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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Patricia Stock

AN INTRODUCTION TO IOBC

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:

Gerben MESSELINK, Sylvia BLÜMEL, Giselher GRABENWEGER, Ilaria PERTOT, Andrea LUCCHI, Philippe C. NICOT

ORGANISATION

ORGANISATION OF IOBC-WPRS

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· IP & Biocontrol in North-African countries
Ahmed MAZIH, Institut Agronomique et Vétérinaire Hassan II, Maroc
· Guidelines for integrated production
Carlo MALAVOLTA, Regione Emilia-Romagna, Italy, *ad interim*

IOBC-WPRS Working Groups

· Benefits and risks of exotic biological agents
Convenor: Olga AMEIXA, University of Aveiro, Portugal
· 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: Bärbel 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, Vladimir Puza and Yolanda Bel

Viruses: Elizabeth Herniou, Miguel López-Ferber and Salvador Herrero

PROGRAMME

Sunday 28th July

SUNDAY 28th JULY

	Press room	Foyer
9:00-17:00	SIP Council Meeting	REGISTRATION
14:00-20:00		
19:00-22:00		MIXER

Monday 29th July

MONDAY 29th JULY

Auditorium 2

8:30-10:30	**Opening Ceremony and Awards Presentations** Juan Ferré and Baltasar Escriche, Chairs of Local Organizing Committee Representatives from Univ. València and Ajuntament de València Zhihong (Rose) Hu, President of the Society for Invertebrate Pathology Eustachio Tarasco, Convenor IOBC WG Microbial and nematode control of invertebrate pests Patricia Stock: Students' Award Ceremony Founder's Lecture Introduction by Neil Crickmore Honoree: Prof Max Bergoin Lecturer: Elisabeth Herniou
10:30-11:00	Coffee Break
11:00-13:00	**Plenary Symposium: Resistance to microbial control agents** Convenors: Raquel Campos-Herrera and Joel González Cabrera Can insects develop resistance to fungal biocontrol agents? Tariq Butt Mechanisms of practical resistance to commercially relevant entomopathogenic bacteria Juan Luis Jurat-Fuentes Lessons told by nature: Resistance of codling moth against granulovirus and its implications for pest control Cydia Pomonella Johannes Jehle Sand crickets and mole crickets are resistant to entomopathogenic nematodes and their bacteria Adler Dillman

	Press room	Auditorium 2	Multispace AB	Multispace CD	Commission R 8
13:00-14:30 LUNCH	JIP & BC editorial meeting		Student workshop P. Stock: The nuts and bolts of grant writing		
14:30-16:30		Cross-divisional Symp. BAC/Virus The multiple layers of host-pathogen interactions	Fungi 1 Isolation, diversity and ecology	Microbial control 1 Bacteria and proteins	Nematodes 1 EPN ecology and behaviour
16:30-17:00	Coffee Break				

Monday 29th July

17:00-19:00		Virus 1 Virus discovery and taxonomy	Cross-divisional Symp. MC/Fungi Microbial control of wireworms	Bacteria 1 Crystal proteins mode of action	Nematodes 2 EPN biocontrol
19:30-21:30	Slugs & Snails DIVISION BUSINESS MEETING	Virus DIVISION BUSINESS MEETING	Fungi DIVISION BUSINESS MEETING	Bacteria DIVISION BUSINESS MEETING	Nematodes DIVISION BUSINESS MEETING

Tuesday 30th July

TUESDAY 30th JULY

7:30-9:00	**5K Run at Turia Gardens (old river bed)** *bus transfer from Valencia Conference Venue to old river bed at 07:00h			
	Auditorium 3	Multispace AB	Multispace CD	Commission R 8
9:30-11:30	Microbial control 2 Control of soil dwelling pests	Virus Symposium Covert virus infections in insects	Fungi 2 Mode of action	Bacteria Workshop (part 1) Domain-based specificity and protein structure-function …
11:30-12:00	Coffee Break			
12:00-14:00	Microbial control 3 Entomopathogenic fungi allone or in combination	Virus 2 Population genetics and ecology	Fungi Symposium Managing ticks populations with fungi: Accomplishments and challenges	Bacteria Workshop (part 2) … to help determine safety in insecticidal proteins
14:00-15:00	Distribution of lunch boxes			
15:00-18:30	**Excursions:** Walking Tour Valencia Historical Center: meeting point 17:00h at Serrano's Tower City of Arts and Sciences and Oceanographic and Albufera Natural Park: bus transfer from Valencia Conference Venue at 15:00h			
20:00-23:00	**BBQ (Jardines de la Hacienda)** *bus transfer from Valencia Conference Venue to BBQ at 19:30			

Wednesday 31st July

WEDNESDAY 31st JULY

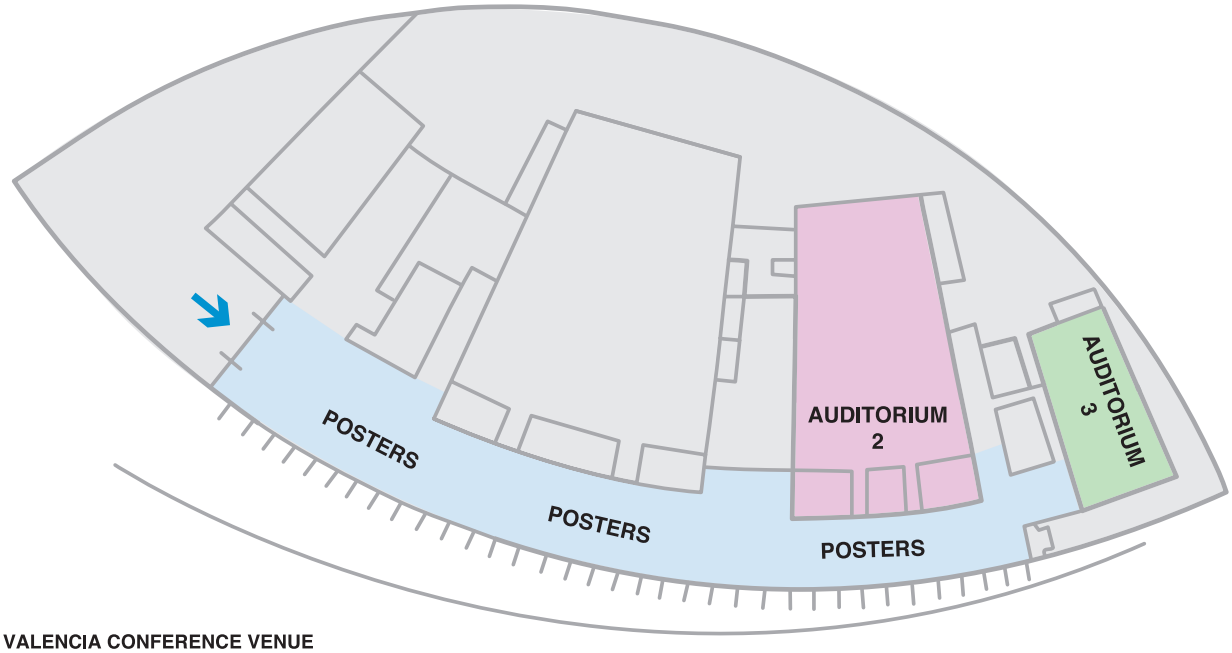
	Auditorium 3	Multispace AB	Multispace CD	Commission R 8
8:30-10:30	Bacteria 2 Molecular insights into Bt toxicity	Virus 3 Pathogenicity and Virulence	Fungi 3 Entomopathogenic fungi as endophytes	Nematodes Symposium Nematode application, what, when, and how?
10:30-11:00	Coffee Break			
11:00-13:00	MC Symposium Biopesticides IV. Realising the potential: Ecological benefits of microbial biocontrol	Virus 4 Infection cycle and morphogenesis	Fungi 4 Control of ticks and piercing-sucking insect pests	Nematodes 3 EPN infection process and bioprocessing
13:00-14:30 LUNCH		Virus Workshop The forthcoming change in virus species naming to a binomial system	Science communication	
14:30-16:30	Bacteria 3 Entomopathogenic bacteria diversity	Virus 5 Immunity and host response	Slugs & Snails 1 IPM Toolkit - Biological Control, Mollusc Behaviour and Mollusc Biology	DBI 1 Important diseases of beneficial invertebrates; from cockles to crickets
16:30-17:00	Coffee Break			
16:30-18:00	POSTER SESSION (Foyer)			
18:00-20:00	Microbial control 4 Formulation and field efficacy	Virus 6 Virus-host Interactions	Slugs & Snails 2 IPM Toolkit - Barriers, Monitoring and Molluscicides	DBI 2 Diseases of managed and wild bees
20:15-22:00	Microbial control DIVISION BUSINESS MEETING	Microsporidia DIVISION BUSINESS MEETING	Slugs & Snails Workshop Identification of molluscs and their associated nematode parasites	DBI DIVISION BUSINESS MEETING

Thursday 1st August

THURSDAY 1st AUGUST

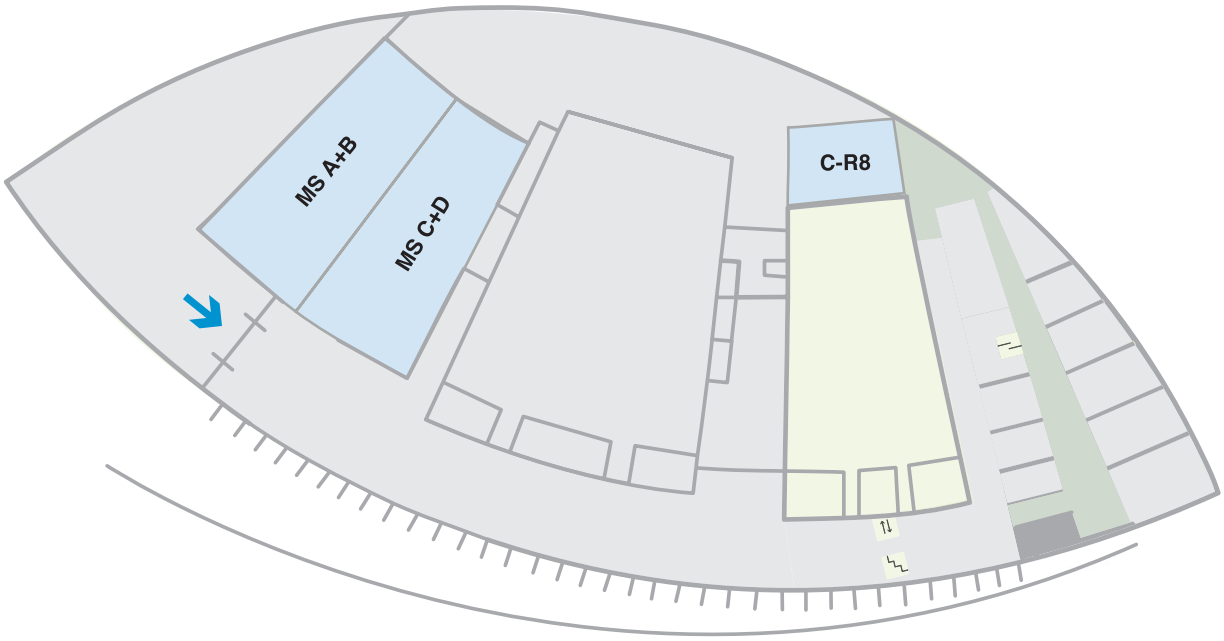
	Auditorium 3	Multispace AB	Multispace CD	Commission R 8
8:30-10:30	Microbial control 5 Microbial control	Virus 7 Biological control	Nematodes 4 Novel approaches in the basic and applied research on EPN	Microsporidia Microsporidia-host interactions: from organism to molecular levels
10:30-11:00	Coffee Break			
11:00-13:00	SIP BUSINESS MEETING			
13:00-14:30 LUNCH	IOBC Business Meeting	JURY student competition		
14:30-16:30	Bacteria 4 Bacterial symbionts of invertebrates	Slugs & Snails Symposium Future of Integrated Pest Management for Mollusc Control	Fungi 5 Control of chewing insect pests	Cross-divis. Symp. DBI/Microsporidia Microsporidia and microsporidia-like cryptomycota infecting micro-eukaryotes and metazoan parasites
16:30-17:00	Coffee Break			
17:00-19:00	Bacteria Symposium Insecticidal Bacteria: Cornerstones for Biological Control and IPM Programs	ICTV study group meeting	Microbial control 6 Entomopathogenic fungi	DBI Symposium Emerging Diseases in Invertebrates as One Health Sentinels
20:00-3:00	**BANQUET (La Cartuja)** *bus transfer from Valencia Conference Venue to Banquet at 19:30			

