgut epithelial cells and therefore can be maintained in hosts for a long time. Although the completed sequences of DpCPV genomic fragments have been determined, little is known about the functions of DpCPV encoding proteins and the molecular mechanisms of CPV infection since lacking of a reliable cell
culture system. Based on the consensus that spike proteins of viruses perform essential functions in receptor binding and cell penetration, in the present study, the turreted proteins of DpCPV (VP3, VP4 and VP5) were selected for investigation, in which VP3 was truncated into GTase (1-386 aa) and MTase (406-1058 aa) fragments according to its structure. The results showed that MTase and VP4 functioned as the viral attachment proteins during the cell entry of DpCPV and Bombyx mori alkaline phosphatase protein (ALP) served as the MTase ligand. These results aug-
ment our understanding of the mechanisms used by cypoviruses to enter their hosts.

**POSTER SESSION. Wednesday, 16:30 PV-31**

The deficiency in nuclear localization signal of Neodiprion lecontei nucleopolyhedrovirus DNA polymerase prevents rescue of viral DNA replication and virus production in *dnaPol*-null Autographa california nucleopolyhedrovirus (AcMNPV) bacmid. Several key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, China

Corresponding author: DNA polymerase (DNApol) is highly conserved in baculovirus and is required for viral DNA replication. However, little is known about gammabaculovirus DNApol. Here DNApol of the gammabaculovirus Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) was cloned into a *dnaPol*-null alphabaculovirus AcMNPV bacmid, creating Bac-GFP-AcDNApol-NlPol. The resulting recombinant bacmid did not spread to neighboring cells, virus growth curve and real-time PCR revealed that NeleNPV *dnaPol* substitution did not rescue AcMNPV DNA replication and virus production. Immunofluorescence microscopy revealed that NeleNPV DNApol was expressed but could not localize to the nucleus. Subsequently NeleNPV DNApol was fused to SpfNPV DNApol nuclear localization signal (NLS) and the fused DNApol could import into nucleus. The NLS-fusing NeleNPV DNApol was further transposed into the *dnaPol*-null AcMNPV bacmid, creating Bac-GFP-AcDNApol-HA:NlPol. The recombinant virus could replicate and produce infectious virus in Sf9 cells, albeit at reduced levels compared to wild type AcMNPV. Taken together, our results suggested that the NLS deficiency of NeleNPV DNApol blocked viral DNA replication and production of infectious virus in *dnaPol*-null AcMNPV bacmid.

**POSTER SESSION. Wednesday, 16:30 PV-32**

Interaction between Autographa california nucleopolyhedrovirus (AcNPV) ME53 and VP80

Özşahin, Emine1; Nagy, Eva2; Doucet, Daniel3; and Krell, Peter J.1
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ME53 and VP80 are conserved lepidopteran baculovirus proteins. Although ME53 translocates to the nucleus late in infection, it lacks a nuclear localization sequence. However we mapped an ME53 nuclear translocation sequence. ME53 and VP80 are conserved lepidopteran baculovirus proteins. Although ME53 translocates to the nucleus late in infection, it lacks a nuclear localization sequence. However, little is known about gammabaculovirus DNApol. Here DNApol of the gammabaculovirus Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) was cloned into a *dnaPol*-null alphabaculovirus AcMNPV bacmid, creating Bac-GFP-AcDNApol-NlPol. The resulting recombinant bacmid did not spread to neighboring cells, virus growth curve and real-time PCR revealed that NeleNPV *dnaPol* substitution did not rescue AcMNPV DNA replication and virus production. Immunofluorescence microscopy revealed that NeleNPV DNApol was expressed but could not localize to the nucleus. Subsequently NeleNPV DNApol was fused to SpfNPV DNApol nuclear localization signal (NLS) and the fused DNApol could import into nucleus. The NLS-fusing NeleNPV DNApol was further transposed into the *dnaPol*-null AcMNPV bacmid, creating Bac-GFP-AcDNApol-HA:NlPol. The recombinant virus could replicate and produce infectious virus in Sf9 cells, albeit at reduced levels compared to wild type AcMNPV. Taken together, our results suggested that the NLS deficiency of NeleNPV DNApol blocked viral DNA replication and production of infectious virus in *dnaPol*-null AcMNPV bacmid.
envenomation and topical fungal infection (Beauveria bassiana) using 16S rRNA sequencing, an analysis of cultivable bacteria and a qPCR analysis of immunity- and stress-related genes. Envenomation led to a predominance shift from enterococci to enterobacteria, an increase in CFUs and the upregulation of AMPs in wax moth midguts. Furthermore, mycosis nonsignificantly increased the abundance of enterobacteria and the expression of AMPs in the midgut. Combined treatment led to an increase in the abundance of Serratia and a greater upregulation of gloverin. Interestingly, that oral administration of predominant bacteria (Enterococcus faecalis, Enterobacter sp. and Serratia marcescens) to wax moth larvae synergistically increased fungal susceptibility. Thus, the activation of midgut immunity might prevent the systemic bacterioses in envenomated larvae, thus permitting the development of parasitoid larvae and entomopathogenic fungi. Moreover, changes in the midgut bacterial community may promote fungal killing.

**POSTER SESSION. Wednesday, 16:30 PMI-3 STU**

**Development of encapsulation techniques for plant extracts as seed treatments to reduce bird damage in agriculture**

Lemke, A.1; Dürger, J.2; Esther, A.2; Diehm, M.2; Neuberger, K.2; Tölcher, R.4; Patel, A.2

1 Bielefeld University of Applied Sciences, WG Fermentation and Formulation of Biologicals and Chemicals, Faculty of Engineering Sciences and Mathematics, Germany
2 Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für Pflanzenschutz in Gartenbau und Forst - Wirbeltierforschung, Münster, Germany
2 Phytosanitary analysis of immunity- and stress-related genes. Envenomation led to a predominance shift from enterococci to enterobacteria, an increase in CFUs and the upregulation of AMPs in wax moth midguts. Furthermore, mycosis nonsignificantly increased the abundance of enterobacteria and the expression of AMPs in the midgut. Combined treatment led to an increase in the abundance of Serratia and a greater upregulation of gloverin. Interestingly, that oral administration of predominant bacteria (Enterococcus faecalis, Enterobacter sp. and Serratia marcescens) to wax moth larvae synergistically increased fungal susceptibility. Thus, the activation of midgut immunity might prevent the systemic bacterioses in envenomated larvae, thus permitting the development of parasitoid larvae and entomopathogenic fungi. Moreover, changes in the midgut bacterial community may promote fungal killing.

**POSTER SESSION. Wednesday, 16:30 PMI-4 STU**

**Slow release of semiochemicals in push-pull-kill strategies for biological psyllid pest control**

Muskat, L.2; Humbert, P.1; Gross, J.1; Görg, L.1; Dippel, C.2; Schulke, J.3; Patel, A.V.1

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Psyllid pests are distributed all over the world and cause damage in crop plants. 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. Volatile organic compounds released by attractive and repellent apple trees offer innovative options for the control of C. picta.

The aim of this research was the development of a hydrophobic gel formulation for the controlled and long-term release of recently identified semiochemicals. The VOC release properties of the formulation were analyzed by GC-FID. The attractive and repellent effects on C. picta were validated in olfactometer experiments. A gel formulation was developed that releases the attractant β-caryophyllene for 96 hours at 20-25°C and 40 – 60 % r.h. By modifications of the gel microstructures, the release was prolonged to 360 hours with the formulation still containing 10 % of the initial loading. After an initial burst release, the structure-modified formulation showed a constant release of 1.42 μg/min (±0.0957; n=25) for the first 144 hours. On-going experiments are using this formulation to identify the attractive concentration range for C. picta. The developed slow-release formulations are evaluated in field trials 2019. Promising attractive formulations will be combined with novel formulations of repellent semiochemicals and of the host-specific Pandora strain ARSEF13372 in order to implement a push-pull-kill strategy for psyllid pest control.

**POSTER SESSION. Wednesday, 16:30 PMI-5**

**Ectoparasites of some passerine birds**

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To detect ectoparasites potential vectors and reservoirs of bird’s pathogens, arthropods are researched and collected on different bird nests

**POSTER SESSION. Wednesday, 16:30 PMI-6**

**What is a ‘relevant metabolite’? A critical examination of the assessment in the EU of potential toxin production by microbial control agents**

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Recent studies indicate that the uptake of antagonistic and entomopathogenic microorganisms (MCAs) for use in biological control...
of pests and diseases has been slower in the EU than in other countries and that strict regulatory conditions contribute to this. A special feature of the data requirements (DRs) for authorisation of MCAs in the EU is the concept ‘relevant metabolite’. We have critically investigated the history behind this term in the regulatory framework, and whether its use might have contributed to unnecessarily complicated evaluations. We find that one problematic aspect is that the term cannot be properly defined for compounds produced by MCAs. It has its origin in the DRs and toxicological risk assessments of chemical pesticides and their degradation products/metabolites, which may have comparable toxicity to the parent chemical.

Another concern is that the use of the term can be misleading since it promotes the idea that microorganisms can be evaluated according to the same criteria as for chemicals, without sufficient attention to the biological nature of MCAs. This can be a substantial concern, since many regulatory experts in the EU have a background in toxicology or ecotoxicology, and not biology or ecology. Our main conclusion is that the assessment of potential production of toxic compounds using the ‘relevant metabolite’ concept has contributed to the slow implementation of MCAs in the EU.

To mitigate the situation, new DRs and evaluation principles that have a stronger basis in the biological and ecological properties and hazards of microorganisms should be developed.

POSTER SESSION. Wednesday, 16:30 PMI-7

Bioactive compounds of Trichoderma spp.: A multifunctional tool for pest management.

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The effect of the volatile organic compounds (VOCs) emitted by Trichoderma spp. on the simultaneous management of plant pathogens and insect pests has been only rarely addressed. The aims of the present research were to evaluate the effect of the VOCs emitted by 15 different strains of Trichoderma spp. on (1) in vitro radial growth of Fusarium oxysporum f. sp. Lycopersici (FoL59), (2) the severity of the disease in planta, (3) the oviposition rate of the greenhouse whitefly Trialeurodes vaporariorum, and (4) on the growth promotion of tomato plants via volatile organic compounds. For the in vitro experiment, one Petri plate with Trichoderma grown on PDA was placed over a FoL59 and the pathogen radial growth was calculated with image j. The same strains were assessed in planta by exposing the roots of tomato seedling to the VOCs emitted by Trichoderma spp in a sealed environment for 21 days. The progress of the disease and the severity were assessed. Finally, the seventh strains that showed a over 30% of disease control were evaluated for insect oviposition management, by allowing a 48 hours interaction between the plant roots and the fungal VOCs, after that, 20 couples of T. vaporariorum were confined in a clip cage and let to lay eggs during 48 hours. The number of eggs per treatment was registered.

We expect that fungal VOCs from Trichoderma spp. induce the defense responses against pathogens and simultaneously modulate the insect oviposition behavior via signals of volatile organic compounds.

POSTER SESSION. Wednesday, 16:30 PMI-9 STU

Mutations in the Voltage Gated Sodium Channel gene associated with deltamethrin resistance in the predatory mite Phytoseiulus persimilis

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The implementation of Integrated Pest Management (IPM) in current agricultural practice is a convenient and very effective strategy to maintain pest populations under control. The use of Biological Control Agents, like Phytoseiulus persimilis, is key for the success of such approach. This predatory mite is widely used since it is very effective for controlling the two spotted spider mite (Tetranychus urticae), one of the most devastating pests worldwide. However, there are times where outbreaks of additional pests occur, and it is necessary to use conventional pesticides as deltamethrin to save the crop. In these times, the combination of Biological Control Agents with selective resistance to certain pesticides can be a timely solution to maintain control of outbreaks, effectively reducing the input of conventional pesticides to the crop.

We identified mutations located in the voltage gated sodium channel (VGSC), the main target of pyrethroids, that correlate with the deltamethrin resistance phenotypes observed in commercial populations of P. persimilis. These mutations are located in a particular region of the channel protein, previously proposed as the binding site for this family of pesticides. Our results suggest it is possible to integrate the use of P. persimilis with pyrethroid applications in an IPM context to reduce the impact of damaging pests while protecting the Biological Control Agents.

POSTER SESSION. Wednesday, 16:30 PMI-8

Assessment of developmental abnormalities and lethality in zebrafish embryos after exposure to the bioinsecticide Pea Albumin 1 subunit b (PA1b)

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PA1b (Pea Albumin 1 subunit b) is a peptide extract from pea seeds (Pisum sativum) and more generally from legume seeds that adopts a cys-teine knot fold. Its insecticidal activity against major pests in stored crops such as cereal weevils (Sitophilus sp.), mosquitoes and certain species of aphids makes it a promising bioinsecticide.

Here, we test the impact of PA1b on Danio rerio (zebrafish) embryos, to evaluate developmental toxicity. Different concentrations of PA1b were used to assess developmental abnormalities and lethality of zebrafish embryos.

Exposure to PA1b at concentrations 200, 120, 90 and 60 µg/ml showed no deformities in zebrafish embryos with viability ranging between 90% and 100% for all exposed embryos through 96h. These results show that PA1b is not toxic and does not induce developmental abnormalities in vertebrates.
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CONTRIBUTED PAPERS. Wednesday, 18:00 MC-25

**Metarhizium brunneum** F52 microsclerotia formulation for the management of the annual bluegrass weevil: compatibility with fungicides and efficacy alone and combined with imidacloprid and hydrogel

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The annual bluegrass weevil (ABW), *Lirronotus maculicollis*, is a serious pest of short-mown turfgrass on golf courses in eastern North America with severe insecticide resistance issues. While products based on the conidial spores of entomopathogenic fungi have thus far given unreliable control of ABW adults and larvae in the field, the use of fungal microsclerotia may improve economy and efficacy of fungus-based products. We studied a microsclerotia-based formulation of *Metarhizium brunneum* F52 for ABW management. Given the intensive use of fungicides on golf course turf, we also tested the compatibility of the formulation with commonly used turfgrass fungicides. Under laboratory conditions, chlorothalonil did not inhibit fungal growth; iprodione showed slight inhibitory effects at higher concentrations; propiconazole, propiconazole + trifloxystrobin, and metconazole + pyraclostrobin strongly inhibited fungal growth except at the lowest concentration of 1 mg/L. Under greenhouse conditions in pots with creeping bentgrass, only propiconazole but not chlorothalonil or iprodione suppressed *M. brunneum* F52 growth. Spores produced from *M. brunneum* F52 microsclerotia granules caused high mortality of ABW adults in Petri dishes in the lab. But in the greenhouse in pots with creeping bentgrass, the fungus had no significant effect on adults and larvae. In the field, the fungus alone did not significantly affect ABW larval populations but provided additive ABW control of up to 64% when combined with the neonicotinoid imidacloprid in simultaneous applications for white grub control. Application of hydrogel granules to stabilize soil moisture did not affect the efficacy of any treatments.

CONTRIBUTED PAPERS. Wednesday, 18:15 MC-26

**Influence of orchard age on the efficacy of a granulovirus: architecture trumps biochemistry**


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Higher levels of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), falsecodling moth, have been recorded in juvenile than mature citrus orchards. Reasons for this were analysed during a 3-year study and were found to be diverse. Here we focus on the role that the Cryptoplebia leucotreta granulovirus (CrleGV) plays in these differences in pest levels. CrleGV occurs naturally in populations of *T. leucotreta* in citrus orchards and is also often applied as a biopesticide. Despite the same pest management programme being followed, a significantly higher proportion of larvae retrieved from mature orchards (7%) were infected with CrleGV than larvae retrieved from juvenile orchards (4%). Both biochemical and architectural reasons for this difference were investigated. Susceptibility of larvae to CrleGV was significantly higher when reared on diet supplemented with fruit from juvenile orchards compared to fruit from mature orchards (61% and 45% mortality). This may relate to the higher ash content in the juvenile tree fruit (3.32% and 0.26%). Clearly, this did not explain the differences in pest and CrleGV levels. In a separate study, persistence and consequently efficacy of applied CrleGV biopesticide was compared under conditions of higher and lower exposure to ultraviolet (UV) radiation from natural sunlight. This represented the denser and sparser architecture of mature and juvenile trees, respectively, and thus the greater and lesser protection against UV-degradation. Significant differences in the rate of virus degradation were recorded at various intervals after application. Tree architecture was thus more important for virus efficacy than was fruit biochemistry.

CONTRIBUTED PAPERS. Wednesday, 18:30 MC-27

**Field testing of two different formulations of Beauveria brongniartii for control of white grubs of Melolontha melolontha in apple orchards**

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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 strategies 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 the biomass in a fluid-bed on millet as carrier. In a field trial a liquid and a granule based formulation was applied in spring of 2017, 2018 and 2019. MelocontTM was applied as positive control. In 2017 soil samples were analysed before, 35 and 196 days after application. Because of the extreme drought in 2018 no soil samples were analysed. In 2019 soil samples were taken before third application for analysing the MPN. The white grub infestation was analysed in 2019. 35 days after the first application in 2017 no difference in the most probable number (MPN) of *B. brongniartii* and the number of infected larvae of *G. mellonella* was assessed. After 196 days after application the MPN and the number of infected larvae of *G. mellonella* was assessed. 196 days after application the MPN was significantly higher in all treatments whereas the best results were obtained with the liquid formulation and Melocont™. These results correspond with the bioassays with *G. mellonella*. Results of 2019 on MPNs, bioassays and the occurrence of white grubs in the field will be presented and discussed.

CONTRIBUTED PAPERS. Wednesday, 18:45 MC-28

**Effectiveness of a Beauveria bassiana formulation, Biolisa Madara, against Pine Wilt Disease after annual application for three years**

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Pine wilt disease is the most serious forest disease in Japan. The wilt is caused by the pine wilt nematode (*Bursaphelenchus xylophilus*) carried by *Monochamus alternatus* (Cerambycidae). The wilt occurs in...
autumn. Generally, chemical controls are conducted on this disease in Japan. Fumigation is conducted in winter to control next generation of the beetle, and other non-chemical methods are expected. Biolisa Madara is a fungal formulation. Conidia of Beauveria bassiana are covering on the both sides of a 2.5′50 cm tape made of non-woven fabric. To kill the next generation adult beetles, dead trees are chopped into about 2m and piled up. The top of the piles are stapled Biolisa Madara, and then covered by biodegradable plastic sheet. This operation is conducted in winter. New emerged adults in early summer would encounter the fungus and killed under the sheets. We tried this application annually for three years and compared with the control effects from the fumigation in both serious and minor damaged coastal pine forests (Pinus thunbergii) in Ibaraki prefecture, Honshu. In the serious damaged forest, mortality rate of pine trees conducted by both Biolisa Madara and fumigation were decreased from 2016 to 2018. In the minor damaged forest, the rate was kept lower. The transmission coefficient by Biolisa Madara was also equal to or lower compared with the control effects from the fumigation in both serious and minor damaged forest. These results suggest that Biolisa Madara has equal control effect to the fumigation.

CONTRIBUTED PAPERS. Wednesday, 19:00 MC-29 STU

Development of a formulation to control psyllid pests in fruit orchards with the entomopathogenic fungus Pandora sp. Muskat, L.; Humbert, P.; Niehaus, K.; Ellenberg, J.; Patel, A. V.

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Psyllid pests are distributed all over the world and cause damage in various crop plants. Psyllid pathogenic fungi from the Entomophthorales bear high potential for psyllid pest control because of their high host specificity and fast speed-to-kill. This is why we set out to develop an effective kill-formulation which can be applied within attract-and-kill strategies against psyllid pests in fruit orchards. It is known that fungi of the Entomophthorales sequentially excrete hydrolytic enzymes in order to escape from the insect hemolymph through the cuticle and to sporulate from the insect cadaver surface. The aim of this study was to mimic the insect host cadaver by addition of chitin and its derivatives to the fungal formulation in order to improve fungal growth and sporulation. The number of discharged conidia was increased 1,89-fold by chitin and 1,67-fold by addition of a complex nitrogen source. Maximal numbers of discharged conidia were observed by combinations thereof (2,75-fold in comparison with the control). The effect of chitin and other nutrients on the chitinolytic and proteolytic enzyme activity of the fungus was analyzed by enzyme assays and by HPLC and GC-MS coupled with microscopic observations of the degradation within the biopolymer beads. In future work, it will be investigated how the chitin derivatives improve virulence of Pandora sp. against the target insect Cacopsylla picta. Additionally, it will be investigated how biobased superabsorbents improve rehydration of the capsule in day-night cycles. This work will pave the way for above-ground application of Entomophthorales.

CONTRIBUTED PAPERS. Wednesday, 19:15 MC-30


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Cutworms can cause significant damage to newly forming buds in vineyards in spring, and currently a single pesticide is available to reduce cutworm numbers in Canada. Previous work by Lowery and Delury (unpublished) has identified two native isolates of Beauveria bassiana which are effective at low temperature against the species responsible for the largest proportion of damage in the Okanagan Valley, Noctua c1mes and Bagatrotis orbit. Subsequent work at the Institute for Sustainable Horticulture has screened the Okanagan isolates and several native coastal B. bassiana isolates for efficacy against other lepidopteran pests in addition to the two cutworm species. At this time, 5 isolates of B. bassiana appear to have high potential as new biocontrol products. Entomopathogenic nematodes are a second biocontrol tool with potential for managing grape cutworm as they attack larvae in the soil. This project is developing an Integrated Pest Management strategy based on these two biological tools. Results from the first year of this project will be presented. Lab bioassays completed with three nematode species, Heterorhabditis bacteriophora, Steinernema carpocapsae, and Steinernema Feltiae and five B. bassiana isolates; 3 from South Coastal BC and 2 from the colder interior Okanagan have been completed. Preliminary results indicate that nematode, S. Feltiae and one of the Okanagan B. bassiana isolates are most efficacious for the control of both species at 15 - 17°C. The most promising nematode species and fungal strains are being examined individually and in combination in potted grape trials at different temperatures.

CONTRIBUTED PAPERS. Wednesday, 19:30 MC-31

The California experience: promoting microbial control through effective outreach Dara, S.K.

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Although microbial control has been in practice for several decades, it is not seen as a mainstream control option, especially in conventional agriculture due to a number of factors, including concerns for efficacy and higher costs compared to chemical control. The $40 billion agriculture industry in California offers an excellent opportunity for improving integrated pest management practices with microbial control and promoting sustainable agricultural practices in both organic and conventional production systems. Developing sound microbial control strategies and effective outreach are key players for promoting microbial control. A few points from the California experiences include developing research data from laboratory, greenhouse, and field studies and disseminating the information through extension events, trade journal articles, client interactions, news and social media.
of the infection. To examine the primary phase of the infection, newly molted 5th instar T. ni larvae were orally inoculated with AcMNPV OBs, and transcription profiles from infected midgut tissue were compared with those from mock infected larvae. AcMNPV gene expression profiles in the midgut were remarkably similar to those observed from infections of the T. ni cell line, Tnms42. However, substantial differences were observed in genes associated with high-level BV production (lp-25k), acceleration of systemic infection (v-1gf), and enhancement of viral movement (anril-1/orfl20), suggesting specific adaptations of viral gene expression in the polarized midgut cells. We also analyzed T. ni transcriptional responses to AcMNPV infection. The numbers of differentially expressed T. ni genes increased as the midgut infection progressed, and we identified a total of 3,372 differentially expressed T. ni transcripts. Genes encoding HMG176, Atlastin, and CPH43 were among the most dramatically upregulated in response to AcMNPV infection. Also, a number of cytochrome P450 genes were downregulated in response to infection. We also analyzed the effects of AcMNPV midgut infection on T. ni genes associated with innate immunity. Detailed knowledge of host midgut responses to baculovirus infection during the primary phase of the infection will be important for understanding how baculoviruses establish productive infection in the midgut and how the infection progresses to the secondary systemic phase.

CONTRIBUTED PAPERS. Wednesday, 18:15 V-42 STU

Neuropeptide Expression in Spodoptera exigua after baculovirus infection. A focus on Proctolin and its relevance in locomotion and digestion.

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Baculoviruses constitute a large group of invertebrate DNA viruses, mainly infecting insects from the order Lepidoptera. It has been demonstrated that during a baculoviral infection, the virus can enter and replicate into the central nervous system cells, but it remains unknown whether this tissue tropism is important for the host behavioural changes that are commonly observed following virus infection. The larval neuropeptidergic system is composed of small protein-like molecules functioning as neurohormones, neurotransmitters or neuromodulators. These peptides are involved in regulating animal physiology and behaviour and could be targeted by baculoviruses in order to achieve host behavioural manipulations leading to increased viral fitness and/or dispersion. In this work, we have studied the effect of a Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) infection on the neuropeptide gene expression of Spodoptera exigua larval brains. Expression of the gene encoding the neuropeptide proctolin, was severely downregulated following infection and was chosen for further analysis. A recombinant Autographa californica multiple nucleopolyhedrovirus (AcMNPV) overexpressing the S. exigua proctolin gene was generated and used in bioassays using S. exigua larvae to study its influence on the viral infection. AcMNPV-proctolin infected larvae showed less locomotion activity and suffered a loss of weight compared to larvae infected with wild type AcMNPV or mock ones. In addition, proctolin expression seemed to increase the pathogenicity and virulence of the virus at low doses. These results provide additional information on the role of proctolin during a baculovirus infection, affecting the viral replication and dispersion.

CONTRIBUTED PAPERS. Wednesday, 18:45 V-44 STU

Transcriptome analysis of Deformed wing virus-infected bumble bees (Bombus terrestris)

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The last decades have seen many reports of the decline of many wild bee species. While a number of factors are thought to be involved in these declines, an increase in the prevalence of viral pathogens is a potentially important cause. A major group of wild insect pollinators is the bumble bees (genus Bombus), comprising ca. 250 species worldwide. The full genome of Bombus terrestris is now available, allowing us to perform broader transcriptomic analyses on this species. By experimentally infecting individuals with the +ssRNA virus Deformed wing virus (DWV), we aimed at highlighting immune pathways triggered by viruses in Bombus terrestris. In a cage experiment, groups of 5 individuals of Bombus terrestris workers were subjected to four treatments: injected with DWV, fed with DWV, injected with buffer, and control (unmanipulated). While many studies in honey bees use injections, mimicking viral transmission through feeding on honey bees by parasitic Varroa destructor mites, the natural transmission route for Bombus species is likely per os, through feeding on contaminated flowers. Ten days after our experimental treatment, total RNA of these 20 individuals were extracted and sent for Illumina next-generation sequencing. Here we explore differences in gene expression between the treatment groups to help identifying genes of importance in the response of bumble bees to viruses. We hypothesise that these genes might be under selection in wild Bombus species because of the increased prevalence of RNA viruses in honey bees and their spillover into wild bee species.

CONTRIBUTED PAPERS. Wednesday, 19:00 V-45

Isolation of ferritin and its response to BmNPV infection in silk-worm, Bombyx mori

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Ferritin is a ubiquitous iron storage protein that plays an important role in host defence against pathogen infections. In the present study, native ferritin was isolated from the hemolymph of Bombyx mori using native-polyacrylamide gel electrophoresis (native-PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results revealed that ferritin consisted of two subunits, designated as Bm-FerHCH and BmFerLCH. Previously integrated previous transcriptome and iTRAQ data showed that the two subunits were down-regulated in resistant silkworm strain BC9 and there was no obvious change in the expression levels of the subunits in susceptible silkworm strain P50 after BmNPV infection. Virus overlay assays revealed that B. mori ferritin as the form of heteropolymer had an interaction with B. mori nucleopolyhedrovirus (BmNPV), but it can’t interact with BmNPV after depolymerisation. What's more, reverse transcription quantitative PCR (RT-qPCR) analysis suggested that BmFerHCH and BmFerLCH could be induced by bacteria, virus and iron. This is the first study to extract B. mori ferritin successfully and confirms their roles in the process of BmNPV infection. All these results will lay a foundation for further research the function of B. mori ferritin.

CONTRIBUTED PAPERS. Wednesday, 19:15 V-46

Transcriptome analysis of Cydia pomonella granulovirus (CpGV) in codling moth (Cydia pomonella L.) larvae

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Cydia pomonella granulovirus (CpGV, family Baculoviridae) has been used as an effective and environment-friendly biological control agent for codling moth (Cydia pomonella L.), which is a serious global pest on pome fruit and a key invasive pest in apple production in China. CpGV-M isolate, the so-called “Mexican isolate”, was wildly used in mainly organic apple plantations in Europe for several decades. However, the detailed picture of CpGV gene expression or even granulovirus gene expression in vivo is still unclear. To gain a CpGV gene expression profile, RNA sequencing using HiSeq-Illumina strategy was performed on 3rd-instar larvae of C. pomonella strain (CpS) challenged with CpGV-M isolate. In this study, we analyzed the temporal patterns of CpGV mRNA levels in CpS larvae at various times following virus infection. Data analyses revealed that the transcript levels of viral genes increased from 48 hours post infection (hpi), revealing very similar gene expression patterns in samples of C. pomonella larvae at various times following virus infection. Our results have revealed direct as well as indirect effects of covert infections with the Spodoptera exigua flavivirus 1 that could contribute to reduce the pest abundence in field conditions.