MAPS



VALENCIA CONFERENCE VENUE

GROUND LEVEL



VALENCIA CONFERENCE VENUE

FIRST FLOOR

VALENCIA

SIP/IOBC

2019

DETAILED PROGRAMME

MONDAY - 29th July

Opening Ceremony Monday, 08:30-10:30
Awards Presentations Auditorum 2
Founders Lecture

Coffee Break Monday, 10:30-11:30 Foyer

PLENARY SYMPUSIUM Monday, 11:00-13:00 Auditorium 2

Resistance to microbial control agents

Organisers / Chairs: Raquel Campos-Herrera / Joel González-Cabrera

PLENARY SYMPOSIUM. Monday, 11:00 **PL-1**

Can insects develop resistance to fungal biocontrol agents?

Butt, T.M.[1]*; Dubovskiy*[2]*, I.; Grizanova*[2]*, E.; Coates*[1]*, 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.***

JIP and BC editorial meeting Monday, 13:00-14:30 Press room

STUDENT WORKSHOP Monday, 13:00-14:30 Multispace AB

The nuts and bolts of grant writing

Organizer: Patricia Stock

Lunch Monday, 13:00-14:30 Foyer

CROSS-DIVISIONAL SYMPOSIUM BACTERIA-VIRUS Monday, 14:30-16:30 Auditorium 2

The multiple layers of host-pathogen interactions

Organisers / Chairs: Umut Toprak / Salva Herrero

SYMPOSIUM. Monday, 14:30 **BVCS-1**

Effect of the peritrophic membrane on toxicity of Bt Cry toxins in insects

Wang, Ping

SYMPOSIUM. Monday, 14:50 **BVCS-2**

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

Erlandson, Martin A*.*

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.[1]

SYMPOSIUM. Monday, 15:30 **BVCS-4**

Insect immunity as affected by stress factors

Pennacchio, F.

SYMPOSIUM. Monday, 15:50 **BVCS-5**

Pathogens associated with invasive or introduced insects threaten the health and diversity of native species

Vilcinskas, Andreas[1,2]

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[1]*, Simone Gasque*[1]*, Hans M. Smid*[2]*, Monique M. van Oers*[1]*,* ***Vera I.D. Ros***[1]

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

Insolation, diversity and ecology

Chairs: Inmaculada Garrido-Jurado / Claudia López-Lastra

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 Entomophtoral pathogen of leaf cutter ants: a possible new species of *Conidiobolus*

Goffré, D. [1]*; Jensen, A. B.* [2]*;Lopez Lastra, C.* [3]*; Valencia Carrasco, C.* [1]*;* ***Folgarait, P. J.*** [1]

CONTRIBUTED PAPERS. Monday, 15:15 **F-4 STU**

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]

CONTRIBUTED PAPERS. Monday, 15:30 **F-5**

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.*[1]*; Sabbatini Peverieri, G.*[1]*; Mazza, G.*[1]*; Bosio, G.*[3]*; Venanzio, D.*[3]*; Giacometto, E.*[3]*; Roversi, P.F.*[1]

CONTRIBUTED PAPERS. Monday, 15:45 **F-6**

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]

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

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

Senthil Kumar, C. M.[1]*; Jacob T. K.*[1]*; Devasahayam S.*[1]*; Geethu C.*[1]*; Hariharan V.*[1]

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

Updated checklist of Entomophthoralean fungi from Argentina

López Lastra, C.C.[1,]*; Manfrino, R.G.2*[1]*; Toledo, A.V.* [2]*; Gutierrez, A.C.,* [1,]*Mendiburu, M.*[1]

CONTRIBUTED PAPERS — Monday, 14:30-16:30
MICROBIAL CONTROL 1 — Multispace CD

Bacteria and proteins

Chairs: Travis Glare / Monika Maurhofer

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

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

Vaughan, A. *[1,2]; Glare, T. [1]; Hurst, M.[2]*

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

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

Huang, T.*; Ma, S.; Yao, J.; Guan, X.*

CONTRIBUTED PAPERS. Monday, 15:00 **MC-3**

Resistance to dsRNA and cross-resistance to Cry3Aa in Colorado potato beetle (*Leptinotarsa decemlineata*)

Mishra S.*[1]; Dee, J.[1]; Moar, W.[2]; Beattie, J.[2]; Jurat-Fuentes, J.L.[1]*

CONTRIBUTED PAPERS. Monday, 15:15 **MC-4**

Is oligomerization an important step in toxicity of the *Bacillus thuringiensis* insecticidal protein Cry1Ia?

Khorramnejad, A.*[1,2]; Domínguez, M.[3]; Caballero, P.[3]; Escriche, B.[1]; Bel, Y.[1]*

CONTRIBUTED PAPERS. Monday, 15:30 **MC-5**

Pseudomonas protegens* CHA0 transcriptome changes in response to root- and insect associated lifestyles *Vesga, P.*[1]; Keel, C.[2]; Croll, D.[3]; Maurhofer, M.[1]***

CONTRIBUTED PAPERS. Monday, 15:45 **MC-6**

Fear no weevil: searching the microbiome for a sweetpotato weevil biocontrol agent

Keyser, C.[1]; *Davis, J.[2]; Hernowo, K.[2]; O. Anyanga, M.[3]; Pepe-Ranney, C.[1]; Bissinger, B.[1]*

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

Double agents in the plant's service: root-colonizing pseudomonads with anti-fungal and anti-insect activities

Maurhofer, M.*[1]; Vesga, P.[1]; Spescha, A.[1]; Flury, P.[1]; Löser, T.[1]; Augustiny, E.[1]; Schneider, J.[1]; Vacheron, J.[2]; Grabenweger, G.[3]; Keel, C.[2]*

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

Interaction between novel insecticidal proteins from plants and lepidopteran pests

Rauscher, Gilda[1]; *Leng, Song[1]; Bowling, Andew[1]; Pence, Heather[1]; Barry Jennifer[1]; Liu, Lu[1]; Schepers, Eric[1]; Lum, Amy[1]; Yalpani, Nasser[1]; Gerber, Ryan[1]; Jimenez, Nuria[1]; Haile, Fikru[1]; Heckert, Matt[1]; Crane, Virginia C. [1]; Kassa, Adane[1]; Pilcher, Carol[1]; Booth, Russ[1]; Nelson Mark[1]; Nowatzki, Timothy M.[1]; Lu, Albert L.[1]; Wu, Gusui[2]*

CONTRIBUTED PAPERS — Monday, 14:30-16:30
NEMATODES 1 — Commission R8

EPN ecology and behaviour

Chairs: Selcuk Hazir / Christine Griffin

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

Do *Photorhabdus temperata* and *Photorhabdus cinerea*, symbionts of *Heterorhabditis downesi*, co-exist at the same site by niche separation?

Maher, A.M.D.[1]; Asaiyah, M.A.M.[1]; Quinn, S.[1]; Wolff, H.[2]; Bode, H.B.[2]; **Griffin, C.T.**[1]

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

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

Půža V.*[1]; Jakubíková H.[1,2];* Čápová *D.[1]; Nermuť J.[1]; Mráček Z.[1]*

CONTRIBUTED 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?

Campos-Herrera R.*[1]; Blanco-Pérez R.[1]; Duncan L.W.[2]*

CONTRIBUTED 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

Glazer I[1]; *Velayudhan S.S[1]; Faigenboim A.[2]; Salame L.[1]; Hetzroni A.[3]; Ment D.[1]*

CONTRIBUTED PAPERS. Monday, 15:30 **N-5 STU**

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

La Forgia, D.*[1]; Jaffuel, G.[2]; Campos Herrera, R.[3]; Turlings, T.C.J.[2]; Verheggen, F.[1]*

CONTRIBUTED PAPERS. Monday, 15:45 **N-6**

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

Hazir, S.*[1]; Ulug,_D.[1]; Cimen, H.[1]; Gulsen, S.H.[1]; Touray, M.[1]; Gulcu, B.[2]; Bode, H.B.[3]; Hazir, C.[4]; Karagoz, M.[5]; Bilecenoglu, D.K.[6]; Kaya, H.K.[7]*

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

Characteization of some entomopathogemic nematodes and fungi from the soil of Afghanistan

Fallahzadeh, H.[1,2]; Shokoohi, E.,[3]; Tarasco, E.[4]; Moravej, G.[1]; ***Karimi, J.[1]***

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

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

Özdemir, ***Esengül[1]****; Bayram, Şerife[1]; Toprak, Umut[1]; Evlice, Emre[2]*

Coffee Break — Monday, 16:30-17:00
Foyer

CONTRIBUTED PAPERS — Monday, 17:00-19:00
VIRUS 1 — Auditorium 2

Virus Discovery and taxonomy

Chairs: Robert Harrison / Elisabeth Herniou

CONTRIBUTED PAPERS. Monday, 17:00 **V-1**

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

Burke, Gaelen R.*[1]*

CONTRIBUTED PAPERS. Monday, 17:30 **V-2**

The variable landscape of nonretroviral RNA virus integrations in worldwide samples of the arboviral vector *Aedes aegypti*

Crava, Cristina M.*[1]; Pischedda, Elisa[1]; Di Mattia, Annamaria; Tancredi[1]; Alessandra, Scolari, Francesca[1]; Afrane, Yaw[2]; Ayala, Diego[3]; Carballar-Lejarazú, Rebeca[5]; Bonizzoni, Mariangela[1].*

CONTRIBUTED PAPERS. Monday, 17:45 **V-3 STU**

***Drosophila suzukii* viruses as potential tool for biological control**

Carrau, Tessa[1], Hiebert, Nils[1], Gemmer, Christina[2], Vilcinskas, Andreas[1,2], Lee, Kwang-Zin[1,2]

CONTRIBUTED PAPERS. Monday, 18:00 **V-4**

The invasive hornet *Vespa velutina* carries both honey bee viruses and new viruses.