CONTRIBUTED PAPERS. Wednesday, 19:30 V-47

Ecological implications of covert infections with RNA viruses in the beet armyworm, Spodoptera exigua

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Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that were not able to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In previous works, we discovered three RNA viruses producing covert infections on laboratory as well as field populations of the beet armyworm, Spodoptera exigua. Interestingly, the viral incidence and abundance in field-collected insects were much lower than in laboratory samples, suggesting that covert infections with these viruses could have a negative impact on this pest in the nature. In the context of the EU-funded research project VIROPLANT we aim to determine the direct and indirect effect of these viruses on the ecology of S. exigua in order to explore its potential to contribute to a substantial reduction of the targeted populations. Impact of infections with the different viruses and viral loads on parameters such as developmental time, survival to pupae, fertility and fecundity have been studies. In addition, the effects of these viral infections on the insect susceptibility to other viruses (nucleopolyhedrovirus), bacterial pathogens (Bacillus thuringiensis) or nematodes (Steinernema carpocapsae) have also been evaluated. Our results have revealed direct as well as indirect effects of covert infections with the Spodoptera exigua flavivirus 1 that could contribute to reduce the pest abundence in field conditions.
Development and use of a barrier system for reducing invertebrate damage in crops

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A patented system using a physical barrier was developed that prevents the migration of invertebrate pests such as slugs, snails and vine weevil into crops. Data from laboratory, glasshouse and field trials will be presented to illustrate the efficacy of the barrier design. The barrier demonstrated high efficacy in pilot trials, which has led to a commercial domestic garden deployable system (Molukski). Discussion of how best to implement the barrier and potential improvements to the design will be presented.

Sluxagon: A pesticide-free, paintable slug fence

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The invasive “Spanish” slug Arion vulgaris is widely spread in Europe and has a voracious nature. We developed a pesticide-free combination of wood oils and tensides that dries to a surface with non-stick properties against all kinds of terrestrial slugs and snails (which we could test so far). It works as a paintable slug fence which is painted on a vertical barrier made of e.g., wood, metal, plastic or stone with a height of at least 20 cm and left to dry for 24 hours. After that, the slug’s mucus will interact with the imperfectly bound tensides in the lacquer matrix, causing it to fail its adhesion. Limited areas may thus be effectively protected by Sluxagon for up to 5 weeks or longer, depending on the amount of rainfalls. The product has been patented and is available in Germany, Austria, Switzerland, France and Luxemburg so far.

LIMACAPT: a self-powered connected sensor for monitoring slugs

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LIMACAPT is a sensor that can automatically count slugs in fields. Images are acquired during the night during slug activity, using a camera and infrared lighting. The computer embedded into the device runs an algorithm which processes several hundred images taken each night. The user receives the results of this data analysis sent by means of a low rate smartphone. The system works with a low error rate, approximately 5%, without any need for user intervention and enables the recognition and identification in the fields of objects appearing and disappearing from the frame without counting the same slug several times. LIMACAPT is a tool in precision farming which detects the present slug populations early and daily, to enable farmers to effectively deal with this pest as soon as the risk becomes apparent, for reasoned interventions. LIMACAPT opens up new scientific perspectives in terms of modelling for better insight into the pest.

Optimising snail and slug management in Australian crops: linking mollusc activity with climate using time-lapse cameras

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Several exotic snail and slug species of European-Mediterranean origin have established in Australia, and become significant pests of grain crops. Both taxa cause economic losses through feeding damage to establishing seedlings and field control costs, while snails also contaminate harvested grain resulting in grain rejection or value loss, grain cleaning costs and a serious threat to market access. Management practices to complement baiting, including physical controls implemented between cropping seasons (stubble burning, cableing, bashing) and alterations to harvest machinery (sieves, dislodger bars), have become less practical for farmers under modern reduced-disturbance and increased-throughput farming systems, creating a more favourable habitat for molluscs and reducing control opportunities. Our recent work highlighted variable control achieved by molluscicide baits against pest snails under field conditions, reflecting complex behavioural and/or physiological responses mediated by environmental conditions. To better understand these effects, we investigated mollusc activity and seasonal phenology in a multi-year study across eight Australian locations. Monthly-collected snails and slugs were examined to determine body moisture content and reproductive development. Continuous video footage of snail and slug activity was collected using fixed time-lapse cameras along with microclimate variables, and analysed using a newly-developed computer vision system to detect and track movement of the target species in video frames. Movement data were analysed with respect to micro-climatic variables to identify environmental triggers for mollusc movement and the optimal timing to implement baiting programmes. We present an overview of current and emerging research directions for mollusc control in Australia, including potential visual sensing applications for snails.

The development of iron-III-phosphate as an integrated slug control method in UK agriculture

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The inability to control slugs in oilseed rape and wheat crops costs the UK agricultural industry some £43.5 million per year. Since it was first launched in 2005 Iron (III) phosphate (FePO4) is now the primary active ingredient used by UK growers for arable mollusc management. Iron (III) phosphate is naturally occurring and present in the soil complex. However, when formulated as an ingestible bait for slugs, it functions as a stomach poison interfering with the calcium metabolism, resorption and digestion of food in the gut. Unlike the previous generation of baits, such as metaldehyde or methiocarb based pellets, dead slugs are not often seen on the soil surface as they will often seek refuge and die in the soil profile, requiring education for the end-user. Proving this concept has been one of the biggest challenges in the UK market. Ongoing studies looking at slug behaviour once Iron (III) phosphate has been consumed have shown efficacy to be at least on par with historical standards providing acceptable crop protection. This paper reviews our experiences and challenges of developing control strategies with a novel molluscicide. We also discuss

CONTRIBUTED PAPERS. Wednesday, 18:00 SS-9

CONTRIBUTED PAPERS. Wednesday, 18:15 SS-10 STU

CONTRIBUTED PAPERS. Wednesday, 18:30 SS-11

CONTRIBUTED PAPERS. Wednesday, 19:00 SS-13
the potential of our latest research into biorational mollusc management, using naturally occurring compounds that function as repellents.

**CONTRIBUTED PAPERS. Wednesday, 19:15 SS-14 STU**

**Can we make changes to slug pellets to improve their efficiency?**

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Gastropod damage to crop plants has a significant economic impact on agriculture and horticultural industries worldwide, with the grey field slug (Deroceras reticulatum (Müller)) considered as the main mollusc pest in the United Kingdom. The prevailing form of crop protection are pellets containing the active ingredient, metaldehyde. Metaldehyde is known to cause paralysis and ultimately death after threshold amounts are ingested. The paralysing effects were suggested to result in inadequate pellet consumption; greater understanding of the interaction between consumption and the paralysing effects of metaldehyde could reveal an area of potential manipulation to be targeted by novel molluscicide formulations. An audio sensor was used to record individual slugs feeding on a variety of pellet types, including commercially available pellets and novel metaldehyde formulations. A graphical.mflapp application was used to quantify the time each bite was taken; the length of each bite and the total number of bites. There was significant individual variation in number of bites on a non-toxic pellet but this was not observed on metaldehyde formulations. Slugs took significantly fewer bites from metaldehyde pellets than from non-toxic pellets. There was no significant difference in the length of the bites between non-toxic and metaldehyde pellets.

**CONTRIBUTED PAPERS. Wednesday, 19:30 SS-15 STU**

**Do molluscicidal control measures allowed in Lithuania kill invasive slugs?**

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The idea behind this study came from frequent Lithuanian complaints that molluscicides do not help. The aim of the present research was to study the effect of commercially available measures in Lithuania against the invasive slug Arion vulgaris (syn. A. lusitanicus). We studied: Will products against pest slugs stop their consumption of food and kill them during one week? How these products change daily slugs’ consumption of food? Which method is the most effective? We tested two metaldehyde-based and one Iron phosphate based molluscicide baits, molluscidal spray and repellent grains. Each of these products were repeatedly tested with 20 slugs collected from gardens, and individually placed in boxes along with the same size piece of lettuce, carrot, oatmeal and cat food pellet. Food consumption was evaluated, and food was changed every other day for one week. Survival of slugs, consumption of food and molluscicide bait, weight of slugs, amount of excrements and visual effects on the slugs were evaluated.

Our results showed that molluscidal control methods are not effective for adult slugs before laying eggs. Molluscidal baits changed consumption of food only partially and we will present this in detail. Some slugs even laid eggs. Our study did not reveal increased mortality of slugs. The molluscidal spray did not change any consumption parameters in comparison with the control group. Repellent grains’ barrier did not stop slugs and they successfully reached, and consumed the food.

We can conclude that more effective control measures need to be developed in Lithuania against invasive slugs.

**CONTRIBUTED PAPERS. Wednesday, 19:45 SS-16 STU**

**Changes to the foraging behaviour of the grey field slug (Deroceras reticulatum) in the presence of molluscicides and implications for control.**

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Deroceras reticulatum is one of the most economically important mollusc crop pests in western European agriculture. Conventional slug control involves regular application of molluscicides metaldehyde and Iron (ferric) phosphate in a grain-based pellet form. Control of slugs with molluscicide pellets is often ineffective, inefficient and presents environmental pollution risks. Understanding the influence of molluscicides on the foraging and feeding behaviour of D. reticulatum may provide insight and a potential explanation for inefficiencies in slug population control, as well as providing knowledge vital for improvement of pellet design and application.

Slug behaviour was recorded in overnight lab based behavioural assays in soil filled arenas, with the use of time lapse, infra-red recording equipment. Molluscicide pellets were added into arenas in order to observe the pest - pellet interaction. The locomotive and feeding behaviour of D. reticulatum in the presence of varying concentrations of metaldehyde and ferric phosphate pellets was modelled in order to describe the effect that encountering a molluscicide has on slug behaviour. Pellet feeding duration, time between pellet encounters, as well as pellet reacceptance and slug mortality, was incorporated into the model in order allow predictions of the likely behavioural outcome of applying a certain concentration of molluscicide to control a slug population. Though a large range of factors are likely to influence the behaviour of D. reticulatum in the field, understanding the direct effect of molluscicide on slug behaviour may provide a basis for improved pellet design. Due to the recent announcement of the banning of metaldehyde for outdoor use in the U.K., it is almost certain that Iron (ferric) phosphate will become the most utilised molluscicide in U.K. agriculture, and therefore gaining knowledge of slug’s interactions with this molluscicide is likely to be a key asset moving forward with the changing world of slug control.

**CONTRIBUTED PAPERS. Wednesday, 18:00 DBI-9**

**Diseases of managed and wild bees**

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Honey bees have a type 1 peritrophic matrix (PM) lining the midgut. It is continuously secreted from midgut epithelial cells and moves to the posterior as concentric sheets. This structure is imaged by fluorescence microscopy after staining with calcofluor white, which binds to chitin. Calcofluor fluoresces vividly, emitting at 440-470 nm, showing the PM as diaphanous blue-white sheets. To determine the effects of *N. ceranae* infection, worker honey bees were inoculated individually with 20,000 *N. ceranae* spores in 50% sucrose or with sucrose not containing spores, and then caged collectively according to treatment. Bees were removed from their cages at 4, 6, 8 and 10 days after inoculation. The midguts were removed from these sampled bees, fixed in formalin, dehydrated with a series of ethanol and xylene solutions and then embedded in paraffin. Embedded tissues were sliced in 5 micron sections, placed on microscope slides, and then rehydrated and stained with calcofluor and Biebrich. Developing spores were observed in epithelial cells by day eight, and changes in PM struc-
ture were observed then also, compared to control bees. Most notably, the PM secretion appeared to cease in infected bees. Also, the layers of PM dissociated from the epithelial cell layer in infected bees, while the PM remained closely applied to the epithelium in control bees. These observations suggest that the PM may be less effective in its putative roles as a protective barrier to the midgut and a substrate for digestive enzymes, following *N. ceranae* infection.

**CONTRIBUTED PAPERS. Wednesday, 18:15 DBI-10 STU**

Elucidating the honey bee immune response at the *Varroa* mite feeding site.

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The European honey bee (*Apis mellifera*) is the most economically important managed pollinator worldwide, contributing billions of dollars to agriculture every year. Honey bee health is under pressure with winter colony losses of 1.8 – 53% across Europe and 30% in the USA. These losses have largely been attributed to the ectoparasitic honey bee mite, *Varroa destructor* and the numerous pathogens it vectors, most notably Deformed Wing Virus. Despite much research, the honey bee immune response to *V. destructor* and its associated pathogens are poorly understood. This could be, in part, due to previous studies of the immune response investigating whole bee homogenates rather than exploring the response more locally at the *Varroa* feeding site. As such, we designed, and present a novel biopsy method to investigate a localised honey bee immune response. Using this method we have found the increased expression of several immune genes including *Dicer* at the *Varroa* feeding site when compared to the systemic response of both parasitised and unparasitised honey bees. This method of exploring localised gene expression at the *V. destructor* feeding site, as opposed to systemically within the whole honey bee, draws a more accurate picture of the honey bee immune response. A greater understanding of this vector-host relationship may aid in the development of new approaches for improving honey bee survival.

**CONTRIBUTED PAPERS. Wednesday, 18:30 DBI-11**

Prevalence and diversity of ssRNA+ honey bee-infecting viruses in wild *hymenoptera*

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Honey bee decline stems from multiple interacting environmental and biotic factors, such as pesticides and management practices, as well as parasites and microbial diseases. In this context, bees are becoming a prominent model in viral ecology. Wild bees have been shown to carry a variety of honey bee viruses, in particular bumble bees that seem particularly sensitive to the diseases. Likewise, ants have been found positive for bee viruses, suggesting they could also be reservoirs. Together this suggests that the host range of the viruses originally described in honey bees might be wider than honey bees and that subsequently the epidemiology of these viruses might involve a broader suite of hosts. But so far evidence of sustained honey bee virus infection in wild bees and ants is scarce. To test whether honey bee viruses are specialists or generalists among ants and bees, we examined the prevalence and phylogenetic relationships of the 6 most pathogenic honey bee viruses (SBV, ABPV, BQCV, CBPV, IAPV, DWV) as well as two sainiviruses in individual insects, including 349 ants, 110 wild bees, and 74 honey bees, sampled in Europe and America. Our results show extremely low virus prevalence in wild *hymenoptera* suggesting honey bee viruses are largely specialists.

**CONTRIBUTED PAPERS. Wednesday, 18:45 DBI-12 STU**

Bacterial diversity of the *Tetragonula carbonaria* microbiome within and between hives

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The native stingless bee *Tetragonula carbonaria* represents a growing source of honey production and pollination in Australia but remains understudied compared to honey bees and bumble bees. Common bee symbionts are known to comprise most of the *T. carbonaria* microbiome, however broader patterns of diversity in microbial community structure and function between and within hives remain under-investigated. Related hives kept by beekeepers in different environments were identified, and the microbiota of bees from these hives was profiled using 16S rRNA amplicon sequencing. This amplicon data was explored at the highest resolution possible, and microsatellite profiling of the bee hosts was used to assess the link between genetic and microbial community relatedness. We use *T. carbonaria* to explore the possibility of phyllosymbiosis at the subspecies level, and contrast patterns observed from known symbiotic bacteria of bees with other, likely environmentally derived, microbiota. Furthermore, we discuss progress in elucidating functional implications of this symbiotic diversity through untargeted metabolomics using gas chromatography-mass spectrometry.
families (Agfidae, Alaninematidae, Alloionematidae, Angiostomatidae, Araeolaimidae, Ascarididae, Cephalobidae, Cosmocercidae, Diplogasteridae, Mermithidae, Panagrolaimidae and Rhabditidae) that are morphologically divergent. They may exist in different life stages, spending part or all of its life cycle in the host, and making efficient and accurate species diagnostics challenging. Thus most often, a combination of morphological, molecular and other tools is required. Various techniques had been used to recover nematodes from gastropods, the choice depending on research goals and available tools. This workshop will explore approaches to gastropod nematode identification, systematics and taxonomy with emphasis on major taxa like *Phasmarhabditis, Agfa, Angiostoma* and *Alloionema*.