*Dalmon, A.[1,2]; **Gayral, P.[3]**; Decante, D.[2,4]; Klopp, C.[5]; Bigot, D.[3,6]; Thomasson, M[1,2]; Herniou, E. A.[3]; Alaux, C.[1,2]; Le Conte, Y.[1,2]*

CONTRIBUTED PAPERS. Monday, 18:15 **V-5 STU**

Characterisation of the RNA virosphere in Australian tephritid fruit flies

***Sharpe, Stephen R.**[1]; Morrow, Jennifer L[1]; Brettell, Laura E[1]; Papanicolaou, Alexie[1]; Chapman, Tony A[2]; Cook, James M[1]; Riegler, Markus[1]*

CONTRIBUTED PAPERS. Monday, 18:30 **V-6 STU**

Identification of new viruses specific of the honey bee mite *Varroa destructor*.

Millán-Leiva, A.; *Herrero, S.; Coll, S.; González-Martínez, R. M.; Parenti, S.; González-Cabrera, J.*

CONTRIBUTED PAPERS. Monday, 18:45 **V-7**

Metatranscriptomics reveals novel RNA viruses of important rice invertebrates in China

*Haoran Wang[1,2]; Shufen Chao[1]; Guoqing Chen[1]; Dawei Song[1]; **Guozhong Feng[1]***

COSS-DIVISIONAL SYMPOSIUM — Monday, 17:00-19:00
MICROBIAL CONTROL-FUNGI — Multispace AB

Microbial control of wireworms

Organisers / Chairs: Dietrich Stephan / Stefan Jaronski

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?

Vidal, S[1]; Laurenz, S[1]; Patel, P[2]; Beitzen-Heineke, W.[3]

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

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

Kabaluk, T.

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

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

***Grabenweger, G.**[1]; Eckard, S.[1]; Reinbacher, L.[1]; Rogge, S.[2]*

CONTRIBUTED PAPERS — Monday, 17:00-19:00
BACTERIA 1 — Multispace CD

Crystal proteins mode of action

Chairs: Marianne Carey / Juan Luis Juart-Fuentes

CONTRIBUTED PAPERS. Monday, 17:00 **B-1**

Insights into the *in vivo* crystallization pathway and mechanism of toxicity of Cyt1Aa, a naturally crystalline mosquitocidal toxin

***Tetreau, G.*[*1]**; Banneville, A.-S.[*1]; Brewster, A.S.[*2]; Andreeva, E.[*1]; Hunter, M.S.[3]; Snigireva, I.[4]; Beaudoin, J.[1]; Burt, A.[1]; Bacia, M.[1]; Sierra, R.G.[3]; Zala, N.[1]; Burke, N.[1]; Bafna, J.A.[5]; Laporte, F.[6]; Fenel, D.[1]; Park, H.-W.[7,8]; Teulon, J.-M.[1]; Boeri-Erba, E.[1]; Rodriguez, J.A.[9]; Després, L.[6]; Vivaudou, M.[1]; Weik, M.[1]; Pellequer, J.-L.[1]; Boutet, S.[3]; Cascio, D.[9]; Signor, L.[1]; Winterhalter, M.[5]; Gutsche, I.[1]; Coquelle, N.[1,10]; Sauter, N.K.[2]; Sawaya, M.R.[9]; Federici, B.[7]; Colletier, J.-P.[1]*

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*

***Pinos, D.**; Martínez-Solís, M.; Herrero, S.; Ferré, J.; Hernández-Martínez, P.*

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

***Güney, G.[1,2,3]**; Hänniger, S.[2]; Heckel, D.G.[2]; Bayram, Ş.[1]; Coutu, C.[3]; Hegedus, D.[3]; Sezen, K.[4]; Güney, E.[4]; Cedden, D.[1]; Toprak, U.[1]*

CONTRIBUTED PAPERS. Monday, 17:45 **B-4 STU**

Receptor analysis of Cry1Ca toxin expressed on Sf9 cells

***Adegawa, S.**[1,2]; Sato, R.[1]*

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.[1,2]; Prentice, K.[3], Vera-Velasco, N.M.[4]; Smagghe, G.[3]; **Hernández-Martínez, P.**[1]; Escriche, B.[1]*

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

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

***Domínguez, M.**[1]; Villanueva, M.[1,2]; Fernández, A.[1]; Caballero, P.[1]*

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.**[1]; Jiménez-Moreno, N.[2]; Orera, I.[3]; Williams, T.[4]; Fernández, A.[5]; Villanueva, M.[1,5]; Ferré, J.[6]; Caballero, P.[5] & Ancín-Azpilicueta, C.[2]*

CONTRIBUTED PAPERS — Monday, 17:00-19:00
NEMATODES 2 — Commission R8

EPN Biocontrol

Chairs: Ralf-Udo Ehlers / Annika Pieterse

CONTRIBUTED PAPERS. Monday, 17:00 **N-9**

Diabrotica v. virgifera* management using genetically improved strains of *Heterorhabditis bacteriophora

***Ehlers, R.-U.[1]**; Molina, C.[1]; Vandenbossche, B.[1]; Dörfler, V.[1]; Barg, M.[1]; Toepfer, S.[2]*

CONTRIBUTED PAPERS. Monday, 17:15 **N-10 STU**

Potential microbial control of xylophagous pests with entomopathogenic nematodes and fungi

***El Khoury, Y.**[1,2]; Noujeim, E.[2]; Ravlić, J.[3]; Oreste, M.[1]; Addante, R.[1]; Nemer, N.[4] Tarasco, E.[1]*

CONTRIBUTED PAPERS. Monday, 17:30 **N-11**

The potential use of native entomopathogenic nematodes isolated in the Italian areas infested by *Popillia japonica* (coleoptera: scarabaeidae)

*Torrini, G. [1]; Paoli, F.[1]; Simoncini, S.[1]; Strangi, A.[1]; Cutino, I.[1]; Benvenuti, C.[1]; Mazza, G.[1], Bosio, G.[2]; Venanzio, D.[2]; Giacometto, E.[2]; Tarasco, E.[3]; Sabbatini Peverieri, G.[1]; Roversi, P.F.[1]; **Marianelli L.[1]***

CONTRIBUTED PAPERS. Monday, 17:45 **N-12**

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]*

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

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*

Toubarro, 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.

Slugs & Snails DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Press room
Virus DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Auditorium 2
Fungi DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Multispace AB
Bacteria DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Multispace CD
Nematodes DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Commission R8

TUESDAY - 30th July

5K Race	Tuesday, 07:30-09:00 Old river bed

CONTRIBUTED PAPERS — Tuesday, 9:30-11:30
MICROBIAL CONTROL 2 — 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]*; Resquín-Romero, G.*[2]*; Yousef, M.*[1]*; Ríos-Moreno, A.*[3]*; 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, P[1,2]*; Karley, A.J*[1]*; Mitchell, C*[1]*; Graham, R.I.* [3]*; 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 fungus gnats, *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-Guimarães, R.*[2]*; Coyne, D.*[3]*; 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.[1,2]*; Humbert, P.*[1]*; Patel, A.*[1]

VIRUS SYMPOSIUM — Tuesday, 9:30-11:30
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.**[1]; Carballo, A.[2]; Herrero, S.[3]; Murillo, R.[4]; Caballero, P.[1,4]*

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

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

CONTRIBUTED PAPERS — Tuesday, 9:30-11:30
FUNGI 2 — Multispace CD

Mode of action

Chairs: Stefan Jaronski / Nemat Keyhani

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.**[1]; Muturi, E.J.[1]; Flor-Weiler, L.[1]; Rooney, A.[1]*

CONTRIBUTED PAPERS. Tuesday, 10:00 **F-11**

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

***Dubovskiy I. M.** [1,2]*, Grizanova E. V. [1], Coates C. J. [3], Butt T. M. [3]*

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.**[1]; Silva, Cárita S.R.[2]; Arruda, Walquíria[3]; Keyhani, Nemat O.[4]; Fernandes,* Éverton *K.K.[2]*

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.**[1,4]; Belaich, M. [2]; Villamizar, L.[3]; Patarroyo, M. A. [4,5]; Barrera, G.[1]*

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.**[1]; Ren, K.[2]; Shao, W.[2]; Ying, S.[2]; Feng, M-g.[2]*

BACTERIA WORKSHOP PART 1 — Tuesday, 9:30-11:10
Commission R8

Domain-based spedificity 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.**[1]; Jiménez Juárez[1], N.; Pérez Ortega[1]; C., Pence, H.[2]; Bowling, A.[2]; and Lu., A.[1]*

WORKSHOP. Tuesday, 10:10 **BWS-3**

WCR-active protein complexes from fungal genus *Pleurotus*

***Sepčić, K.**[1]; Panevska, A.[1]; Razinger, J.[2]; Modic, Š.[2]*

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.[1] ; Milligan, J.[1] ; Bean, G.[1] ; Slightom, R.[1] ; Howe, A.[1] ; Werner, B.[1] ; Moore, R.[1] ; Pleau, M.[1] ; Nance, A.[1] ; Yin, Y.[1] ***; Bowen, D.[1]***

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 spedificity 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.**[1]*

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[a], Ruby H.P. Law[a], Charles Bayly-Jones[a], Stephanie Kondos[a], Siew Siew Pang[a], Hari Venugopal[a], Tom T. Caradoc-Davies[b], James C. Whisstock[a], Michelle A. Dunstone[a]

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.**[1]; Quan, Y.[1]; Banyuls, N.[1]; Van Rie, J.[2]*

CONTRIBUTED PAPERS Tuesday, 12:00-14:00
MICROBIAL CONTROL 3 Auditorium 3

Entomopathogenic fungi allone or in combination

Chairs: Giselher Grabenweger / Waqas Wakil

CONTRIBUTED PAPERS. Tuesday, 12:00 **MC-17**

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.*[2]*; Ment, D.*[2]

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

Biological control of pollen beetles with Beauveria bassiana

Kaiser, D.[1]*; Grabenweger, G.*[1]*; Bacher, S.*[2]

CONTRIBUTED PAPERS. Tuesday, 12:30 **MC-19 STU**

Compatibility of the parasitoid *Hyposoter didymator* and the entomopathogenic fungus *Metarhizium brunneum* for the control of *Spodoptera littoralis*

Miranda-Fuentes, P.*; Yousef, M.; Quesada-Moraga, E.*

CONTRIBUTED PAPERS. Tuesday, 12:045 **MC-20 STU**

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

González-Mas, N.*[1]*; *Sánchez-Ortiz, A.*[2]*; Valverde-Garcia, P.*[1]*; Quesada-Moraga, E.*[1]

CONTRIBUTED PAPERS. Tuesday, 13:00 **MC-21**

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

Wakil. W[1,2]*; Tahir, M*[2,3]*; Usman, M*[1]*; Gulzar, S*[1]

CONTRIBUTED PAPERS. Tuesday, 13:15 **MC-22 STU**

Ecological control of Japanese pine sawyer beetle, *Monochamus alternatus* vectoring pine wilt nematode using *Metarhizium anisopliae*

Kim, Jong Cheol; *Shin, Tae Young; Kim, Sihyeon; Lee, Mi Rong; Park, So Eun; Li, Dong Wei; Beak, Sehyeon; Jo, Min Sung; Gasmi, Laila; Kim, Jae Su*

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 robertsii* and avermectins on mosquito larvae: survival and immune responses

Noskov, Yu.A.[1,2]*, Polenogova, O.V.*[2]*, Yaroslavtseva, O.N.*[2]*, Belevich, O.E.*[2]*, Yurchenko, Yu.A.*[2]*, Chertkova, E.A.*[2]*, Kryukov, V.Yu.*[2]*, Glupov V.V.*[2]