**WORKSHOP. Wednesday, 20:45 SSW-2**

**Identification of molluscs**

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Molluscs can often be a challenge in terms of species identification due to similar external morphology and general appearance. This is especially true for many slug species. In this workshop we will be running a mollusc identification clinic including dissections of genitalia. Participants are encouraged to bring specimens of molluscs for species identification. Thus we will have a unique opportunity to discuss methods for identification of molluscs and which morphological characteristics we use for deciding on species and possible sub-species, hybrids or other types of operational taxonomic units.
Unspecialised endophytic fungi protect herbaceous plants against insects, but experimental design is critical – a meta analysis

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Herbaceous plants harbour species-rich communities of endophytic fungi, distinct from those seen in grasses. These fungi are asymptomatic, ubiquitous, and are generally termed ‘unspecialised endophytes’. While some of these endophytes are specialised insect-killers (thus called entomopathogenic), many are not, and remarkably little is known about how these fungi interact with insect herbivores when colonizing plant tissues. Through a meta-analysis we demonstrate that both entomopathogenic and non-entomopathogenic unspecialised endophytes significantly reduce a range of growth parameters of insect herbivores, belonging to different orders. Insect growth and performance of both sucking and non-feeding herbivores was improved when the strain was re-isolated from the plant after the third passage. After each passage, a conidial suspension of each isolate was used in bioassays to evaluate both virulence against 4th instar larvae of the model insect Galleria mellonella and endophytic behavior on each respective plant host.

Differences related to the tissue colonization percentages of the samples among the three species tested were detected at the first re-isolation. Likewise, higher endophytic colonization rates were observed in melon (96.6%), followed by tomato (73.3%), and the lower was detected in cotton plants (40.0%). However, endophytic colonization rate improved when the strain was re-isolated from the plant after the third passage in tomato and cotton, 90.0% and 76.7%, respectively. Isolates obtained after third passage from tomato and melon lost virulence if compared with the original isolate, whereas three passages through cotton plants did not cause a significant reduction of virulence. These results are discussed in terms of the possible evolution of the endophytic behavior of entomopathogenic fungi.

CONTRIBUTED PAPERS. Thursday, 8:45 MC-33

Microbial cues to induce grooming in Drosophila melanogaster to resist their infection

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Grooming is one of hygiene behaviors, which serves to clean contaminat-ed microbes from their body surface. The microbial cues to induce insect hygiene behavior are still relatively unexplored, and hence, we have studied the microbial cues, which can be important to induce grooming behavior. It seems that olfactory cues from microbes enhance insect grooming behavior, and gustatory signals even induce the behavioral reflex. With gram-negative bacteria, gram-positive bacteria and fungi, we have examined the cues that Drosophila uses to remove microbes through grooming behavior. Our results suggested that the different cues depending on the type of microbe were served to induce the grooming to remove the microbes from the body surface in Drosophila melanogaster.

CONTRIBUTED PAPERS. Thursday, 8:30 MC-32

Is there a trade-off between virulence and endophytic behaviour of the entomopathogenic fungus Beauveria bassiana?

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Systemic crop protection by the use of endophytic entomopathogenic fungal strains is on the forefront of IPM. However, the possible origin of this endophytic behaviour remains poorly studied, and hence, the possible occurrence of a trade-off between virulence and endophytic behaviour. In this work, the possible adaptation to the endophytic behavior of a Beauveria bassiana strain after being successively recovered from melon, tomato and cotton tissues was investigated. Plants were sprayed with a fungal suspension of the B. bassiana endophytic strain EABb 04/01-Tip to achieve an endophytic fungal colonization of the plant. Once the colonization was established, the strain was re-isolated from the plant during three passages. After each passage, a conidial suspension of each isolate was used in bioassays to evaluate both virulence against 4th instar larvae of the model insect Galleria mellonella and endophytic behavior on each respective plant host.

Purpureocillium lilacinum is also a mycopathogen, although highly specific

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Purpureocillium lilacinum is commonly known as a pathogen of nemato-des. However, recently it was shown to behave as an entomopathogen as well. This fungus exhibited high control efficiency against leaf cutter ants (LCA). Considering that many fungi have been shown to have multiple functions we examined whether P. lilacinum could act as a mycopathogen against the symbiotic cultivar of LCA. Therefore, we cultivated on petri dishes and on slides both fungi together to evaluate their interaction, using cultivar strains from two Acromymex and one Atta species. P. lilacinum stopped the growth of the cultivars from Acromymex LCA but not from Atta LCA. Furthermore, we observed cell wall destruction of the cultivar hyphaes from Acromymex but not from Atta. These results are in agreement with a traditional view that cultivar strains differ with LCA. However, they disagree with the latest molecular phylogeny of the cultivars of LCA as our cultivars should fail on Clade B and we expected a similar response by the pathogen. Clade B, however, includes 6 different fungi lineages therefore it could be that our P. lilacinum strains are responding to differences found among these lineages or are responding to local differences. Irrespective of the reason behind these results, our information is essential from a biological control perspective as 1) P. lilacinum seems to control Acromymex but not Atta LCA’s cultivars and 2) P. lilacinum behaves as a multifunctional fungi against Acromymex leaf-cut-ters positioning itself as an excellent candidate for their biological control.
Roles of peritrophic matrix in Cry1Ac resistance of cotton bollworm Helicoverpa armigera

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Crystalline (Cry) proteins from Bacillus thuringiensis (Bt) are widely used for pest control as sprays and in transgenic crops. However, evolution of insect resistance to Bt toxins threatens the prolonged use of Bt as efficient biopesticide. To date, different resistant mechanisms have been identified, but some are still not completely elucidated. Here, a comparative analysis of the proteome of peritrophic matrix (PM) and transcriptome of midgut were performed to identify potential resistant mechanism to Cry1Ac in the laboratory-selected XJ10 strain of Helicoverpa armigera. This strain had a 146-fold resistance to Cry1Ac toxin and 45-fold resistance to Cry1Ac activated toxin. In comparison to the Bt susceptible XJ strain, several trypsin were down-regulated in both mRNA and protein levels in the XJ10 strain. Furthermore, 215 proteins were identified from PM and nearly all of them had corresponding mRNAs in the midgut. These results provided a new insight that the PM may participate in Bt resistance besides its function in digestion.

CONTRIBUTED PAPERS. Thursday, 09:45 MC-37

NoVil: The hunt for weevil control-Update

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Characterization of novel baculovirus isolates for potential development and application as biopesticides against agricultural pests in South Africa

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Several baculovirus biopesticide products are currently used as components of integrated pest management programmes for the control of lepidopteran crop pests in South Africa. Many of these are based on foreign isolates and imported into South Africa. To date, this has led to intensive research over the last 10 years and challenges, as well the requirement for additional products targeting other potential host resistance development and slow speed of kill. These challenges, as well the requirement for additional products targeting other crop pests, have led to intensive research over the last 10 years and bioprospecting for novel South African baculovirus isolates. To date, this research has resulted in discovery of genetically distinct viruses infecting several crop pests either from field-collected insects or from laboratory reared insect colonies. Three novel baculoviruses, namely CpGV-SA, CpeNPV and HarpNPV-SA, are of particular interest given their resistance breaking potential, broader host range and virulence against host insects respectively. Here, the genetic and biological characterisation of these novel isolates together with ongoing projects that aim to secure the future of baculovirus biopesticide development and application in South Africa is presented.

CONTRIBUTED PAPERS. Thursday, 10:00 MC-38

IDH-α-mediated metabolic disorders disrupted active immunization in eusocial termites

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Active immunization is a vital colony-level disease defense to improve the survival of social insects, but its underlying regulatory mechanisms are almost unexplored. Here, we chose the eusocial termite as a model and silenced an important metabolic gene, isocitrate dehydrogenase subunit alpha (IDH-α), to address how metabolism influenced active immunization. Our results showed that dsIDH-α-injected termites exhibited significantly reduced IDH-α at mRNA and protein levels and altered levels of isocitrate and NADH, indicating the impaired NAD+–IDH reaction. IDH-α silenced termites displayed metabolic disorders, which were revealed by significant changes in several metabolites from the carbohydrates and amino acids. When grooming towards fungus-exposed termites, IDH-α silenced nestmates showed the significantly increased activity of caspase 3, expression of four apoptosis-related genes and rate of apoptosis in vivo, suggesting that IDH-α-mediated metabolic disorders intensified apoptotic lesions. Especially, the apoptotic lesions caused high-level infections as revealed by growth of the significantly increased number of colony forming units from dissected gut contents. Furthermore, the increased disease susceptibility led to the significantly lower antifungal activity and higher mortality of the silenced nestmates, which indicated that IDH-α-mediated metabolic disorders disrupted active immunization against fungal pathogens in termites. Our findings illustrate the important biological functions of the key metabolic gene IDH-α in active immunization of termites, thereby providing a clear picture of the molecular linkage between metabolism and social immunity. (Unpublished)
Step-by-step acquired resistance of Adoxophyes honmai passed by nucleopolyhedrovirus: resistance mechanism and inheritance trait

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Resistance to Adoxophyes honmai nucleopolyhedrovirus (AdhoNPV) was developed in the smaller tea tortrix, Adoxophyes honmai, by selecting survivors from AdhoNPV-fed larvae in the laboratory. Resistance ratio of this selected strain (R-strain) increased to more than 400,000 folds as compared to non-selected strain (S-strain), though that of a strain (R-N21-strain) stopped selection at 21st generation (G21) was approximately 100 to 400 folds and did not change without passaged by virus administration until 168th generation. Mode of resistance was examined in R-N21-strain larvae as compared to that of R- and S-strain. R-N21-strain larvae did not decrease binding and fusion efficiency for occlusion derived viruses (ODVs) to midgut epithelial cells, but the gene expression in the midgut cells and hemocoelic susceptibility against budded viruses were less than that of S-strain. These results suggest that the resistant mechanisms for reduced expression of viral genes and decreased hemocoelic susceptibility were acquired at early generations until G21, but afterward prevention of binding-fusion of midgut cells to ODVs were acquired at late generations. The mode of inheritance of resistance was also examined for reciprocal F1, and backcrossed hybrids obtained from the R- and S-strain larvae nearly 20th (early generations) and 140th generations (late generations). Comparison of dose-response of those crosses against AdhoNPV revealed that the resistance of A. honmai larvae to AdhoNPV was controlled by polygenes located on autosomal chromosomes in both of early and late generations.

Insecticidal activity of granulovirus and nucleopolyhedrovirus isolated from a natural co-infection in Spodoptera ornithogalli larvae

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The yellow-striped armyworm, Spodoptera ornithogalli (Guenée) (Lepidoptera: Noctuidae) is a polyphagous pest widely distributed from Canada to Argentina, including the Caribbean islands. It has been reported on a variety of crops including alfalfa, sorghum and soybean. In Colombia, S. ornithogalli has been described mainly as a pest of cotton, citrus and cut-flowers. Although the damage caused to crops is mainly due to leaf herbivory, damage to tomato fruits and cotton bolls has also been reported. In this work, the insecticidal activity of a granulovirus (SporGV) and a nucleopolyhedrovirus (SporNPV) isolated from a naturally occurring co-infection in a S. ornithogalli larvae in Colombia was studied. The mean lethal concentration (LC50) of each isolate and the experimental mixtures of SporGV and SporNPV at different proportions were evaluated in S. ornithogalli neonate larvae by using five concentrations between 1x103 and 1x105 occlusion bodies (OBs)/mL. The mean lethal concentration of the SporNPV and SporGV evaluated independently was greater than that obtained using the natural co-infection mixture. On the other hand, the mean time to death of SporGV was four times higher than SporNPV alone or mixtures. The best insecticidal activity was obtained when the proportion 97.5% of SporNPV and 2.5% of SporGV was used. The results demonstrated a positive effect of co-infection between SporNPV and SporGV in insecticidal activity over Spodoptera ornithogalli larvae. The co-infection between two baculoviruses could be used for the development of more efficient biopesticides.

CONTRIBUTED PAPERS. Thursday, 09:00 V-51

Tutavir – a new control tool for the tomato leafminer Tuta absoluta

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The tomato leafminer Tuta absoluta is considered a key pest in tomato plants. It originates from South America, but in the meantime, T. absoluta is considered a key pest in tomato and cut-flowers. Although the damage caused to crops is mainly due to leaf herbivory, damage to tomato fruits and cotton bolls has also been reported. In the framework of the EU project BIOCOMES, Andermatt Biocontrol developed the product Tutavir, which is based on Phthorimaea operculella granulovirus. Tutavir is in the registration process in the EU and is extensively tested in different countries. An emergency authorization already allows the use in a limited area. Field trials are performed for example in Italy as well as in Germany.

In a greenhouse trial in summer 2018 in Italy, Tutavir showed an efficacy of 75 % concerning mined leaflets and was as effective as Btk and nematodes. A second trial in Italy in open field confirmed the good efficacy of Tutavir. With three different application rates, a dose-response effect was found whereas the two higher rates (100 ml/ha & 200 ml/ha) were comparable to the Btk reference product. When looking at the number of damaged fruit, Tutavir showed an efficacy up to 83 %. In a practical trial, Tutavir was even more efficient than Chlorantraniliprole concerning fruit damage. The different trials demonstrate that Tutavir is a valuable additional tool for a successful management strategy of T. absoluta.

CONTRIBUTED PAPERS. Thursday, 09:30 V-53

Genome sequence and biological activity of a new group II alphabaculovirus from Chrysodeixis includens with tetrahedral occlusion bodies

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A new alphabaculovirus isolate, termed Chrysodeixis includens nucleopolyhedrovirus #1 (ChinNPV#1), was recovered from dead larvae of the soybean looper (Chrysodeixis includens). Electron microscopy revealed that the occlusion bodies (OBs) of this isolate were distinctly tetrahedral in shape, in contrast with OBs from other C. includens alphabaculoviruses that have exhibited a more typical polyhedral morphology. Sequencing of ChinNPV#1 DNA identified a genome of 130, 540 bp with a 37.3% G+C nucleotide distribution and 126 annotated ORFs. Two copies of the he65 ORF (AcMNPV ac105) were identified, as well as dna ligase III, which has been found in only five other baculovirus genomes. The ChinNPV#1 polyhedrin amino acid sequence shared 99.6% sequence identity with the polyhedrin of Thysanoplusia orichalcea single nucleopolyhedrovirus (ThorSNPV), which also produces tetrahedral OBs, but 100% sequence identity with the polyhedrin of a Trichoplusia ni single nucleopolyhedrovirus (TnSNPV) isolate that had not been reported to form tetrahedral OBs. Phylogenetic inference based on alignments of the baculovirus core gene amino acid sequences placed ChinNPV#1 in the same clade as Pseudoplusia includens single nucleopolyhedrovirus-IE, Chalcites chrysoeides nucleopolyhedrovirus, and TnSNPV. Like these three viruses, ChinNPV#1 does not contain homologous repeat regions (hrs) found in other baculovirus genomes. In bioassay of C. includens neonates, ChinNPV#1 had a similar potency and speed of kill to other ChinNPV isolates with the typical polyhedral shape. The host range of the isolate was found to be very narrow and was non-infectious.
to Trichoplusia ni larvae and six other tested noctuid species.