CONTRIBUTED PAPERS Tuesday, 12:00-14:00
VIRUS 2 Multispace AB

Population genetics and ecology

Chairs: Adly Abd-Alla / Gealan Burke

CONTRIBUTED PAPERS. Tuesday, 12:00 **V-9**

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

Yang, J.; Zhang, H.; Zuo, Y.; Li, Lu.; Wu, W.; Yuan, M.; ***Yang, K.***

CONTRIBUTED PAPERS. Tuesday, 12:15 **V-10 STU**

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

Hinsberger, A.[1] *; Blachère-Lopez, C.* [1,2] *; Theulier, S.*[3] *; Guerrero, P.*[3] *; Lopez-Ferber, M.*[1] *; Bayle, S.*[1]

CONTRIBUTED PAPERS. Tuesday, 12:30 **V-11**

Canonical 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, Jiangbin[1,2]; Siripuk Suraporn[3]; Gani, M.[4]; Jehle, Johannes A.[1]

CONTRIBUTED PAPERS. Tuesday, 12:45 **V-12 STU**

Genetic variability in Chrysodeixis includens nucleopolyhedrovirus

Aguirre, Eduardo[1]*; Beperet, Inés*[2]*; Williams, Trevor*[3]*; López-Ferber, Miguel*[4]*; Caballero, Primitivo*[1,2]

CONTRIBUTED PAPERS. Tuesday, 13:00 **V-13**

A fish perspective of a shrimp disease: yellow head virus in two polyculture ponds

Minardi, D.[1]*; Bass, D.* [1]*; Sritunyalucksana, K.* [2]*; Itsathitphaisarn, O.* [3,4]*; Stentiford, G.D.* [1]

CONTRIBUTED PAPERS. Tuesday, 13:15 **V-14 STU**

Discovery, full-genome sequencing and phylogenetic analysis of a novel Deformed Wing Virus variant found in the dwarf honeybee, *Apis florea*

Hroobi, A.[1,2]*; Campbell, E.*[1]*; Bowman, A.*[1]

CONTRIBUTED PAPERS. Tuesday, 13:30 **V-15**

Seasonal pattern of viral load in colonies of *Apis mellifera* from Italian and French apiaries

Molinatto, G.[1,3]*; Peruzzi, M.*[2]*; Diévart, V.*[2]*; Mondet, F.*[2]*; Alaux, C.*[2]*; Kretzschmar, A.*[2]*; Marzachì, C.*[3]*; Bosco, D.*[1]*; Manino, A.*[1]

CONTRIBUTED PAPERS. Tuesday, 13:45 **V-16**

Occurrence and molecular phylogeny of honeybee viruses in hornets

Yang, Sa[1,2#]; Gayral, Philippe[3#]; Zhao, Hongxia[4#]; Wu, Yanyan[1,2]; Bigot, Diane[3†]; Wang, Xinling [1,2]; Yang, Dahe[1,2]; Herniou, Elisabeth A.[3]; Deng, Shuai [1,2]; Li, Fei [1,2]; Diao, Qingyun [1,2]; Darrouzet, Eric [3*]; **Hou, Chunsheng** [1,2*]

FUNGI SYMPOSIUM Tuesday, 12:00-14:00
Multispace CD

Managing ticks populations with fungi: Accomplishments and challenges

Organisers / Chairs: Jae Su Kim / Stefan Jaronski

SYMPOSIUM. Tuesday, 12:00 **FS-1**

Managing Tick Populations with Fungi: The Korean Experience

Lee, M. R.[1]*; Kim, J. C.*[1]*; Kim, S.*[1]*; Park, S. E.*[1]*; Li D.*[1]*; Jo, M.*[1]*; Shin, T. Y.*[1]*; Lee, D. H.*[2]*;* ***Kim, J. S.***[1]

SYMPOSIUM. Tuesday, 12:24 **FS-2**

Managing Ticks with Fungi: The Israeli Experience of tick-*Metarhizium* interaction

Ment, D.

SYMPOSIUM. Tuesday, 12:48 **FS-3**

Advances and challenges on the use of entomopathogenic fungi for tick control in Brazil

Fernandes, Éverton K.K.[1]*; Bittencourt, Vânia R.E.P.*[2]

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[1]

[1]Novozymes 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

WEDNESDAY - 31st July

CONTRIBUTED PAPERS — Wednesday, 08:30-10:30
BACTERIA 2 — Auditorium 3

Molecular insights into Bt toxicity

Chairs: OP Pereia / Colin Berry

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

Determination of critical regions for toxicity of the Vip3A protein in European, American, African and Asian pests

Gomis-Cebolla, J.[1]*; Bel, Y.*[1]*, Ferreira Dos Santos, R.*[2]*; Wang, Y.*[3]*; Caballero, J.*[4,5]*; Caballero, P.*[4,5]*; He, K.*[3]*; Jurat-Fuentes, J.L*[2]*; Ferré, J.*[1]

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

Characterization of *vip3* positive *Bacillus thuringiensis* isolates and toxicity of Vip3Aa65 against lepidopteran pests

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

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

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

CONTRIBUTED PAPERS. Wednesday, 09:15 **B-12**

Role of Sigma54 on sporulation in *Bacillus thuringiensis*

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

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

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

Zhang, R.[1,2]; *Slamti, L.*[2]; *Verplaetse, E.*[2]; *Zhang, J.*[1]; *Song, F.*[1]; *Lereclus, D.*[2]

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

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

EL Khoury, N*.*[1,2]*; Bennaceur, I.*[1]*; Majed, R.*[1,2] *; Kallassy, M.*[2]*; Gohar, M*[1]*.*

CONTRIBUTED PAPERS. Wednesday, 10:00 **B-15**

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

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

CONTRIBUTED PAPERS. Wednesday, 10:15 **B-16**

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

Placidi de Bortoli, C.[1]*; Banerjee, R.*[1]*; Meagher, R.*[2]*; Abdelgaffar, H.*[1]*; Yang, F.*[3]*; Kerns, D.*[3]*; Huang, F.*[4]*; Komivi, A.*[5]*; Rao, T.*[1]*; Jurat-Fuentes, J.L.*[1]

CONTRIBUTED PAPERS — Wednesday, 08:30-10:30
VIRUS 3 — Multispace AB

Pathogenicity and Virulence

Chairs: Cristina del Rincón / Mariano Belaich

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

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

Pigeyre Laetitia[1,2]*, Shatz Malvina Laetitia*[1,2]*, Ravallec Marc*[2]*, Gasmi Leila*[3]*, Clouet Cécile*[2]*, Guerardel Yann*[4]*, Cot Didier*[5]*, Dupressoir Thierry*[1]*, Gosselin-Grenet Anne-Sophie*[2]*,* ***Ogliastro Mylene***[2]

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
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.[1]*; Beperet, Inés*[2]*; Williams, Trevor*[3]*; López-Ferber, Miguel*[4]*; Caballero, Primitivo*[1,2]

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[1]*; Vu, Quynh*[2]*;* ***Bigot, Diane***[1]*; Gogol-Döring, Andreas*[3,4]*; Koch, Peter*[4]*; Jenkins, Christina*[1]*; Doublet, Vincent*[1,5]*; Theodorou, Panagiotis*[1]*; Paxton, Robert J.*[1,3]

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
Vlak, Justinus M.[1]*, Ali, Ghulam*[1]*, van Strien E.A.*[3]*, van der Werf, Wopke*[2]*, Schijlen, Elio G.W.M.*[4] *and*
Zwart, Mark P.[3]

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
Carpenter, Alexis[1]*; Bryant, William B.*[1,2]*;* ***Clem, Rollie J.***[1]

CONTRIBUTED PAPERS. Wednesday, 10:00 **V-23**
The effect of light signal on the climbing behavior of cotton bollworms infected with NPV
Liu Xiaoming[1]*, Tian Zhiqiang*[1]*, Shen Zhongjian*[1]*, Yu Jian*[1]*, Liu Xiaoxia*[1]

CONTRIBUTED PAPERS. Wednesday, 10:15 **V-24**
Characterization of Novel RNA viruses isolated from tsetse fly *Glossina morsitans morsitans*
Meki, I.K.[1,2]*; Kariithi, H.M*[1,2]*.; Rezapanah, M*[1,3]*.; van der Vlugt, R.A.A*[2]*.;* ***Abd-Alla, A.M.M***[1]*.; van Oers, M.M*[2]*.; and Vlak, J.M*[2]*.*

CONTRIBUTED PAPERS — Wednesday, 08:30-10:30
FUNGI 3 — 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*
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.[1]*; Jensen, B.*[1]*; Saleem Akhtar, S.*[1]*; Roitsch, T. G.*[1]*; Meyling, N. V*[1]

CONTRIBUTED PAPERS. Wednesday, 09:00 **F-19**
Endophytic *Metarhizium robertsii* Affects Maize Growth and Gene Expression and Growth of Black Cutworm
*Ahmad****, I.,***[1] *Jimenez-Gasco, M.M.,*[2] *Luthe, D.S,.*[3] ***Barbercheck, M.E.***[1]

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

CONTRIBUTED PAPERS. Wednesday, 09:30 **F-21**
Interaction between *Beauveria bassiana* and the nitrogen fixing bacterium *Sinorhizobium meliloti*.
Ortiz-Urquiza, A.[1]

CONTRIBUTED PAPERS. Wednesday, 09:45 **F-22**
Effect of endophytically-colonized tomato and nightshade host plants on life-history parameters of *Tuta absoluta* (Lepidoptera: Gelechidae)
Akutse K. S.[1]*, Khamis F. M.*[1]*, Ekesi S.*[1]*, Wekesa S. W.*[1]*, and Subramanian S.*[1]

NEMATODES SYMPOSIUM — Wednesday, 08:30-10:30
Commission R8
Nematode application, what, when and how?
Organisers / Chairs: David Shapiro-Ilan / Raquel Campos-Herrera

SYMPOSIUM. Wednesday, 08:30 **NS-1**
Scaling up EPN production for sugarcane pest management in Brazil
Leite, L.G.[1]*; Shapiro-Ilan, D.I.*[2]*; Hazir, S.*[3]*; Chacon-Orozco, J.G.*[1]

SYMPOSIUM. Wednesday, 08:54 **NS-2**
Entomopathogenic nematode application: tools left in the box
Hiltpold, I.

SYMPOSIUM. Wednesday, 09:18 **NS-3**
Enhancing the aboveground efficacy of entomopathogenic nematodes
Shapiro-Ilan, D.I.[1,]*; Goolsby, J.A.*[2]

SYMPOSIUM. Wednesday, 09:42 **NS-4**
Entomopathogenic nematode application against root-damaging *Diabrotica* larvae in maize: what, when, and how?
Toepfer, S.[1]***;*** *Toth, S.*[1,2]

SYMPOSIUM. Wednesday, 10:06 **NS-5 STU**
A novel strategy to control fall armyworm with entomopathogenic nematodes
Fallet, P.[1]*; De Gianni, L.*[1]*; Kajuga, J.*[2]*; Waweru, B.*[2]*; Glauser, G.*[3]*; Toepfer, S.*[4]*; Turlings, T.C.J.*[1]

Coffee Break — Wednesday, 10:30-11:00
Foyer

MICROBIAL CONTROL SYMPOSIUM — Wednesday, 11:00-13:00
Auditorium 3
Biopesticides IV. Realising the potencial: Ecological benefits of microbial biocontrol
Organisers / Chairs: Roma Gwynn / Mike Brownbridge

SYMPOSIUM. Wednesday, 11:00 **MCS-1**
Realising the potential: Ecological benefits of microbial biocontrol
Gwynn, RL[1]***; Brownbridge, M***[2]***; Glare, TR***[3]

SYMPOSIUM. Wednesday, 11:30 **MCS-2**
Entomopathogens as endophytes: Their broader contribution to IPM
Quesada-Moraga, E.