CONTRIBUTED PAPERS. Thursday, 09:45 V-54

The effect of nucleopolyhedrovirus inoculum purity on the microbial load of Helicoverpa armigera larval cadavers

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In vivo propagation is usually used in the production of baculovirus biopesticides because it is an economical and effective way to propagate baculoviruses. However, the in vivo propagation process results in contamination of the biopesticide with the microbial flora of the host larvae. High levels of microbial contaminants not only adversely affect the shelf life of a biopesticide but may impede regulatory approval of the product. Although there is relatively little that can be done to prevent contamination of a baculovirus biopesticide with the microbial flora of the host cadavers, it would be reasonable to assume that a higher concentration of microbial contaminants in the baculovirus inoculum would be associated with a higher concentration of microbial contaminants in the biopesticide. To test this assumption, a study was performed to determine whether Helicoverpa armigera nucleopolyhedrovirus (HearNPV) inoculum purity has a significant effect on the microbial load of Helicoverpa armigera larvae infected with HearNPV, and whether microbial contamination is exacerbated by storage time and storage temperature. Culture-based methods were used to detect and enumerate the microbial contaminants in the larval homogenates and representative isolates were identified using 16S rDNA sequencing. The inoculum purity did not have a significant effect on the concentration of microbial contaminants in larval cadavers at the time of harvest, but interesting differences in microbial contamination levels became apparent upon storage at temperatures above 25 °C. The implications of these findings for the in vivo production of HearNPV biopesticides are discussed.

CONTRIBUTED PAPERS. Thursday, 10:00 V-55

Application of a novel cell line derived from Thaumatotibia leucotreta eggs to for the manipulation and production of alpha and beta baculoviruses.

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Thaumatotibia leucotreta, more commonly known as the false cobbing moth, is a significant pest of several high value crops in sub-Saharan Africa. Control of this pest is achieved using an integrated pest management (IPM) programme, which combines several techniques including cultural practices, chemical insecticides and chemical pesticides among others. The betabaculovirus Cryptophlebia leucotreta granulovirus (CrleGV) forms part of the biological control options used against this pest and has been applied successfully in the field for more than 15 years. Furthermore, the alphabaculovirus Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV) was recently identified in the liftho moth, Cryptophlebia peltastica, and is being developed into a biological pesticide. This virus was found to have a broad host range, which includes T. leucotreta, making it a promising candidate as a biopesticide for use against this pest. Although the use of these viruses provides a strong foundation for the continued control of T. leucotreta, continued development is necessary to ensure long term management can be achieved. Recently, a novel insect cell line derived from T. leucotreta eggs was established and has been maintained for more than 30 passages. Inoculation with either CrleGV or CrpeNPV budded virus resulted in the formation of occlusion bodies 144 h post infection. The susceptibility of the cell line to the betabaculoviruses Cydia pomonella granulovirus, Phthorimaea operculella granulovirus and Plutella xylostella granulovirus, is currently being evaluated. Initial results indicate the formation of granules within the cells. Analysis of mRNA in the cells is underway to confirm whether viral genes are being expressed.

CONTRIBUTED PAPERS. Thursday, 8:30 N-25

Antiprotozoal activity of Xenorhabdus and Photorhabdus bacteria mutually associated with entomopathogenic nematodes

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Millions of people die or lose their life quality because of human parasitic diseases or parasite related complications every year. As parasitic diseases are contagious, the patients constitute a risk for society as well. Most of these diseases are caused by protozoa. Due to the fact that vaccines against parasites are generally not successful and resistance occurs to synthetic therapeutics in time, it is extremely crucial to search for alternative solutions. There are a great number of studies to discover novel and effective compounds from various plant and animal extracts and the secondary metabolites of microorganisms against parasites. As a result of these studies, identified bioactive novel therapeutics provide opportunities to improve new medicines against parasites. The effect of 27 cell-free bacterial supernatants of various Xenorhabdus and Photorhabdus spp. were tested in vitro against the human parasitic protozoa Acanthoemoeba castellanii, Entamoeba histolytica, Trichomonas vaginalis, and Leishmania tropica. Five-day-old bacterial cultures were centrifuged at 10,000 rpm for 10 minutes and filtered through a 0.22 μm syringe filter to obtain cell-free supernatants. The anti-protozoan effects of supernatants were evaluated using a microdilution method. The cell-free bacterial supernatants were added to culture medium resulting in concentrations of 10%, 5%, 2.5% and 1.25%. There was substantial variation among in bacterial efficacy among treatments. Some of the bacterial supernatant produced 100% mortality, whereas others had no effect. In general, Xenorhabdus bacteria showed greater efficacy than Photorhabdus bacteria. The anti-protozoal bioactive compounds produced by Xenorhabdus bacteria were identified as Fabclavine, Xenocoumacine and PAX peptide.

CONTRIBUTED PAPERS. Thursday, 8:45 N-26

Steinernema carpocapsae secrete/excreted proteins modulate insect immune responses

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The entomopathogenic nematode Steinernema carpocapsae possess...
many of the attributes of an effective biological control agent of insect pests. The ability this nematode has to infect and to kill D. melanogaster provides an excellent tool to study the molecular interplay between this useful parasite and the host. So far, it was shown that S. carpocapsae releases secrete/excreted proteins (ESPs) that participates in host invasion and in host evasion by targeting immune recognition proteins and effector proteins in the insect. Now we discourse ESP regulatory functions of the insect immune system.

The infection of Drosophilia by axenic S. carpocapsae induces the activation of haemocytes by the over expression of upd3 cytokine, which in turn leads to the activation of Jak/Stat. The injection of ESPs with axenic nematodes did not interfere with haemocyte activation, but significantly downregulates the activator domeless and the effector genes toa4 and toaM. Thus causing the negative regulation of the proliferation of hemoocytes and the differentiation of lamellocytes. Moreover, in flies challenged with inactivated X. nematophila, nematode ESPs strongly suppress key genes in immune response, namely the pgrp-lc receptor and the polyphenol oxidase genes, pop1 and pop2, with the consequent reduction in the production of antimicrobial peptides and a decrease in the reaction of melanization. The modulation caused by ESPs of S. carpocapsae in the host immune system must be an important feature in the interaction nematode - insect opening new avenues in the discovery of specific targets to promote a more efficient insect control agent.

CONTRIBUTED PAPERS. Thursday, 9:15 N-28

Extending the survival of Heterorhabditis bacteriophora dauer juveniles through phenotypic selection and marker-assisted breeding

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To enhance the entomopathogenic nematode (EPN) Heterorhabditis bacteriophora biocontrol potential molecular marker-assisted genetic improvement was applied. Breeding nematode strains with prolonged dauer juvenile (Dj) longevity is of utmost priority to extend shelf life and increase field persistence. For this purpose, we largely enhanced the repertoire of phenotypic and genotypic information concerning stress tolerance and survival. Within this frame, more than 80 H. bacteriophora wild type (WT) strains and inbred lines were extensively characterized for their Dj-longevity and EMS-mutants with extended survival have been generated through selection. Concerning genomic information, RNA-seq analyses assessed the expression of more than 22,000 transcripts in long- and short-living nematodes and a large set of WT strains and inbred lines has been genotyped by sequencing (GBS) yielding more than 700 reproducible single nucleotide polymorphisms (SNPs). All the generated phenotypic and genotypic information has been subsequently combined to determine genes, DNA polymorphisms and genotypes with high potential for improvement of DJ-longevity in H. bacteriophora. The resulting hybrid strains, selected inbred lines and EMS-mutants with extended survival have been tested for their general performance with satisfactory results. Combination of traits, like longevity, stress resistance and liquid culture production potential can now be supported by high throughput genotyping screens. Interestingly, several of the genes identified through our approach have not been characterized in the model nematode Caenorhabditis elegans.

CONTRIBUTED PAPERS. Thursday, 9:30 N-29

Excreted/secreted products of entomopathogenic nematodes and their effect on insect immunity

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Entomopathogenic nematodes (EPNs) are the obligate parasites of insects. In the fight against the immune system of the host, nematodes use their excreted/secreted products (ESPs). These products are a mixture of molecules with distinct functions. We identified several functions of the ESPs and examined them in detail - specifically their influence on phenoloxidase activity (PO) and coagulation. ESPs were obtained from two isolates of Heterorhabditis bacteriophora from different environments. Firstly, we purified the samples based on their charge. There were 5 fractions obtained from each isolate of H. b. in which their activity in the inhibition of G. mellonella PO was verified. Inhibition activity was also observed for the coagulation in both isolates. For the active fractions in PO inhibition were used a second purification based on their size. We obtained 18 new fractions in which we found very highly activity which was manifested in the inhibition of phenoloxidase and coagulation. Phenoloxidase and coagulation inhibition were also tested on ESPs isolated from EPN Steinernema carpocapsae. The inhibition of both immune responses was confirmed. Potential use of these results is mainly in the biological control of insect pests. This study was supported by grant GAČR 17 - 03253S.

CONTRIBUTED PAPERS. Thursday, 9:45 N-30

Identification of molluscs and their associated nematode parasites

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Molluscs can often be a challenge in terms of species identification due to similar external morphology and general appearance. This is especially true for many slug species. In this workshop we will be running a mollusc identification clinic including dissections of genitalia. Participants are encouraged to bring specimens of molluscs for species identification. Thus we will have a unique opportunity to discuss methods for identification of molluscs and which morphological characteristics we use for deciding on species and possible sub-species, hybrids or other types of operational taxonomic units.

CONTRIBUTED PAPERS. Thursday, 8:30-10:30

Microsporidia

Microsporidia-host interactions: from organism to molecular levels

Chairs: Ronny Larsson / Yuliya Sokolova

CONTRIBUTED PAPERS. Thursday, 8:30 MS-1

Co-infection of paramyxoid and microsporidian parasites in femi

ised amphipod crustaceans

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Within populations of the amphipod crustacean Orchestia aestivalis, co-infection with the paramyxoid Paramarteilia orchestiae and the microsporidian Dictyoceola cavimamin occurs more frequently than expected by chance. Both parasites occur more frequently in female than in males, suggesting that one or both of these parasites causes feminisation of O. aestivalis. O. aestivalis was resampled from the type locality of P. orchestiae in France and from another population at Dale in the UK. When only single infections are considered, P. orchestiae still exhibits higher prevalence in female hosts than in males but D. cavimamin does not. This indicates that the paramyxoid, rather than
the microsporidian, is the cause of feminization in *O. aestivalis*.

CONTRIBUTED PAPERS. Thursday, 8:45 MS-2

***Firing of the harpoon-like polar tube in microsporidian parasites***

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Microsporidia are parasites that have a unique infection mechanism. In order to initiate infection, microsporidia employ a harpoon-like organelle called the polar tube that is propelled out of the spore to pierce host cell membrane. The polar tube serves as a conduit, allowing the translocation of infectious material into the host. The molecular events driving polar tube firing are still poorly understood. We have optimized high speed, wide-field, phase-contrast live-cell imaging to capture the events during the firing process. These studies begin to provide insights into the initial stages of the microsporidium proliferation.

CONTRIBUTED PAPERS. Thursday, 9:00 MS-3

Cancelled

CONTRIBUTED PAPERS. Thursday, 9:15 MS-4

Ptdlns(3)P-binding Protein NbSWP12 is Significant for microsporidia proliferation in insect cells

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Microsporidia are obligate eukaryote intracellular parasites and infect a variety of hosts from protozoa to mammals, especially invertebrate like insects and aquatic arthropod. Here we identified a spore wall protein NbSWP12 encoding BAR (Bin/Amphiphysin/Rvs) domain from microsporidia Nosema bombycis. Sequence analysis suggested homologous genes of NbSWP12 are in a widespread distribution among microsporidia. Heterologous expressed NbSWP12 can form homodimer and interact with Ptdlns(3)P. Indirect immunofluorescence assay showed NbSWP12 located at the meront membrane, partly co-localized with microtubule and then mainly gathered at the two poles of splitting meront. RNA interference and expressing a single-chain variable fragments against NbSWP12 in Sf9-III cell lines can inhibit proliferation of *N. bombycis* in insect cell lines. NbSWP12-GFP fusion protein expressed in yeast cells located at cytoplasm membrane and vesicle membrane. Transform NbSWP12 into the *gvp36a* strain, a BAR protein knocks out strain of *Saccharomyces cerevisiae*, could recover the phenotype as vacuole biogenesis deletion. These findings suggest that NbSWP12 may serve as an important regulator of membrane in microsporidia proliferation. Further research will uncover how NbSWP12 bound with Ptdlns(3)P participate in meront proliferation.

CONTRIBUTED PAPERS. Thursday, 9:30 MS-5

Pathological analysis of silkworm infected by two microsporidia

*Nosema bombycis* CQ1 and *Vairimorpha necatrix BM*

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Microsporidia *Nosema bombycis* CQ1 can be vertically transmitted in silkworm *Bombyx mori* but *Vairimorpha necatrix BM* cannot. Therefore, the pathological differences in silkworm infected with these two microsporidia were required to be clarified. Here, we compared the pathological characteristics of silkworm being infected by *N. bombycis* CQ1 and *V. necatrix BM* separately. Our data firstly showed that the typical symptom of *V. necatrix BM* infection is making xenomas, which are full of microsporidia in different stages, at the posterior of intestine. However, no xenomas were formed surrounding intestines infected with *N. bombycis* CQ1. Secondly, *N. bombycis* CQ1 can infect the epithelial cells and connective tissues of silkworm ovaries at the larval stage, while *V. necatrix BM* did not. It is worth noting that the oocytes of silkworm larvae cannot be infected by *N. bombycis* CQ1. Thirdly, at pupal stage, the follicle cells and oocytes can be infected by *N. bombycis* CQ1. In addition, there are dramatical increase of microvilli and the formation of synapses on the follicle cell membrane. The *N. bombycis* CQ1 sporigen was wraped by the synapse and meantime the oocyte was invaded by the sporont. This study is the first report about the comparing infection features of *N. bombycis* CQ1 and *V. necatrix BM* in silkworm tissues and it provided elaborate and visual information of pathological characteristics which can help to explain the different transmission strategies of these two microsporidia.
CONTRIBUTED PAPERS. Thursday, 14:30 B-25

Constraints on the evolution of increased parasitism in a caterpillar symbiont

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Many pathogenic microbes are believed to have evolved from commensal or mutualistic symbionts, and theory predicts that symbionts can readily switch to more parasitic strategies. However, many symbionts have stable interactions with hosts that improve nutrient assimilation or confer protection from pathogens. Here we investigated a midgut microbe, Enterobacter cloacae, that is mildly parasitic to its insect host (Plutella xylostella) in the laboratory, and tested whether we could increase or decrease levels of parasitism through experimental evolution. We focused on whether this symbiont might evolve increased protection to larval hosts (from the well-know bacterium Bacillus thuringiensis -Bt) and whether E. cloacae could evolve to be more parasitic and facilitate Bt infection.