SYMPOSIUM. Wednesday, 12:00 **MCS-3**
Integrating different strategies: Can we predict the outcome of multiple agents at different scales?
Meyling, NV.[1]

SYMPOSIUM. Wednesday, 12:30 **MCS-4**
***Quo Vadis*, Commercial Microbial Control?**
Where have we been, where are we going, and how do we get there?
Dimock, M.B

CONTRIBUTED PAPERS Wednesday, 11:00-13:00
VIRUS 4 Multispace AB

Infection cycle and morphogenesis
Chairs: David Thielman / Manli Wang

CONTRIBUTED PAPERS. Wednesday, 11:00 **V-25**
Autographa californica multiple nucleopolyhedrovirus *ie2* is critical for virus replication
Hepat, Rahul[1]; Willis, Leslie G.[1]; Sokal, Nadia[1]; Harrison, Robert L.[2]; Erlandson, Martin, A.[3];
Theilmann, David A.[1]

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. didymator* by RNA interference
Lorenzi, Ange *; Jouan, Veronique ; Ravallec, 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, Fengqiao[1,2] ; Kuang, Wenhua[1,3]; Wang, Xi[1,2]; Hou, Dianhai[1]; Huang, Huachao[1]; Sun, Xiulian [1];
Deng, Fei[1]; Wang, Hualin[1]; van Oers, Monqiue M.[4]; ***Wang, Manli[1]****; Hu, Zhihong[1]*

CONTRIBUTED PAPERS. Wednesday, 12:15 **V-30**
NSP2 forms viroplasms during Dendrolimus punctatus cypovirus infection
Congrui, Xu[1]; Jia, Wang[1]; Jian, Yang1; Chengfeng, Lei[1]; Jia, Hu[1]; Xiulian, Sun[1]

CONTRIBUTED PAPERS. Wednesday, 12:30 **V-31**
Virus biology of *Euscelidius variegatus* iflavirus 1: towards the production of an infectious viral clone
Marzachì, Cristina[1]*; Ottati, Sara[1]; Persico , Alberto[1,2]; Abbà, Simona[1]; Rossi, Marika[1]; Vallino, Marta[1]; Turina, Massimo[1]; Galetto, Luciana[1]*

CONTRIBUTED PAPERS. Wednesday, 12:45 **V-32**
Development of autofluorescent baculoviruses to follow infection in living cells
Hinsberger, A.[1]*; Graillot, B.[2]; Blachère-Lopez, C.[1,3] ; Juliant, S.[4]; Duonor-Cerutti, M.[4]; King, L.A.[5]; Possee, R.D.[5,6]; Gallardo, F.[7]; Lopez-Ferber, M.[1]*

CONTRIBUTED PAPERS Wednesday, 11:00-13:00
FUNGI 4 Multispace CD

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 longhornef tick, *Haemaphysalis longicornis*
Mi R. L.*, Dongwei L., Jong C. K., Sihyeon K., Tae Y. S. and Jae S. K.**

CONTRIBUTED PAPERS. Wednesday, 11:15 **F-26 STU**
e-Biopesticide: Management of silverleaf whitefly, *Bemisia tabaci* using entomopathogenic *Beauveria bassiana*
Baek, S*; Kim, JC; Kim S; Lee, MR; Li, D; Shin, TY; Kim, JS**

CONTRIBUTED PAPERS. Wednesday, 11:30 **F-27 STU**
isScreening Potential Entomopathogenic Fungi for the Control of the Greenhouse Whitefly (*Trialeurodes vaporariorum*)
Spence, E.[1,2]; *Hesketh, H.[1]; Svendsen, C.[1]; Chandler, D.[2]; Martin, G.[3]; Berry, S.[3]; Edgington, S.[4]*

CONTRIBUTED PAPERS. Wednesday, 11:45 **F-28**
Development of a biological tick control agent based on an innovative attract-and-kill strategy
Patel, A.[1]*; Lorenz, S.-C.[1]; Humbert, P.[1]; Wassermann, M.[2]; Mackenstedt, U.[2]; Przyklenk, M.[3]; Beitzen-Heineke, E.[3]; Beitzen-Heineke, W.[3]; Büchel, K.[4]; Dautel, H.[4]*

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.[1]*; Salimova, D.[1]; Dalinova, A.[1]; Stepanycheva, E.[1]*

CONTRIBUTED PAPERS Wednesday, 11:00-13:00
NEMATODES 3 Commission R8

EPN infection process and bioprocessing
Chairs: Li Xingyue / 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 beicherriana*)
Li, X.[1,2]*; Zhang, H.[1]; Cao, A.[2]; Yang, W.[1]; Liu,Q.[3]*

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.[1]*; Belur, P.D.[2]; Malan, A.P.[1]*

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.[1]; Enow, E.[2]; Tetteh, A.D.[2]; Molina, C.[1]; ***Ehlers, R.-U.[1,2]***

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.[1]*; Molnár, I.[2]; Stock, S.P. [1,3]*

CONTRIBUTED PAPERS. Wednesday, 12:30 **N-23**

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

Vandenbossche, B.[1]; *Molina, C.[1]; Barg, M.[1]; Dörfler, V.[1]; Consoli, E.[2]; Centurion Carrera, A.[2]; Ayral, S.[2]; Strauch, O.[1]; Ehlers, R.-U.[1]*

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 COMUNICATION — Wednesday, 13:00-14:30 — Multispace CD

Science Comunication

Organizer: A. Lorena Passarelli

SCIENCE COMUNICATION. Wednesday, 13:00

Science communication: How does it help the public, science education, research, and the scientist?

Passarelli, A. Lorena

Lunch — Wednesday, 13:00-14:30 — Foyer

CONTRIBUTED PAPERS BACTERIA 3 — Wednesday, 14:30-16:30 — Auditorium 3

Entomopathogenic bacteria diversity

Organisers / Chairs: Shuyuan Guo / Christina Nielsen-Leroux

CONTRIBUTED PAPERS. Wednesday, 14:30 **B-17**

Analysis of *Bacillus thuringiensis* diversity from different environments

Fernandez, A.B.[1]; *Caballero, P.[1]*

CONTRIBUTED PAPERS. Wednesday, 14:45 **B-18**

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

Wang, K. *[1]; Shu,C.[1]; Zhang, J.[1]*

CONTRIBUTED PAPERS. Wednesday, 15:00 **B-19**

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

Villanueva, M.[1,2]; *Valtierra-de-Luis[1], D.; Caballero, P.[1]*

CONTRIBUTED PAPERS. Wednesday, 15:15 **B-20 STU**

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

Kawahara, A.[1]; *Iiyama, K.[2]; Asano, S.[3]; Nishi, O.[1]; Yasunaga-Aoki, C.[1]*

CONTRIBUTED PAPERS. Wednesday, 15:30 **B-21**

Novel mosquitocidal toxins from Paraclostridia

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

CONTRIBUTED PAPERS. Wednesday, 15:45 **B-22 STU**

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

Hiebert, N.[1*]; Carrau, T.[1]; Bartling, M.[2]; Vilcinskas, A.[1,2]; Lee, K.-Z.[1,2]

CONTRIBUTED PAPERS. Wednesday, 16:00 **B-23**

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

Gholmie, M.A.R.[1]; Levy, S.M.[2]; Falleiros, Â.M.F.[2]; Lopes, I.O.N.[4]; Neiva, M.M.[3]; ***Sosa-Gómez, D.R.***[4]

CONTRIBUTED PAPERS. Wednesday, 16:15 **B-24**

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

Perera, O.P.[1]*; Polpitiya, A.D.*[2]

CONTRIBUTED PAPERS VIRUS 5 — Wednesday, 14:30-16:30 — Multispace AB

Inmunity and host response

Chairs: 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. Zaghloul[1], Robert Hice[2], Peter Arensburger[3], and Brian A. Federici[2,4]

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*

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

The role of baculovirus P26 in suppressing the insect melanization response

Yin, Mengyi[1,2]; *Kuang, Wenhua[1,3]; Wang, Qianran[1,2]; Yuan, Chuanfei[1,4]; Lin, Zhe[4]; Gong, Peng[1];*
Zou, Zhen[4#]; Hu, Zhihong[1#]; Wang, Manli [1#M]

CONTRIBUTED PAPERS. Wednesday, 15:15 **V-36**

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

Kokusho, R.[1,2]; *Katsuma, S.[1]*

CONTRIBUTED PAPERS. Wednesday, 15:30 **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., Akhanaev Yuriy B., Kasianov Nikita S., Martemyanov Viatcheslav V.*

CONTRIBUTED PAPERS. 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, Qianquan*

CONTRIBUTED PAPERS. Wednesday, 16:00 **V-39**

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

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

CONTRIBUTED PAPERS SLUGS & SNAILS 1 — Wednesday, 14:30-16:30 — Multispace CD

IPM Toolkit - Biological Control, Mollusc Behaviour and Mollusc Biology

Chairs: R Rae / Solveig Haukeland

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

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

Nermuť, J.[1]; *Holley, M.[1,2]; Půža, V.[1]*

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.****[2,3] Zając[4], K.; Lachowska-Cierlik, D.[5]; Antzée-Hyllseth[2], H.; Trandem, N[2]; Hatteland, B.A[6,7]*

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
Laplanche, 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]*, Cordsen 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] *; Galinier, R.*[1]*; Gourbal, B*[1] *& Duval, D*[1]

CONTRIBUTED PAPERS. Wednesday, 16:00 **SS-7**
Morphological indicators of reproduction in the garden snail *Cornu aspersum*
Ortega-Hidalgo, M.M.[1]*; Rodriguez-Zaldua, 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, 14:30-16:30
DBI 1 — 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
Maciel-Vergara, G.[1,5,6]*; Tanga CM.*[2]*; Aoko, E.*[3]*; Beckers, E.*[4]*; Jensen, AB.*[1]*; van Loon, JJA.*[5]*; van Lent, JWM.*[6]*; Ros, VID.*[6]*; Eilenberg, J.*[1]*; van Oers, MM*[6]*.*

CONTRIBUTED PAPERS. Wednesday, 14:45 **DBI-2**
Addressing the health of *Macrobrachium rosenbergii* in Bangladesh aquaculture
Hooper, Ch.[1]*; Bateman, KS.*[1]*; Ross, S.*[1]*; Stentiford, GD.*[1]*; Rahman, MM.*[2]*; Basak, SK.*[2]*; Bass, D.*[1]

CONTRIBUTED PAPERS. Wednesday, 15:00 **DBI-3**
Diseases of the Caribbean spiny lobster, *Panulirus argus*
Atherley, NAM.*; Dennis, MM.; Freeman, MA.