Selection for parasitism led to symbionts increasing pathogen-induced mortality but reduced their competitive ability with pathogens and their in vitro growth rates. Symbionts did not evolve to confer protection from pathogens. However, several lineages evolved reduced parasitism, primarily in terms of moderating impacts on host growth, potentially because prudence pays dividends through increased host size. Overall, the evolution of increased parasitism was achievable but was opposed by trade-offs likely to reduce fitness. The evolution of protection may not have occurred because suppressing growth of B. thuringiensis in the gut might provide only weak protection. In general then transitions to increased parasitism may not occur as readily as theory predicts.

CONTRIBUTED PAPERS. Thursday, 14:45 B-26

Microevolutionary alterations in midgut bacterial community of Galleria mellonella resistant to Bacillus thuringiensis

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During selection of greater wax moth Galleria mellonella for resistance to Bacillus thuringiensis (Bt) (R line) the microevolutionary alterations in midgut bacterial community have been detected as compare with insects of susceptible line (S line). Uninfected R line insect had less percentage of genus Enterobacteriaceae than the S larvae, however, upon infection with Bt the percentage were significantly elevated up to 80 % in R line. Unlike the S insects, Bt infection significantly in 2-3 times reduced the percentage of the genus Enterococcus in the R insects but in 2-3 times elevated in S line. As compare with insects of S line we have detected less percentages of potentially pathogenic bacteria genus Serratia and Bacillus in midgut of R line larvae during Bt infection. These tendency has been detected in cadavers of the larvae from R line. In the uninfected state, resistant insects exhibited enhanced basal expression of antibacterial (AMP) genes in the midgut. Following oral infection with Bt, the expression of these genes was elevated in the midgut of S line larvae. RNA interference of Gloverin gene resulted in elevated susceptibility of insects of S line to Bt infection. These observations suggest that the R line not only has a more intact midgut but is secreting antimicrobial factors into the gut lumen which not only mitigate Bt activity but also affects the viability of other gut bacteria. Also we have found that epigenetic mechanisms operating at the pre-transcriptional and post-transcriptional levels contribute to the transgenerational inherited transcriptional reprogramming of stress and immunity-related genes. This work was supported RFBR №18-316-20007 mol_a_ved.

CONTRIBUTED PAPERS. Thursday, 15:00 B-27

Effect of symbionts on gene expression in the kissing bug Rhodnius prolixus

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Kissing bug (Hemiptera: Reduviidae: Triatominae) is vector of Trypanosoma cruzi, the causative agent of Chagas disease. The insects are obligately hematophagous and rely on bacteria for development to adulthood, presumably due to provisioning of B vitamins that are deaparate in vertebrate blood. Removal of symbionts results in delayed development, increased mortality, and ultimately failure to develop into adults. Paratransgenesis, the exploitation of microbiota to control a pathogen, has been developed to control Trypanosoma cruzi in the gut of the kissing bug Rhodnius prolixus. In this system, the bacterial symbiont Rhodococcus rhodnii has been altered to express anti-trypanosome molecules, attacking the parasite within the insect gut. Despite significant progress on developing the technology, several fundamental aspects of kissing bug symbiosis remain unexplored including how the symbiont and host cooperate to establish the symbiosis. This gap in our knowledge may hinder application of paratransgenesis technology. To identify host factors that contribute to the establishment of the symbiosis, we sought to characterize changes in gene expression in R. prolixus reared with and without symbionts using RNAseq. Our results indicated that several immune genes are differentially expressed in response to symbiont infection, as are a number of lipid metabolism genes. We are now employing RNAi to understand the effect of silencing these genes on host development and symbiont titer. These experiments will shed new light into how these associations persist and potentially offer valuable insights into improving paratransgenesis in kissing bugs.

CONTRIBUTED PAPERS. Thursday, 15:15 B-28

Alteration of the honeybee gut microbiota after chronic exposures to different families of insecticides and infection by Nosema ceranae

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Germany

During selection of greater wax moth Galleria mellonella for resistance to Bacillus thuringiensis (Bt) (R line) the microevolutionary alterations in midgut bacterial community have been detected as compare with insects of susceptible line (S line). Uninfected R line insect had less percentage of genus Enterobacteriaceae than the S larvae, however, upon infection with Bt the percentage were significantly elevated up to 80 % in R line. Unlike the S insects, Bt infection significantly in 2-3 times reduced the percentage of the genus Enterococcus in the R insects but in 2-3 times elevated in S line. As compare with insects of S line we have detected less percentages of potentially pathogenic bacteria genus Serratia and Bacillus in midgut of R line larvae during Bt infection. These tendency has been detected in cadavers of the larvae from R line. In the uninfected state, resistant insects exhibited enhanced basal expression of antibacterial (AMP) genes in the midgut. Following oral infection with Bt, the expression of these genes was elevated in the midgut of S line larvae. RNA interference of Gloverin gene resulted in elevated susceptibility of insects of S line to Bt infection. These observations suggest that the R line not only has a more intact midgut but is secreting antimicrobial factors into the gut lumen which not only mitigate Bt activity but also affects the viability of other gut bacteria. Also we have found that epigenetic mechanisms operating at the pre-transcriptional and post-transcriptional levels contribute to the transgenerational inherited transcriptional reprogramming of stress and immunity-related genes. This work was supported RFBR №18-316-20007 mol_a_ved.
Gut microbes positively affect the physiology of many animals but the molecular mechanisms underlying these benefits remain poorly understood. We recently reported that bacteria-induced gut hypoxia functions as a signal for growth and molting of the mosquito *Aedes aegypti*. In this study, we tested the hypothesis that transduction of a gut hypoxia signal requires hypoxia-induced transcription factors (HIFs). Expression studies showed that HIF-a was stabilized in larvae containing bacteria that induce gut hypoxia but was destabilized in larvae that exhibit normoxia. However, we could rescue growth of larvae exhibiting gut normoxia by treating them with a prolyl hydroxylase inhibitor, FG-4592, that stabilized HIF-a, and inhibit growth of larvae exhibiting gut hypoxia by treating them with an inhibitor, PX-478, that destabilized HIF-a. Using these tools, bioassays provided evidence that HIF signaling activates several pathways with growth functions. HIF signaling also affected midgut stem proliferation and nutrient transport. Altogether, our results indicate that HIF signaling affects multiple processes in *A. aegypti* larvae with conserved functions in growth and metabolism.

**CONTRIBUTED PAPERS. Thursday, 16:00 B-31**

Baculovirus and *Bacillus thuringiensis* based biopesticides in Brazil: Challenges and opportunities

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Biological control agents are used in 10 million hectares (an increase of 77%) in Brazil, with approximately 70 industries manufacturing biological products. The use of Baculovirus and *Bacillus thuringiensis* (Bt) based biopesticides has increased in Brazil in the last few years. Embrapa Maize and Sorghum Research Center has a Bt and Baculovirus Collection with more than 4.600 strains of Bt and more than 100 baculovirus isolates that have been tested against: Spodoptera frugiperda, S. cosmioides, S. eridiania, Helicoverpa zeae, H. armigera and *Chrysodeixis includens*. Some NPV baculovirus have been developed and registered as biopesticides (WP-wettable powder, water and oil emulsion) to control fall armyworm (Cartuchovit®, VirControl Sf®, Baculomip-Sf®). *Baculovirus* *spodoptera* isolates 6NR and 19 were used in these formulations. Baculovirus biopesticides for soybean caterpillar and cotton bollworm are currently under development. *B. thuringiensis* strains have been used and registered for fall armyworm (Crystal®) and some Bt strains are effective against these insect pests and the metallic beetle (*Euchroma gigantea*), boll weevil (*Anthonomus grandis*) and is promising against *Sphenophorus levi*, a sugar-cane pest. These strains harbor some cry1, 2, 6 genes and also vip1, 2 and vip3Ah, vip3Ba1, vip3Aa2, vip3Af1 genes.

**CONTRIBUTED PAPERS. Thursday, 14:30-16:30 FUNGI 5**

Control of chewing insect pests

Chairs: Meelad Yousef / Herman Sttasser

**CONTRIBUTED PAPERS. Thursday, 14:30 F-33**

Below-ground inoculation with *Metarhizium brunneum* for long-term control of the cabbage root fly *Delia radicum*

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Soil-borne pests are particularly challenging to target with control agents that work by contact, such as entomopathogenic fungi (EPF), since the agent must be applied below-ground. Larvae of the cabbage root fly,
Delia radicum, are important pests attacking roots of brassica crops in many countries and farmer have limited or no chemical control agents available. The EPF Metarhizium brunneum is well-adapted to the soil environment and can proliferate in the rhizosphere of many plants. This fungal species is therefore an attractive candidate for application and establishment in the root zone of brassica crops prior to the arrival of D. radicum. We produced granular inocula of two strains of M. brunneum on rice and applied to the peat substrate while sowing cauliflower seeds under greenhouse conditions. Plantslets were repotted into field soil in large pots and transferred to semi-field where 40 D. radicum eggs were manually added around each stem base. Four weeks later plants were harvested and soil sieved for recovery of pupae which were incubated for infections. Both M. brunneum strains persisted in the rhizosphere and were able to infect larvae and pupae at the end of the experiment, although similar amounts of pupae were recovered at harvest in fungus and control treatments. The fungal application was therefore efficient in causing infections of D. radicum below-ground, but the effect on the larval population was likely insufficient to reduce damage significantly. However, the fungi reduced the numbers of emerging flies in the following generation, thus the inoculations may have long-term control potential.

CONTRIBUTED PAPERS. Thursday, 14:45 F-34

Possibilities for use of Metarhizium robertsi C25 in baits against Drosophila suzukii
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Entomopathogenic fungi are widespread in nature where they have frequent contact with various anthropogenic pollutants. Although, the mechanisms regulating the pathogenesis of insects are relatively well known, there are only few reports regarding the interaction of toxic contaminants with these microorganisms. In our previous studies we demonstrated that Metarhizium sp. possess the ability to degrade toxic contaminants such as nonylphenol, dibutyltin or ametryn and all of those compounds influence fungal metabolism. The aim of this study was to determine whether the insecticide acetamiprid has deleterious effect on entomopathogenic fungi. Herein, we determined ability of Metarhizium sp. to produce secondary metabolites, the destruxins, which have great importance in the infection process. In culture filtrates we determined by HPLC-MS/MS the presence of 19 destruxins (Dx) in six Metarhizium strains. Our results showed that acetamiprid in concentrations 5 to 50 mg/L does not have any effect on biomass formation by Metarhizium species but has deleterious effect on destruxin production. This toxic compound decreased significantly D.tx production in all tested strains, even at a concentration of 5 mg/L. The most sensitive strain was M. brunneum which decreased DtxA and DtxB production by 56.4% and 41.9% in comparison to control. These results point to deleterious effect of acetamiprid on the secondary metabolism in Metarhizium sp.

This research was financed by a grant from the National Science Center in Krakow (Poland), contract number UMO-2016/23/B/NZ9/00840.

CONTRIBUTED PAPERS. Thursday, 15:30 F-37

The potential for Helicoverpa armigera to evolve resistance against fungal biopesticides can be mitigated by using heterogeneous combinations of fungal isolates and crop plants
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Insects evolve resistance against conventional control agents with predictable regularity. Fungal biopesticides increasingly offer alternative pest control solutions; however, as biopesticide use increases, so does selection pressure on target insects to evolve resistance. We investigated how entomopathogenic fungi can control the major agricultural pest Helicoverpa armigera while proactively limiting resistance evolution. We draw on evolutionary science and features of host-pathogen interactions, noting that the optimum host genotype to defend against infection often depends on both pathogen strain identity and the pest’s environment. We assessed how simultaneous manipulation of fungal pathogen strain and crop plant decreases selection consistency to prevent resistance
evolution.
First, we identified multiple fungal isolates that kill *H. armigera* and studied the impact of field conditions on viability and virulence of spores. Then we quantified host genetic variation for fungal isolate susceptibility using 2198 *H. armigera* larvae from 32 females mated to 18 males. Larvae were reared on 1 of 3 plants (soybean, maize, or tomato) and inoculated with 1 of 3 fungal isolate treatments (*Metarthizium, Beauveria* or a control). We demonstrate that *H. armigera* populations harbour extensive genetic variation for fungal pathogen resistance, which if not appropriately managed could facilitate biopesticide resistance evolution. However, we show that selection for resistance is inconsistent between different fungal isolates, an effect enhanced by applying spores to larvae feeding on different crop species. We argue that application of multiple biopesticide isolates, an effect enhanced by applying spores to larvae feeding on different fungal isolate treatments (*Metarthizium, Beauveria* or a control), represents a practical solution for long term proactive biopesticide resistance management.

**CROOSS-DIVISIONAL SYMPOSIUM**

**DBI-MICROSPORIDIA**

**Microsporidia and microsporidia-like cryptomycota infecting micro-eukaryotes and metazoan parasites**

Organisers / Chairs: Mark Freeman / Joe Ironside

**SYMPOSIUM. Thursday, 14:30 DMCS-1**

**Hyperparasitic Microsporidians of Myxosporeans**

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Microsporidians are amongst the most abundant of any group of hyperparasitic organisms, with over 80 hyperparasitic species described. They are hyperparasitic in a diverse range of parasitic organisms from unicellular endoparasites like gregarines to large multicellular ectoparasitic arthropods such as copepods. Thelohan (1895) described the first hyperparasitic microsporidian, *Nosema marionis*, from a myxosporean infecting the gallbladder of a marine fish. Since then, two further microsporidians infecting myxosporeans in marine fish have been described; *Nosema notabilis* (Kudo, 1939) and *Nosema ceratomyxae* (Diamant et Paperna, 1985). In addition, the microsporidian, *Neoflabelliforma aurantiae*, has been described hyperparasitising developing actinospores infecting a freshwater oligochaete (Morris et Freeman, 2010).

Three further hyperparasitic microsporidians infecting myxosporeans have been reported but await full descriptions; two are found infecting intestinal myxosporeans in tiger puffer in Japan and another from the urinary system of rabbitfish in the Arabian Sea. This study details the molecular phylogeny of some of these microsporidians, two from the intestinal myxosporeans of tiger puffer in Japan. *Nosema ceratomyxa*, *Neoflabelliforma aurantiae* and the hyperparasite from the Arabian Sea. Ribosomal RNA gene sequence data demonstrates that these microsporidians are not closely related to each other and that they have independently evolved as hyperparasites of myxosporeans on multiple occasions throughout microsporidian evolution. However, all phylogenetic analyses place the microsporidians with others that infect fish and aquatic invertebrates and not with members of the genus *Nosema*. These findings suggest that hyperparasitic microsporidians were once parasites of the primary host and have opportunistically infected myxosporeans on multiple occasions, in some cases to the apparent exclusion of the original primary host.

**SYMPOSIUM. Thursday, 14:54 DMCS-2**

**Hyperparasitic microsporidia in trematode hosts: two new species of Unikaryon that infect microphallids from crabs inhabiting Florida coasts**

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Fifteen microsporidia species have been described from larval trematodes parasitizing fishes and molluscs. Nine of those in fish flukes were assigned to the genus *Nosema* based on morphology; however, their true systematic position remains undetermined. Another microsporidium, *Ovipleistophora diplomotorni*, infects flukes and ovaries of a fish, and is closely related to members of *Pleistophora* and *Ovipleistophora*. The only genus associated exclusively with trematodes is *Unikaryon*, with four species described to date and all infecting molluscs. We report here two new species of *Unikaryon*, but they infect encysted metacercariae of microphallids from crabs in Florida. The first *Unikaryon* sp. 1 was isolated from *Microphallus* sp. in Panopeus herbstii from Tampa Bay and the second *Unikaryon* sp. 2 from *Diacetabulum* sp. in *Pachygrapsus transversus* from Molasses Key. Both species display characteristic for *Unikaryon*: arrangement of spores in sets of two, large posterior vacuole, and eccentric position of a polar filament. Spores of *Unikaryon* sp. 1, unlike those of *Unikaryon* sp. 2, assemble in large membrane-bound agglomerates containing hundreds of organisms and have a larger number of polar filament coils – 7–8, compared to 4-5 in *Unikaryon* sp. 2, also suggesting two separate species. The SSUrDNA-infery phylogenetic analysis place *Unikaryon* sp. 1 in one clade with *Unikaryon* legeri (with 94% of SSUrDNA similarity), the only known species of *Unikaryon* with the sequenced rDNA. Microsporidia of trematodes with their complicated multiple host life cycles may have played an exceptional evolutionary role in disseminating microsporidia among different host groups, particularly within marine ecosystems.

**SYMPOSIUM. Thursday, 15:18 DMCS-3**

**Cytology and Development of Metchnikovellideans and Related Organisms.**

**Larsson, J.I.R.**

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Metchnikovellideans (genera Metchnikovella, Caulleryetta, Amphiambylys and Amphiacantha) is a group of hyper-parasitic microsporidia, living in gregarines (Apicomplexa) of mostly marine hosts (Polychaeta, Priapulida and Echiurida). The development comprises one free and one sac-bound sporogony, normally yielding rounded spores. The genus *Amphiambylys* is an exception, producing rod-shaped spores in the sac-bound sporogony. The free sporogony is by plasmodotomy or budding. The sac-bound sporogony proceeds as vacuolation, in the cytoplasm of the sporent, and the cell wall of the sporent remains as a spore-sac. In the genus *Amphiacantha* the presence of synaptonemal complexes indicates meiotic division. The wall of the sporent grows into a stratified structure, with a layering apparently characteristic for the genus. The spore has a unique construction, traversed by a rod-like, straight or slightly bent, polar filament (manubrium), at one end connecting to a polar cap. The opposite pole ends with a swollen, bulbous, layered structure, from which a lamellar prolongation extends. Sac-bound spores have a thicker spore wall than free spores. Sporogony by vacuolation, and a unique connection between polar filament and polar cap, are shared by metchnikovellideans and some non-hyper-parasitic organisms (chytridiopsids and the genus *Mitosporidium*). These characteristics distinguish them from the typical microsporidia. Chytridiopsids produce spherical spores both in a free and an enclosed sporogony. Spores have a coiled polar filament, carrying for the genus characteristic, often honeycomb-like, surface structures. *Mitosporidium* produces ovoid or slightly bent spores in the single sporogonial sequence. The spores have a coiled polar filament with a wide anterior, posteriad tapering, section, and a polaroplast-like system of lamellae, folded differently from the lamellae of a typical polaroplast, surrounding the anterior part of the polar filament.
Early evolution of Microsporidia: lessons from molecular phylogeny, phylogenomics and genomics of metchnikovellids

**Nassonova, E.**1,2; Paskerova, G.2; Frolova, E.3; Galindo, L.J.2; Torruella, G.1; Moreira, D.1; López-García, P.1; Smirnov, A.2
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Metchnikovellidae are the parasites of gregarines inhabiting the gut of polychaetes and some other marine invertebrates. These tiny and scarce hyperparasitic organisms escaped the molecular studies for the long time. Recent advances in single-cell genomics, whole genome amplification and high throughput sequencing opened the opportunities to sequence their genes and genomes. Molecular phylogeny and phylogenomics confirmed the long-held suspicion that the metchnikovellids are deeply branching microsporidia. They occupy an intermediate position between higher microsporidia with fast-evolving sequences and mitosomes and their less derived mitochondrial relatives belonging to Rozellomycophora (Rozellomycota, Cryptomycota). Comparative analysis of gain and loss of protein families showed that metabolic capacities and the gene content of metchnikovellids are reduced to a level, characteristic of higher microsporidia. However, unlike the latters metchnokivellids possess no ATP/ADP translocases of bacterial origin, completely lost nucleotide excision repair components but retained the clathrin-mediated endocytosis machinery. Genes of metchnikovellids demonstrate a high evolutionary rate, which resulted in the significant divergence between their genera and species in molecular phylogenetic trees. In contrast to Amphiamblis spp. and Amphicocantha spp. the sequenced species of Metchnikovia do not form a monophyletic clade, thus supporting the earlier predictions that this most abundant and numerous genus is an assemblage of genetically heterogeneous species. Phylogenomics and comparative genomics is a promising way to study the systematics and early evolution of microsporidia. Supported by RFBR grant 18-04-01359.

**SYMPOSIUM. Thursday, 16:06 DMCS-5**

**Intranuclear parasites of free-living amoebae: The Rozellomycota and the origins of the Microsporidia**

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Almost 30 years ago, intranuclear eukaryotic parasites were discovered in free-living amoebae *Vannella* isolated from water and inflamed eyes of a keratitis patient; and later from *Saccamoeba* and *Thecamoeba* recovered from environment. The parasites were addressed as microsporidia, as they possess non-flagellate microsporidia-like spores. Molecular rDNA-based phylogenetic analysis showed that they belong to *Rozella* spp. clade of species composed mainly of chytridi-like endoparasites of water molds and algae. Amoeba parasities, namely *Paramicrosporidium* and *Nucleophaga*, and *Mitosporidium* infecting water fleas, emerged in rDNA phylogenies as distinct lineages forming “missing links” with the Microsporidia. Another congruent trait has been found in the organization of the rDNA operon. All members of Rozellomycota have a standard eukaryotic SSU rDNA, but the microsporidia-like organisms show a narrowing of ITS-2. A greater reduction of ITS-2 is also present in “primitive microsporidia” (*Chytridiopsis* and metchnikovellids) which, however, retain standard SSU rDNA secondary structures. The complete loss of ITS-2 resulting in 5.8S/LSU rDNA fusion occurred in derived microsporidia (which also have a reduced SSU rDNA both in size and secondary structure). Genomic (vs. morphological) studies support the “missing link” scenario, revealing that both *Mitosporidium* and *Paramicrosporidium* have fungal-like genomes, less reduced in size and complexity comparatively to microsporidia, including the presence of mitochrondrial genomes. We isolated several strains within various amoebae, and the actual diversity of these organisms is certainly underestimated. Our recent studies of *Paramicrosporidium saccamoebae* confirmed amazing similarity of spore ultrastructure to metchnikovellids, and revealed its life cycle within the nucleus of *Saccamoeba lacustris*.

**SYMPOSIUM. Thursday, 15:00 SSS-2**

**Slime time: Frontiers in slug and snail management in North America**

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Invasive slugs and snails are among the most damaging pests of agriculture throughout the world, including North America. In the Pacific North West (PNW) region of the United States these invertebrates are particularly problematic. For example, gastropods cause an estimated $100 million worth of damage to the grass seed industry annually in western Oregon. Despite this economic impact, molluscicidal baits are the mainstay of pest gastropod control throughout the region. However, recent surveys showed that only 30% of growers are satisfied with bait performance while 98% stated that they would be willing to use alternative approaches for managing these pests if they were available. Biological control using nematodes is a tantalizing option given the success of Nemaslug® in Eu-
The Problem with Pellets; will we ever be able to eradicate slugs pests?

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Most molluscicides for terrestrial slugs and snails are applied as bait pellets. Farmers and growers usually express concerns about the attraction to such baits, their durability and the number of baiting points. These issues are important, but there are many other factors that will affect the impact of bait pellet applications. These include; the likelihood of molluscs being active, the proportion of the population active, the timing of the pellet application, the presence of alternative food, the palatability of the bait and the time taken for toxins to have an impact on the animal. These aspects will be discussed using information from field and laboratory studies on the ecology and behaviour of the field slug, Deroceras reticulatum.

Slug control using Phasmarhabditis hermaphrodita

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Slugs are voracious eaters and highly pestiferous across northern Europe. They are difficult to control due to their high reproductive rate and subterranean nature. Also metaldehyde based chemical pellets are due to be banned for use in the U.K. by 2020. This means that the only credible method of control is the slug parasitic nematode Phasmarhabditis hermaphrodita that has been developed as a biological control agent (Nemaslug®) since 1994 available from BASF Agricultural Specialities. Nematodes are mixed with water and sprayed onto soil where they seek out slug hosts and penetrate and kill them in 4-21 days. Although P. hermaphrodita can provide significant levels of protection from slugs comparable to metaldehyde pellets there are variable reports of its success in the field. The overall aim of my research is to improve P. hermaphrodita for use in slug control. To do this we have isolated numerous wild strains of P. hermaphrodita (and other Phasmarhabditis species) to studying their pathogenic potential and investigate how slugs and snails respond to P. hermaphrodita upon infection. We have shown that wild strains of P. hermaphrodita have superior pathogenicity and host seeking behaviour than the current commercial strain of P. hermaphrodita. We have also demonstrated that P. hermaphrodita has an unusual ability to change the behaviour of slugs making infected slugs move towards areas where in the nematodes are present, potentially due to the manipulation of biogenic amines such as serotonin and dopamine. Our recent studies have also highlighted a reason why P. hermaphrodita cannot kill snails – due to the presence of the snail shell. Upon infection by P. hermaphrodita snails (and slugs) can trap, encase and kill invading nematodes using their shell which acts as a formidable defence mechanism. Taken together, the development of wild strains of P. hermaphrodita (and studies on how they affect slug and snail behaviour and physiology) could provide a more efficient slug control product for use in the field by farmers and gardeners.
spore surface proteins and homologous Cry genes was also highlighted. Taken together these findings support a complex insecticidal mechanism of action leveraging a variety of available virulence factors. Different levels of toxicity can be associated with differences in the gene arsenal of a specific bacterial strain. A recent phylogenetic study confirmed a significant sharing of putative toxicity or virulence related proteins among examined genomes, however some isolates appeared distant from the others and exhibited additional subsets of specific genes.

SYMPOSIUM. Thursday, 17:50 BS-3
Insect pathogenicity determinants of environmental Serratia, Yersinia and Pseudomonas species and implications for biological pest control
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Several environmental isolates of Serratia, Yersinia and Pseudomonas are capable of infecting and eventually killing plant pest insects. Among environmental pseudomonads, plant-colonizing \(P\). protegens and \(P\). chlororaphis stand out because of their potent insecticidal activities towards Dipteran and Lepidopteran pest insects that they exhibit in addition to their manifold plant-beneficial activities. Upon ingestion by herbivorous larvae, these pseudomonads colonize the gut, breach the intestinal barrier, invade the hemocoel, proliferate and ultimately kill the insects. Insect pathogenicity determinants include an arsenal of particular toxins (e.g., Fit toxin) and lytic products. Contractile injection system (CIS)-based strategies provide the pseudomonads with a competitive advantage over members of the resident gut microbiota and aid insect colonization. Particular LPS O-antigen decorations of the cell surface serve as armor against host antimicrobials and weaponry of bacterial competitors. Similarly, the broad host range entomopathogen \(Y\). entomophaga releases a degradative toxin complex (Tc) enabling dissolution of the insect gut enabling pathogen ingress to then overwhelm the insect immune system. In contrast, chronic strains of \(S\). entomophila remain confined within the gut, while other \(S\). proteamaculans isolates can actively breech the gut. The identification of several grass grub (Scarabaeidae) active Tc’s and CIS like Afp variants with altered host range and efficacy within Serratia spp. raises questions to the evolution of different toxins. The diverse infection potentials, host range specificities and equipment with multifarious insect pathogenicity determinants makes these environmental bacteria promising candidates for targeted applications in biological control of plant pests.

SYMPOSIUM. Thursday, 18:40 BS-5
Bacillus thuringiensis: 50 years of safety to vertebrates
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The Bacillus cereus group contains vertebrate pathogen such as \(B\). anthracis and \(B\). cereus as well as the invertebrate pathogen \(B\). thuringiensis (Bt). Microbial biopesticides based on Bt are widely recognised as being among the safest and least environmentally damaging insecticidal products available. Nevertheless, a food-poisoning incident in 2012 prompted a European Food Safety Authority review in which it was argued that Bt poses a health risk equivalent to \(B\). cereus, a known causative agent of diarrhoea. However, a critical examination of available data, and this latest incident, provides no solid evidence that Bt causes diarrhoea. Although relatively high levels of \(B\). cereus-like spores can occur in foods, genotyping demonstrates that these are predominantly naturally occurring strains rather than biopesticides. Moreover, MLST genotyping of >2000 isolates show that biopesticide genotypes have never been isolated from any clinical infection. MLST data, and recent whole genome sequencing studies, demonstrate that the \(B\). cereus group is heterogeneous and formed of distinct clades with substantial differences in biology, ecology and host association. The group posing the greatest risk (the anthracis clade) is distantly related to the clade containing all biopesticides. These recent data support the long-held view that Bt and especially the strains used in Bt biopesticides are very safe for humans.