CONTRIBUTED PAPERS. Wednesday, 15:15 **DBI-4**
Discovery of Marteilia parasites in UK common cockle (*Cerastoderma edule*) fisheries and comparison with *Marteilia cochilliallze S.*[1]*;*
Bass D.[2]*; Villalba García, A.*[3]*; Carballal Durán, M.*[3]*; Cao Hermida, A.*[3]*; Iglesias Estepa, D.*[3]*; Macarie, A.*[1]*, Shaw, P.*[1]*; Feist, S.*[2]*; Hooper, Ch.*[2]*; Kerr, R.*[2]*; Ironside, J.*[1]

CONTRIBUTED 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.[1]*; Aranguren, F. L.*[1]*, Mai, H. N.*[1]*, Kanrar, S.*[1]*, Cruz-Flores, R.*[1]

CONTRIBUTED PAPERS. Wednesday, 15:45 **DBI-6**
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.*[2,3]*; Daniels, C.*[4]*; Van Eynde, B.*[5]*; Smagghe, G.*[5]*; Bojko, J.*[6]*; Stentiford, GD.*[1,2]*; Bass, D.*[1,2]*; van Oers, M.*[7]*; van Aerle, R.*[1,2]

CONTRIBUTED 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.[1,2]*; Verbruggen, B.*[1]*; Bickley, LK.*[1]*; Bateman, KS.*[2]*; Stentiford, GD.*[2]*; Tyler, ChR.*[1]*; van Aerle, R*[2]*; Santos, EM.*[1]

CONTRIBUTED PAPERS. Wednesday, 16:15 **DBI-8 STU**
Comparative genomics analysis of White Spot Syndrome Virus (WSSV) isolates from different geographical regions.
Al Arimi, WSM.[1,2*]*.; Bass, D.*[1,3]*; Stentiford, GD.*[1]*; Wilfert, L.*[4]*; Longdon, B.*[2]*; Tschirren, B.*[2]*; van Aerle, R.*[1]*; Bateman, KS.*[1]

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

POSTER SESSION

POSTER SESSION — Wednesday, 16:30-18:00
BACTERIA — 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]

POSTER SESSION. Wednesday, 16:30 **PB-5**
Identification and characterization of a new *cry* gene of *Bacillus cereus sensu lato*.
Castillo-Esparza, J.F.; Luévano-Borroel, J.; ***Ibarra, J.E.***

POSTER SESSION. Wednesday, 16:30 **PB-6**
Mosquitocidal 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.*

POSTER SESSION. Wednesday, 16:30 **PB-7**
Microbial protein toxins that are toxic to apple nails and mosquito larvae
Nakagawa, N.[1]; Nishikaku, S.[1]; Azuma, Y.[1]; Hayakawa, T.[2]; ***Takebe, S.[1]***

POSTER SESSION. Wednesday, 16:30 **PB-8**
Isolation and characterization of the insect juvenile hormone antagonists from *Streptomyces* sp.
Kim, J. H. *[1]; Choi, J. Y.[1]; Park, D. H.[1]; Park, M. G.[1]; Kim, J. Y.[1]; Wang, M. H.[1]; Cho, H. Y.[1]; Kim, C. J.[2]; Je, Y. H.[1]*

POSTER SESSION. Wednesday, 16:30 **PB-9 STU**
Mosquitocidal activities of actinomycetes with insect growth regulatory activities
Park, D. H.; *Choi, J. Y.; Kim, J. H.; Park, M. G.; Kim, J. Y.; Wang, M. H.; Cho, H. Y.; Je, Y. H.*

POSTER SESSION. Wednesday, 16:30 **PB-10**
Suppression of Sacbrood virus by virus-derived dsRNA produced from *Bacillus thuringiensis* toxic to *Galleria mellonella*
Park, M. G.*; Kim, J. H.; Park, D. H.; Kim, J.Y.; Wang, M. H.; Cho, H. Y.; Je, Y. H.; Choi, J. Y.*

POSTER SESSION. Wednesday, 16:30 **PB-11**
The presence and diversity of insecticidal proteins in metagenomes from various environments
Shilova, I.S.*[1]; Narayan, N.R.[1]; Johnson, A.J.[1]; Davis, I.W.[2]; Haas, J.A.[2]; Loriaux, P.[1]; Rutherford, E.[1], Skennerton, C.T.[1]; Wegener, K.M.[2]; Weinmaier, T.[1]; Williams, R.J.[2]; Wu, Y.[1]; DeSantis, T.Z.[1]; Dabbagh, K.[1]*

POSTER SESSION. Wednesday, 16:30 **PB-12**
***Bacillus thuringiensis*`s plant disease control effect and plant growth promotion effect**
Tomita, Y.*[1]; Yamazak, K.[1]; Aiuchi, D.[2]; Asano, S.[3]; Koike, M.[1]*

POSTER SESSION. Wednesday, 16:30 **PB-13**
***Photorhabdus* lectins disrupt the activity of insect and human immune system**
Dobeš, P.*[1,2]; Fujdiarová, E.[2,3]; Houser, J.[2,3]; Jančaříková, G.[2,3]; Hyršl, P.[1]; Wimmerová, M.[2,3]*

POSTER SESSION. Wednesday, 16:30 **PB-14**
Sex-specificity in innate immunity of insect larvae
Belousova, I.*[1]; Pavlushin, S.[1]; Rudneva, N.[1]; Martemyanov, V.[1]*

POSTER SESSION. Wednesday, 16:30 **PB-15 STU**
Specificity determination in *Bacillus thuringiensis* Cry2A toxins
Barqawi, A*.; Joseph, L.; Rao, T.; Crickmore, N.*

POSTER SESSION. Wednesday, 16:30 **PB-16 STU**
Mode of action of the Cry41Aa parasporin against human cancer cells
Nasiri, M.*; Souissi, W.; Domanska, B.; Crickmore, N.*

POSTER SESSION. Wednesday, 16:30 **PB-17**
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.***

POSTER SESSION. Wednesday, 16:30 **PB-18**
Type I-C CRISPR-Cas system mediates *Bacillus thuringiensis* pathogenicity and environmental adaptation
Peng, D*.; Zhang, Y.; Dong, Z.; Tan, Z.; Sun, M.**

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
García-Robles, I.; de Loma, J.; Capilla, M.; Roger, I.*; Boix, P.*; Carrión, P.*; Vicente, M.; López-Galiano, M.J.; Real, MD. **; Rausell, C. **

POSTER SESSION. Wednesday, 16:30 **PB-20**
Identification and localization of insecticidal genes through the genomic analysis of a new strain of *Bacillus thuringiensis*
Carvalho, K.S.*[1]; Galvão, S.F.A.[2]; Noda, R.W. [3]; Valicente, F.V.[3]*

POSTER SESSION. Wednesday, 16:30 **PB-21**
Development of a Bacterial Pesticidal Protein Resource Center
Pannerselvam, S.*[1]; Crickmore, N.[2]; Berry, C.[3]; Connor, T.[3]; Mishra, R.[1]; Bonning. B.C. [1]*

POSTER SESSION. Wednesday, 16:30 **PB-22 STU**
Molecular taxonomic characterization of *Bacillus thuringiensis* isolates from Kazakhstan
Sagdyeva, K.[1]; ***Schuster, C.[2]****; Leclerque, A. [2]; Uspanov, A.M.[1]*

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
Lazarte, JN; Poliero, A; ***Berón, CM.***

POSTER SESSION. Wednesday, 16:30 **PB-24**
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.***

POSTER SESSION. Wednesday, 16:30 **PB-25**
Populational and Genetic Analysis of *Wolbachia* Symbionts in some Pests of Russia
Ilinsky Yu*[1,2]; Demenkova M.[1,2]; Bykov R.[1]; Yurlova G.[1]; Vyatkin Yu.[2]; Dubatolov V.[3]; Kerchev I.[3]; Tokarev Yu.[4]*

POSTER SESSION. Wednesday, 16:30 **PB-26 STU**
No synergism of Cry1Ca and Vip3Aa by Lepidoptera and Coleoptera fragments of cadherin in *Spodoptera exigua* and *Grapholita molesta*
Andrés-Garrido, A.; *González-Martínez, R.M.; Ramos, S.; Escriche, B.*

POSTER SESSION. Wednesday, 16:30 **PB-27**
Potentially pathogenic microbiota in bacterial communities of the aquatic invertebrates from freshwater waterbodies of different trophic states
Kashinskaya, E.N.*; Simonov, E.P.; Solovyev, M.M.*

POSTER SESSION. Wednesday, 16:30 **PB-28**
Occurrence of entomopathogenic bacteria in fruit flies reared in laboratory condition
Baldo, F.B.*[1]; Chacon-Orozco, J.G.[1]; Leite, L.G. [1]; Raga, A. [1]; Harakava, R.[2]*

POSTER SESSION. Wednesday, 16:30 **PB-29**
Characterization of *Bacillus thuringiensis* isolates from Jamaica
Marshall, D.; ***Brown, S.***

POSTER SESSION. Wednesday, 16:30 **PB-30 STU**
Critical structural and functional amino acids of Vip3Af from *Bacillus thuringiensis*
Quan, Y.[1]*; Banyuls, N.*[1]*; Van Rie, J.*[2]*; Ferré, J.*[1]

POSTER SESSION. Wednesday, 16:30 **PB-31 STU**
Characterization of two highly insecticidal endophytic strains of *Bacillus thuringiensis*
García-Suárez, R.; *Armendáriz-García, D.D.; Luévano-Borroel, J.; Ibarra, J.E.*

POSTER SESSION. Wednesday, 16:30 **PB-32**
Identification of antimicrobial peptides in wheat stink bug, *Aelia rostrata* (Hemiptera: Pentatomidae)
Doğan, Cansu; *Bayram, Şerife; Toprak, Umut*

POSTER SESSION. Wednesday, 16:30 **PB-33**
Identification of novel hemiptericins in sunnpest, *Eurygaster maura* (Hemiptera: Scutelleridae)
Cedden, Doğa[1]***;*** *Bayram, Şerife*[1]*; Babaroğlu, Numan*[2]*; Toprak, Umut*[1]

POSTER SESSION. Wednesday, 16:30 **PB-34 STU**
Cultivation of entomopathogenic fungi in orbitally shaken bioreactors – Investigation of respiratory activity in scale-up experiments
Senn, Yannick[1]*; Dr. Grabenweger, Giselher*[2]*; Dr. Poggendorf, Iris*[1]*;*

POSTER SESSION - DESEASES OF BENEFICIAL INVERTEBRATES	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PBDI-1**
Application of polyamine carbon quantum dots (CQDs) to aquatic viral disease control: taking shrimp white spot syndrome (WSS) as an example
Chen, LL.*; Huang, HT.*

POSTER SESSION. Wednesday, 16:30 **PBDI-2**
Decrease of NOS/NO is involved in BH4 deficiency-dependent lethality of the *Bombyx mori* mutant *lem*[l]
Meng, Y.[1,2]***;*** *Feng, MW.*[1]*; Rui, S.*[1,2,3]*; Wu, SJ.*[1]*; Wang, Y.*[1]*; Ye, Ch.J.*[1,3]*; Jiang, S.*[1,2]

POSTER SESSION FUNGI	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PF-1**
New Isolates of Beauveria bassiana from Kyrgyzstan and their Entomopathogenic Potential
[1]*Tinatin D.,* [1]*Saikal B., Aijamal K.*[1]*,* [2]*Christina S.,*[2]*Leclerque, A.*

POSTER SESSION. Wednesday, 16:30 **PF-2**
***First record of entomopathogenic fungus Entomophaga aulicae* in the populations of browntail moth in Bosnia and Herzegovina**
Tabaković-Tošić, M.[1,2]*; Milosavljević, M.*[2]*; Radovan, L.*[3]