SYMPOSIUM. Thursday, 18:20 BS-4
Recent advances in \(B\). thuringiensis physiology and infection features
Chen, X.\(^1\), Jin, L.\(^1\), Peng, Q.\(^1\), Zhang, J.\(^1\), Song, F.\(^1\)
Candela, T.\(^2\), Gilios, N.\(^2\), Nielsen-Leroux, C.\(^2\), Lereclus, D.\(^2\), Gohar, M.\(^2\)
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China\(^1\)
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The presentation is from two teams and will deal with two aspects related to Bt physiology. One addresses the role of CalY, a structural biofilm protein and highlights its role in infection. The second aspect describes the role of cell wall hydrolases in mother cell lysis (MCL). In vitro in actively dividing bacteria, CalY is located on the surface but accumulates outside the bacteria as fibers at stationary growth and in biofilms. The Bt 407 CalY mutant was affected in biofilm formation and the mutant showed reduced adhesion to Hela cells and Galleria mellonella haemocytes and decreased mortality in this insect. A transcriptional \(gfp\) reporter fusion revealed caly expression in the intestine. Whether the role of CalY in vivo is as adhesin and/or biofilm remains to be elucidated. Bt toxin crystals are often dissociated from the spores following (MCL), thereby exposing it to UV damage. Therefore blocking MCL is of interest for Bt efficacy. Here we analyse the MCL process in \(B\). thuringiensis Kurstaki HD 73. CwB and cwiC hydrolases gene transcription are controlled by SigmaK and positively regulated by the transcriptional factor GenE. The disruption of cwiB delayed MCL while the mutation of cwiC completely blocked MCL. Suggesting that CwIC is essential for MCL in this strain. The sigK mutation also blocked the MCL in HD73. In addition the SpoIIID positively regulates sigK transcription. However, MCL in the spoIIdD mutant was medium-dependent. CwIC is conserved in all sequenced \(B\). cereus group strains but the regulation can be strain dependent.

SYMPOSIUM. Thursday, 17:00-19:00
ICTV STUDY GROUP
Organizer: Robert Harrison

CONTRIBUTED PAPERS. Thursday, 17:00-19:00
MICROBIAL CONTROL 6
Entomopathogenic fungi
Chairs: Todd Kabaluk / Edith Ladunder

CONTRIBUTED PAPERS. Thursday, 17:00 MC-39
Efficacy of Beauveria bassiana strain ATCC 74040 (Naturalis\(^9\)) against the leafhopper Scaphoideus titanus Ball under open-field conditions
Ladurner, E.; Benuzzi, M.; Florentini, F.
CBC (Europe) S.r.l. – BIOGARD Division, AREA TECNICA, Cesena, Italy
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The leafhopper, Scaphoideus titanus Ball (Cicadellidae), native to North America, was introduced accidently to Europe, where it has become a key pest by acting as the principal vector of the grapevine flavescence dorée phyllotasis (FD). FD is a quarantine disease (EPPO A2 List), and insecticide sprays against its vector are mandatory in several European countries. These sprays aim at killing nymphs before they become reproductive, i.e. before they reach the 4\(^{\text{th}}\)-5\(^{\text{th}}\) instar. However, due to the
Effects of entomopathogenic fungi against the crape myrtle bark scale and its natural enemies

Franco, G.M.1; Chen, Y.2; Diaz, R.1
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Crape myrtles, Lagerstroemia sp., are one of the most common ornamental plants in Southeastern U.S. In 2004 the crape myrtle bark scale (CMBS), Acanthococcus lagerstroemiae was detected, leading to an increase in management costs. CMBS directly impacts crape myrtle’s aesthetic and is being managed with pesticides, which threat natural enemies (NE). Entomopathogenic fungi are frequently used to control soft-bodied insects and might not impact NE population, but there is no information on its efficacy against CMBS. Studies regarding direct infection and scale mortality were conducted, where infested plants were sprayed with commercial formulations of Isaria fumosorosea 97 strain, Beauveria bassiana GHA strain, and B. bassiana ANT-03 strain. Scale mortality was assessed daily during a week period by removing scales from the tree and checking leg movements. Percentage of scale mortality was significantly higher in B. bassiana ANT-03 strain (46.5 ± 4.47) and I. fumosorosea (41.8 ± 4.23) when compared to water control (26.1 ± 3.79). NE population was sampled from the treated trees and entomopathogens were recovered from their bodies, but no infection was observed, leading to the conclusion that NE help dispersing the tested entomopathogens. Further investigation is needed to build a comprehensive Integrated Pest Management of CMBS.

Contributed papers. Thursday, 17:45 MC-42
promising new biocontrol organism against wireworms.

CONTRIBUTED PAPERS. Thursday, 18:15 MC-44

Towards *Dactylopus opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) biological and integrated management in Cadiz Province (Spain)

**Yousef Naef, M.; Quesada-Moraga, E.**
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The false carmine cochineal *Dactylopus opuntiae* has been originally used as biological control agent of the prickly pear cactus *Opuntia ficus-indica* Miller, considered as an exotic invasive species in many countries including Spain. However, the extremely high level of infestation has resulted in great inconvenience to the population of local residents and a serious threat to wipe out a plant that is already part of the Andalusian landscape.

In the present research, different control means were evaluated towards this cochineal at field condition in a site in the province of Cadiz (Spain). The populations of the insect in the study site were confirmed to belong to *D. opuntiae*. We detected this insect throughout the study area with a very high population density in terms of number of colonies per cladode and the extent of the infestation. From other hand, we evaluated the efficacy of four different methods (Chlorpyrifos-methyl, Potassium soap, *Crytolaemus montouzieri* larvae, the entomopathogenic fungus * Beauveria bassiana*) for the control of *D. opuntiae*. Our results demonstrated that the Potassium soap and Chlorpyrifos-methyl were the best treatments in reducing *D. opuntiae* populations, with relative population density values being negative only in these two treatments, and efficacies of 91.5 and 76.7% respectively. The results of this investigation showed that the current situation of false carmine cochineal is worrisome, but nevertheless, the use of Potassium soap for its control is recommended because of its high efficacy and low Environmental Impact.

CONTRIBUTED PAPERS. Thursday, 18:30 MC-45

Fitness effects of the newly-discovered microsporidian species *Tubulinosema sp.* on its host *Drosophila suzukii*

**Biganski, S.; Jehle, J.A.; Kleesspies, R.G.**
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A most likely new microsporidian species was discovered and isolated from the invasive Spotted Wing Drosophila (SWD), *Drosophila suzukii* MATSUMURA. Laboratory infection experiments of early stages of SWD larvae resulted in significantly decreased life span and hatching rates depending on applied spore concentration. Furthermore, major impacts on the ability to lay eggs could be found in single pair infection experiments. Infected SWD adults that had been inoculated with *Tubulinosema* spores in second larval stage laid over 70% less eggs compared to controls. The strongest negative effect on fecundity was achieved when both male and female parents were infected (77% less eggs), and secondly for pairs with only infected female parent (59% less), whereas male infection only showed the lowest impact (42% less). When microsporidia transmission from parents to offspring was investigated, a negligible fraction of infected F1 (<4%) was recorded, most likely caused rather by horizontal than vertical transmission. In contrast, inoculation of adults instead of larvae revealed a significant lower effect on fecundity and fertility. The hatching rate as well as the sex ratio of the resulting offspring was not affected at all, irrespectively whether larvae or adults were infected. Life time was also reduced through infection in larval stage but not after inoculation of adult flies. These experiments show that infection with *Tubulinosema* sp. have a strong impact on the population of SWD.

Simplifying insect pathology by means of the Foldscope

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Given that insect pathology starts with and revolves around diagnosis, simplifying the process of identification and characterisation of the disease-causing agent is highly desirable. We found the Foldscope (Foldscope Instruments, Inc.), which is a paper-based, origami-style microscope, extremely useful to study the signs and symptoms, as well as the etiology and pathology, of disease in tiny insects. Multiple entomopathogenic fungal infections were studied in aphids, thrips, whiteflies, scale insects, mealybugs, leafhoppers, planthoppers and other small insects. Sections of diseased larger insects could also be examined and investigated with the help of the Foldscope. At the maximum obtainable magnification of 140x, fungal spores, bacterial cells or nematodes could be examined with, or in most cases without, staining. The 2-micron resolution obtainable was sufficient to differentiate several genera, or even known species, of entomopathogens, particularly fungi. Images of entomopathogens were captured by connecting the Foldscope to a smartphone, which also enabled digital zooming as well as image enhancement. Live specimens, such as entomopathogenic nematodes and fungal spores, suspended in water or other liquids could be visualized using a smartphone. Besides insects, we were also able to investigate fungal diseases of phytophagous mites, including tetranychids, tenuipalpids and eriophyoids. The greatest advantage of this pocket-sized gadget is its in-situ use to examine quickly made slides under available or ambient light conditions, especially in the field. In a series of farmer-oriented or student-specific workshops across India, we have introduced insect pathology to the participants with the aid of the Foldscope.

Fitness effects of the newly-discovered microsporidian species *Tubulinosema sp.* on its host *Drosophila suzukii*

**Biganski, S.; Jehle, J.A.; Kleesspies, R.G.**
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A most likely new microsporidian species was discovered and isolated from the invasive Spotted Wing Drosophila (SWD), *Drosophila suzukii* MATSUMURA. Laboratory infection experiments of early stages of SWD larvae resulted in significantly decreased life span and hatching rates depending on applied spore concentration. Furthermore, major impacts on the ability to lay eggs could be found in single pair infection experiments. Infected SWD adults that had been inoculated with *Tubulinosema* spores in second larval stage laid over 70% less eggs compared to controls. The strongest negative effect on fecundity was achieved when both male and female parents were infected (77% less eggs), and secondly for pairs with only infected female parent (59% less), whereas male infection only showed the lowest impact (42% less). When microsporidia transmission from parents to offspring was investigated, a negligible fraction of infected F1 (<4%) was recorded, most likely caused rather by horizontal than vertical transmission. In contrast, inoculation of adults instead of larvae revealed a significant lower effect on fecundity and fertility. The hatching rate as well as the sex ratio of the resulting offspring was not affected at all, irrespectively whether larvae or adults were infected. Life time was also reduced through infection in larval stage but not after inoculation of adult flies. These experiments show that infection with *Tubulinosema* sp. have a strong impact on the population of SWD.

Invertebrate health as a sentinel of global ‘One Health’

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Most of the animals on Earth are invertebrates - with 1.3 million species described and many millions more undiscovered. Inhabiting all major biomes, they perform critical roles as pollinators, degraders of organic material, predators, prey, parasites and in numerous cases, as direct food sources for humans. Widely publicised declines in terrestrial insects are mirrored by epidemics in aquatic invertebrate populations (e.g. starfish, lobsters) and, by the emergence of disease in domesticated stocks (e.g. bees, shrimp). Cold-blooded, short-lived, with relatively non-adaptive immune systems, invertebrate health outcomes directly reflect the status of their habitats; poor health outcomes in turn implicating plant, animal and human wellbeing. Although generally under-studied (and under-appreciated) invertebrate health can thus be considered a sentinel for ecosystem (and planetary) health. In the context of ‘One Health’ (the collaborative efforts of numerous deep specialists, working at multiple scales to attain optimal health outcomes for people, animals and the environment) it is proposed that invertebrate health outcomes offer an excellent metric to assess environmental status and, departure of habitats from their environ-
mental optima. Here, I will illustrate this thinking using the example of the global emergence of the Microsporidia – opportunistic parasites that infect everything from protozoa to humans. In particular, the Enterocytozoon group Microsporidia (EMG) have emerged in shrimp, fish, mammals, birds and humans over the past 3 decades, associated with intensive aquaculture (fish, shrimp), terrestrial farming (pigs, cows, chickens) and, due to the rise of underlying immune-suppressive conditions such as AIDS and an ageing population (humans).

**SYMPOSIUM. Thursday, 17:30 DBIS-2**

**Crustaceans as Models for Understanding the Unique Application of One Health to a Changing Sea**

Behringer, DC.

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The very nature of the sea makes it an effective medium for distributing pathogens – much more so than the terrestrial realm. The differences between marine and terrestrial environments explain the rapid spread and wide distribution of many marine pathogens relative to their terrestrial counterparts. Considering fewer marine pathogens appear zoonotic relative to their terrestrial counterparts, the threat to human populations from pathogens has seemed to come primarily from threats to food security. However, our environment is changing and so is this viewpoint. It is therefore increasingly important for us to better understand the epidemiology and ecology of marine diseases so that we might account more explicitly for their impacts and gain predictive capability with regards to their spread and distribution.

Crustaceans present particularly valuable models to understand disease dynamics in the sea, but much remains unknown. Many crustacean hosts have long pelagic larval periods that connect their populations across vast expanses of ocean, but we know little about how their pathogens do the same. The properties of seawater that lend it so well to pathogen transport also make it effective for transporting chemosensory cues and crustaceans use chemoreceptors for a multitude of ecological functions, including disease avoidance. But again, the environment is in flux and we need a better grasp on how this might tip the balance and change pathogen-host relationships, change pathogen or host distributions, and change outcomes for fisheries, aquaculture, and human health.

**SYMPOSIUM. Thursday, 18:00 DBIS-3**

**Exploring the transmission of diseases between pollinators at flowers**

Bailes, EJ

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Global food security is highly dependent on pollinators. Pollinators such as bees and hoverflies (Syphidae) increase the yield and quality of the majority of our crops, especially those high in micronutrients vital for human health. However, many of these pollinator populations are known to be in decline, which could have a strong impact on future food production.

In recent years, it has come to light that pathogens that are well known in managed honey bees are shared much more widely within the wild pollinator community. In particular, positive-stranded RNA viruses are potential emerging diseases that have been implicated in the declines of wild bee populations. Understanding the spread of emerging infectious diseases in terrestrial invertebrates is therefore of great importance to global food security.

Interspecific transmission of these bee diseases is thought to occur when pollinators forage on the same flowers. Here, I will highlight some of our recent work that investigates the transmission of disease between pollinators visiting flowers, including to non-bee species. In particular, I will focus on how management interventions, such as wildflower strips planted as part of agri-environment schemes, can alter the transmission of diseases between pollinators.

**SYMPOSIUM. Thursday, 18:30 DBIS-4**

The contribution of extension services to the monitoring of crop pests and to the uptake of augmentative biocontrols in selected low to lower-middle income countries

Edgington, S

CABI, Bakeham Lane, Egham, Surrey, UK

Corresponding author: s.edgington@cabi.org

Monitoring the movement of crop pests and the uptake of augmentative biological control agents (BCAs) to manage them, is limited in many low to lower-middle income countries. This study details how national extension partners (NEPs) in Plantwise - an agricultural development programme facilitating networks of plant clinics where farmers can obtain free, plant health advice, can contribute to both the monitoring of pest outbreaks and, their management using BCAs. Using data generated by NEPs, BCA recommendations that appeared in Plantwise pest management decision guides and/or were given by extension workers at plant clinics in Ghana, Kenya, Zambia, India, Nepal and Pakistan were analysed. For a 12-month period (2015-2016) 45,757 recommendations were given to farmers at the plant clinics, of which around 17,000 were for arthropod pests. BCA recommendations appeared in 13% (Zambia) to 61% (India) of the decision guides assessed; and were present in 0% (Zambia) to 18% (India) of the written recommendations given. Knowledge, availability and price were identified as the main factors affecting the uptake and inclusion of BCA recommendations by NEPs.

**BANQUET**

Thursday, 20:00-3:00

La Cartuja

**We hope to see you in Mérida, México, for SIP 2020!**
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