POSTER SESSION. Wednesday, 16:30 **PF-3**
***New Beauveria bassiana* strain (Bals.-Criv.) Vuill., pathogenicity against weevil pests and physiological characterization**
Moldovan, A.[1,2]*; Munteanu-Molotievskiy, N.*[1]

POSTER SESSION. Wednesday, 16:30 **PF-4**
Evaluation of entomopathogenic fungi to control *Stenoma cecropia* (Lepidoptera: Elachistidae), insect pest of oil palm in Colombia
Montes-Bazurto, L.G.[1]*, Bustillo-Pardey, A.E.*[1]*, Medina-Cardenas, H.C.*[1]

POSTER SESSION. Wednesday, 16:30 **PF-5 STU**
Differential response of *Beauveria bassiana* isolates to growth on hydrocarbons and its potential association with virulence to the soybean pest *Piezodorus guildinii*
Sessa, L.[1]*; Abreo, E.*[1]*; Altier, N.*[1]*; Pedrini, N.*[2]

POSTER SESSION. Wednesday, 16:30 **PF-6**
Spanish mycoviral population of the entomopathogenic fungus *Beauveria bassiana*
Garrido-Jurado, I.[1,3]*; Filippou, C.*[1,2]*; Meyling, N. V.*[4]*; Quesada-Moraga, E.*[3]*; Coutts, R. H.A.*[2]*, Kotta-Loizou, L.*[1]

POSTER SESSION. Wednesday, 16:30 **PF-7**
Comparative analysis of oxidative stress and peroxisomal biogenesis in microsclerotia produced by *Beauveria bassiana* and *Metarhizium robertsii*
Pedrini, N.[1]*; Paixão, F.R.S.*[1]*; Huarte-Bonnet, C.*[1]*; Mascarin, Gabriel M.*[2]*; Fernandes, Éverton K.K.*[3]

POSTER SESSION. Wednesday, 16:30 **PF-8**
Down regulation of chitin synthase 1 gene elevates insecticidal activity of *Beauveria bassiana* ANU1 against *Solenopsis invicta*
Youngjin P*.; Seiha S.*

POSTER SESSION. Wednesday, 16:30 **PF-9 STU**
Differential susceptibility towards UV-B radiation among *Metarhizium* spp. isolates from different ecosystem compartments
Couceiro, J. C.[1,2]*; Fatoretto, M. B.*[3]*; Meyling, N. V.*[2]*; Delalibera Jr., I*[1]

POSTER SESSION. Wednesday, 16:30 **PF-10**
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.*

POSTER SESSION. Wednesday, 16:30 **PF-11**
Virulence of entomopathogenic fungus *Beauveria bassiana* ARP14 against egg of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)
Jang, L. E.; Rahman, M. A.; ***Lim, U. T.***

POSTER SESSION. Wednesday, 16:30 **PF-12**
Comparison of infection dynamics using two strains of *Beauveria bassiana* which has different virulence in *Anopheles stephensi*
Aiuchi, D.[1,2]*; Hatanaka, R.*[1]*; Koike, M.*[1]*; Kanuka H.*[3]

POSTER SESSION. Wednesday, 16:30 **PF-13**
Effect of *Lecanicillium* spp. against eggs of greenhouse whitefly, *Trialeurodes vaporariorum* and sweetpotato whitefly, *Bemisia tabaci*
Ishikura, S.[1]*; Moyo, D. R.*[1]*; Koike, M.*[1]*; Aiuchi, D.*[1,2]

POSTER SESSION. Wednesday, 16:30 **PF-14**
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.[1]*; Bohatá A.*[1]*; Konopická J.*[1,2]*; Hoštičková I.*[1]*;* Čurn *V.*[1]

POSTER SESSION. Wednesday, 16:30 **PF-15**
Modified Adamek's medium renders high yields of *Metarhizium robertsii* blastospores that are desiccation tolerant and infective to cattle-tick larvae
Iwanicki, N.S.A.[1]*;* Ferreira*, B.O.*[1]*; Mascarin, G.M.*[2]***; Delalibera Jr., I.***[1]

POSTER SESSION. Wednesday, 16:30 **PF-16**
Current trend of microbial insecticides: Challenges and Opportunity in fungal insecticides
Lee, M. R.*; Kim, J. C.I; Kim, S.; Park, S. E.; Li D.; Jo, M.; Shin, L. G.; Tae Y.;* ***Kim, J. S.***

POSTER SESSION. Wednesday, 16:30 **PF-17**
Single Cell Encapsulation via Pickering Emulsion for Biopesticide

Applications: *Metarhizium brunneum* against foliar pests
Yaakov N.[1]; Ananth M.K.[1]; Felfbaum R.[1]; Lahat M.[1]; Da Costa N.[2]; Belausov E.[3]; ***Ment D.[2];***
Mechrez 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]; Gogebashvil, M. [2]; Ivanishvili, N. [2]; Arjevanidze, M.[1]; 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*[1,2]; Zemek, Rostislav[2]; Bohatá, Andrea[1]; Nermuť, Jiří[2]; Mráček, Zdeněk[2]; Palevsky, Eric[3]*

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
Parys, 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. [2], Tomilova, Oksana G. [1], Tyurin, Maksim V. [1], Akhanaev, Yuri B. [1], Polenogova, Olga V.[1], Alikina, Tatyana Yu.[3], Kabilov, Marsel R.[3], 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.[3], 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[1], Cisneros, J.[1], ***Jaronski, S.T.****[2], Vitek, C[1], Flores, D.[3]*

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.****[2]; Vitek, C.[1]; Flores, D.[3]*

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, Cárita S.R.[1]; Arruda, Walquíria.[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. [1,2]; Chehab, R..[1]; Tawil, C.[1]; Malek, S.[1]; Noujeim, .[3]; Guillot, J.[2]; ***Nemer, N.****[1]*

POSTER SESSION. Wednesday, 16:30 **PF-28**
Histological changes in the *Culex pipiens* mosquito larvae treated by the entomopathogenic fungus *Cladosporium sp.*
Hamid, S.*[1,2] ;Sahir-Halouane, F.[1]; Benzina, F.[1]*

POSTER SESSION. Wednesday, 16:30 **PF-29**
Natural occurrence of entomopathogenic fungi in apple orchards in Germany
Ehrich, C.A.[1]; Spitzer, J.[2]; Popova, E.[2]; ***Stephan, D.****[1]*

POSTER SESSION. Wednesday, 16:30 **PF-30 STU**
Can nematotoxic proteins of *Coprinopsis cinerea* be used to protect plants against plant-parasitic nematodes?
Moradi, A. *[1], Annageldi, T.[2], El-shetehy, M.[1], Wieczorek, K.[3], Mauch, F.[1] and Künzler, M.[2]*

POSTER SESSION. Wednesday, 16:30 **PF-31**
Effect of Entomopathogenic Fungi onPopulations of *Euschistus heros* (F.) in Soybean Crops (*Glycine max* (L.) Merril) in Paraguay
Alarcón-Ramírez, F.A[1].; Velázquez, C[1]; ***Resquín-Romero, G****[2].*

POSTER SESSION. Wednesday, 16:30 **PF-32 STU**
Using Entomopathogenic Fungi to Control the Greenhouse Whitefly (*Trialeurodes vaporariorum*): Developing a Standardised Bioassay
Spence, E.*[1,2];* *Hesketh, H.[1]; Svendsen, C.[1]; Chandler, D.[2]; Martin, G.[3]; Berry, S.[3]; Edgington, S.[4]*

POSTER SESSION. Wednesday, 16:30 **PF-33**
Insecticidal activity of entomopathogenic fungi against *Gonipterus platensis* under laboratory conditions
Montalva, C.*[1] Rojas, E.[2]; González, A.[3]; González, C.[1]*

POSTER SESSION. Wednesday, 16:30 **PF-34**
Insecticidal effect of entomopathogenic fungus, *Beauveria bassiana* ANU1, with hydramethylnon to a red imported fire ant, *Solenopsis invicta*, worker
Park, Y. and Sung, S.

POSTER SESSION	Wednesday, 16:30-18:00
MICROBIAL CONTROL	Foyer

POSTER SESSION. Wednesday, 16:30 **PMC-1**
The oak processionary moth (*Thaumetopoea processionea*) in climate change – bionomy, phenology and natural antagonists
Halbig, P.[1]; **Kleespies, R.G.**[2]; Koch, U.[2]; Schumacher, J.[1]; Mühlfeit, M.[3]; Plašil, P.[3]; Lobinger, G. [4]; Möller, K.[5]; Delb, H.[1]

POSTER SESSION. Wednesday, 16:30 **PMC-2 STU**
Endophytic effect of *Beauveria bassiana* in tomato on greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)
Pourtaghi, E.; *Talaei-hassanloui, R.*

POSTER SESSION. Wednesday, 16:30 **PMC-3**
Virulence of entomopathogenic fungi *Beauveria bassiana* isolates on the Asia citrus psyllid, *Diaphorina citri*
Jiang, R.X.[1,2]; Jiang, H.B.[1,2]; Wang, J.J.[1,2]*

POSTER SESSION. Wednesday, 16:30 **PMC-4 STU**
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
Miranda-Fuentes, P.*; Quesada-Moraga, E.; Garrido-Jurado, I.; Yousef, M.*

POSTER SESSION. Wednesday, 16:30 **PMC-5**

First report of *Conidiobolus coronatus* in Paraguay as biological control of leaf cutting ants

Resquín-Romero, G.[1]; Sarubbi-Orue, H.[1]; Pino-Quintana, D.; Amarilla, S.P.[2]; Butt, T.[3]

POSTER 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.[3], Holderegger, R.[4]; Widmer, F.[1]; Enkerli, J.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-7**

Behavioural abilities of EPNs to search the insect host inside sugarcane culm

***Chacon-Orozco, J.G.**[1]; Silva, M.S.O.[1]; Cardoso, J.F.M.[1]; Leite, L.G.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-8**

Effect of the successive passes of *Metarhizium anisopliae* on insecticidal activity to *Demotispa neivai*

*Cuartas-Otálora, P.E.[1]; Montes-Bazurto, L. G.[2]; Borrero-Echeverry, F.[1]; Quiroga, G.M.[1]; **Grijalba, E.P.**[1]; Buitrago, L.F.[2]; Bustillo, A.E.[2], Gómez, M.I.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-9**

***Beauveria bassiana* against leaf cutter ants: are all strains equally effective to different ant pest species?**

Goffré, D.; Osorio, A.; ***Folgarait, P.J.***

POSTER SESSION. Wednesday, 16:30 **PMC-10**

Antifungal activity of entomopathogenic *Bacillus thuringiensis.*

***Yamazaki, K.**[1]; Tomita, Y.[2]; Asano, S.I.[3]; Aiuchi, D.[4]; Koike, M.[2]*

[1]Hokkaido Sugar CO., Ltd., Tokyo, Japan

POSTER SESSION. Wednesday, 16:30 **PMC-11**

Development of Attract-Kill granule for white grubs control

***Liu, Q.**[1]; Shu, C.; Zhang, J.*

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

Langner, M.[1,2]; ***Stephan, D.***[2]

POSTER SESSION. Wednesday, 16:30 **PMC-13**

Host response of *Lymantria dispar dispar* to *Chromobacterium* spp. infections

Sparks, M.E.; Kuhar, D.; Farrar, R.R.; Blackburn, M. B.; Gundersen-Rindal, D. E.

POSTER SESSION. Wednesday, 16:30 **PMC-14 STU**

Biological Control: Fighting below ground insect pests with *Pseudomonas* bacteria

***Spescha, A.**[1]; Schneider, J.[1]; Gilliéron, F.[1]; Flury, P.[1]; Grabenweger, G.r[2]; Maurhofer, M.[1]*

POSTER 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.[3]; Desidério, J. A.[2]; Lemos, M.V. F.[2]*

POSTER SESSION. Wednesday, 16:30 **PMC-16**

Biological control of the Japanese beetle (*Popillia japonica*) with entomopathogenic fungi

***Sostizzo, T.**; Grabenweger, G.*

POSTER SESSION. Wednesday, 16:30 **PMC-17**

POSTER SESSION. Wednesday, 16:30 **PMC-18**

Cellulase improves the endophytism of encapsulated *Metarhizium brunneum* on potato plants

***Jakobs-Schoenwandt, D.**[1]; Krell, V.[1]; Unger, S.[2] and Patel, A.V[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-19 STU**

Influence of inundative mass application of *Metarhizium brunneum* BIPESCO 5 on indigenous *Metarhizium* strains in maize fields

***Zottele, M.**[1]; Gruber, A.[1]; Enkerli, J.[2]; Strasser, H.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-20**

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.[2,4]; ***Lemos, M.V.F.***[2]

POSTER SESSION. Wednesday, 16:30 **PMC-21**

Defense mechanism of ixodid ticks to fungal infection: cuticle, the first barrier

***Silva, C.S.R.**[1]; Golo, P.S.[2]; Castro, R.N.[3]; Ângelo, I.C.[4]; Arruda, W.[5]; Fernandes, É.K.K.[1].*

POSTER SESSION. Wednesday, 16:30 **PMC-22**

Blastospores and conidia supplemented or not with secondary metabolite of plants to control flies

***Filgueiras, M.D.G**; Christian L.;* Éverton *K K.F.*

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

***Jankevica, L.**[1], Strike Z.[2]; **Smits, A.**[2]*

POSTER SESSION. Wednesday, 16:30 **PMC-24 STU**

Effect of arbuscular mycorrhizal fungi on the susceptibility of *Spodoptera exigua* to viral and bacterial entomopathogens

***Frattini, A.**[1]; Rivero, J.[2]; Pozo, M.J[2]; Herrero, S.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-25 STU**

Management of Colorado potato beetle overwintering adults with entomopathogenic nematodes

Čačija, M.; ***Mrganić, M.**; Kolenc, M.; Lemić, D.; Drmić, Z.; Virić Gašparić, H.; Bažok, R.*

POSTER SESSION. Wednesday, 16:30 **PMC-26**

First report of entomopathogenic nematodes *Heterorhabditis bacteriophora* from Croatia and its virulence against *Lasioptera rubi*

***Majić, I.**[1]; Sarajlić, A.[1]; Lakatos, T.[2]; Tóth, T.[2]; Raspudić, E.[1]; Laznik, Ž.[3]*

POSTER SESSION. Wednesday, 16:30 **PMC-27**

Impact of Moisture on the Viability of *Metarhizium* Microsclerotia

***Behle, R.W.;** Goett, E.J.*

POSTER SESSION. Wednesday, 16:30 **PMC-28**

Solid State Fermentation at Bayer CropScience Biologics – Ready for the next step

***Steinwender, B.M.**; Marba-Ardebol, A.M; Schink, S.*

POSTER SESSION. Wednesday, 16:30 **PMC-29**

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

***Beitzen-Heineke, E.**[1]; Dreyer, W.[2]; Hermann, K.[3]; Humbert, P.[3]; Landzettel, C.[4]: Laurenz, S.[5]; Przyklenk, M.[1]; Meßmer, H.[6]; Patel, A.[3]; Vidal, S.[5]*

POSTER SESSION. Wednesday, 16:30 **PMC-30**

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.[3];* Lázaro-*Berenguer, M.*[2]*; González-*

Martínez, R.M.[2]; Gomis-Cebolla, J. [2]; Ferré, J.[2]

POSTER SESSION. Wednesday, 16:30 **PMC-31 STU**

A new method of microsporidia metabarcoding

***Trzebny A.**[1]; Slodkowicz-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

***Kereselidze, M.**[1,2]; Pilarska, D.[3,4]; Linde, A.[5]*

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.

***Quiles, A.**[1,2]; Bacela-Spychalska, K.[2]; Teixeira, M.[1]; Lambin, N.[1]; Grabowski, M.[2]; Rigaud, T.[1]; Wattier, R.A.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-34**

Development of *Anncaliia algerae* in *Drosophila melanogaster*

*Weidner. E.[1], **Sokolova Y.** [1,2], DiMario P.[1]*

POSTER SESSION. Wednesday, 16:30 **PMC-35**

The Enterocytozoonidae: Emergent Microsporidia in the aquatic-terrestrial food chain

***Trew, J.**[1]; Aldama-Cano, 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 SLUGS AND SNAILS	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PSS-1**

Characterization of two *Phasmarhabditis* (Nematoda) earthworm isolates and susceptibility of earthworms and invasive slugs to EM434

*Leung, N.[1]; Fitch, D.[2]; Paine, T.[1]; **Tandingan De Ley, I.**[1,3]*

POSTER SESSION NEMATODES	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PN-1**

New isolates of the nematophagous fungus *Arthrobotrys oligospora* for biocontrol of garlic nematodes

***Doolotkeldieva, T.**[1]; Bobushova, S.[1]; Schuster, C.[2]; Leclerque, A.[2]*

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

***Liu, Q.-Z.;** Yang Y.-Q.*

POSTER SESSION. Wednesday, 16:30 **PN-3 STU**

SNP analysis for generation of trait-related molecular markers in the entomopathogenic nematode *Heterorhabditis bacteriophora*

***Godina, G.**; Kirsch, 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*

***García-Montelongo, M.**; González-Villarreal, S.E.; Ordoñez-Acevedo, L.G.; Lule-Chávez, 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-Pérez, R.** [1,2]; Bueno-Pallero, F.Á.[1]; Vicente-Díez, I.[2]; Marco-Mancebón, V.S.[3]; Pérez-Moreno, I. [3]; 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]; Cortés-Martínez, C. I[2]*

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.[1]; **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], Dobeš Pavel[1], Hurychová J.[1], Maguire R.[3], Hyršl 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.[3]; Lortkipanidze, M.[2]; Arjevanidze, M.[1]; De Luca, F.[4]; Troccoli, A.[4]; Fanelli, E.[4]*

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 *Rhynchophorus ferrugineus* in response to bacterial challenge and entomopathogenic nematode infection

***Torrini, G.[1];** Cappa, F.[2]; Mazza, G.[1]; Inghilesi, A. F.[2]; Benvenuti, C.[1]; Viliani, L.[2]; Roversi, P. F.[1]; Cervo, R.[2]*

POSTER SESSION. Wednesday, 16:30 **PN-13**

Recombinant *Bacillus thuringiensis* for intestinal nematodes

***Flanagan, KA[1];** Abraham, A[1]; Gazzola, D[1]; Li, H[1]; Kellog, T[1]; Kim, Y[1]; Rus, F[1]; Ostroff, G[1]; and Aroian, RV[1]*

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,

*H.T[4].; Perret-Gentil, A[2]; **Shapiro-Ilan, D.I.**[1]*

POSTER SESSION. Wednesday, 16:30 **PN-15**
Evaluation of potential of entomopathogenic nematodes for control of box tree moth – *Cydalima perspectalis* (Walker, 1859) in laboratory conditions
*Miladinović, Z.; **Nježić, B.***

POSTER SESSION. Wednesday, 16:30 **PN-16**
Management of black vine weevil (*Otiorhynchus sulcatus*) by entomopathogenic nematodes in Georgia
Mikaia, N.

POSTER SESSION. Wednesday, 16:30 **PN-17**
Molecular and phenotypic characterization two strains of *Photorhabdus luminescens* associated with Iraqi *Heterorhabditis bacteriophora*
*Al-Zaidawi, J.B.[1]; **Karimi, J.[1]**; Moghadam, E.M.[2]*

POSTER SESSION. Wednesday, 16:30 **PN-18**
Comparison of entomopathogenic nematode and insecticide management of western corn rootworm larvae
***Razinger, J.[1]**; Žigon, P.[1]; Kolmanič, A.[2]; Modic, Š.[1]*

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]*

POSTER SESSION. Wednesday, 16:30 **PN-20**
Fighting parasitic nematodes with natural products and microbial crystals
***Fahs, H.[1]**; Refai, F.[1]; White, R[1]; Gopinadhan, S.[1]; Kremb, S.[1]; Page, A[2]; Cipriani, P.[1]; Butterfoss, G.[1]; Twaddle, A.[1]; Piano, F.[1]; Kallassy, M.[2]; Gunsalus, K.[1]*

POSTER SESSION	Wednesday, 16:30-18:00
VIRUSES	Foyer

POSTER SESSION. Wednesday, 16:30 **PV-1**
Phylogenetic analysis of six strains of baculovirus with activity towards *Spodoptera frugiperda*
*Zanella-Sainz, Ingrid[1]; León-Galván, Ma. Fabiola[1], Ibarra-Rendón, Jorge E.[2] **Del Rincón-Castro, Ma. Cristina[1]***

POSTER SESSION. Wednesday, 16:30 **PV-2 STU**
Functional and genomic approaches to study the evolution of recently domesticated viruses in *Campopleginae* parasitoid wasps
***Cerqueira de Araujo, A.[1]**; Leobold, M.[1]; Bézier, A.[1]; Drezen, J-M.[1]; Josse, T.[1]; Huguet, E[1]*

POSTER SESSION. Wednesday, 16:30 **PV-3 STU**
Survey of CpGV mixed-genotype infection occurrence in treated orchards
***Hinsberger, A.[1]**, Blachère-Lopez, C.[1,2], Lopez-Ferber, M.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-4 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 domesticus*
***Maciel-Vergara, G.[1,2,3]**; Ros, V.I.D.[2]; van Oers, M.M.[2]; van Loon, J.J.A[3].*

POSTER SESSION. Wednesday, 16:30 **PV-5**
First results of the virome of *Scaphoideus titanus*, *Frankliniella occidentalis* and *Thrips tabaci*
***Abbà, Simona[1]**; Chiapello, Marco[1]; Ottati, Sara[1]; Galetto, Luciana[1]; Tavella, Luciana[2]; Turina, Massimo[1]; Marzachì, Cristina[1]*

POSTER SESSION. Wednesday, 16:30 **PV-6**
Unraveling the multiple nudiviral integration traces within insect genomes
***Bézier, A.[1]**; Leobold, M.[1]; Gayral, P.[1]; Drezen, J-M.[1]; Herniou E.A.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-7**
Construction of a novel baculovirus expression system with increased foreign protein production and expression time
***Gwak, W.S.[1]**; Kim H.S.[1]; Lee J.Y.[1]; Bae J.S.[1]; Kim T.H.[1]; Choi C.J.[1]; Woo S.D.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-8 STU**
Construction of novel baculovirus inducible vectors for rapid production of foreign proteins
***Kim H.S.[1]**; Gwak, W.S.[1]; Lee J.Y.[1]; Bae J.S.[1]; Kim T.H.[1]; Choi C.J.[1]; Woo S.D.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-9 STU**
***Chrysoperla carnea´s* performance when fed with two nucleopolyhedroviruses (SeMNPV and AcMNPV) *Spodoptera exigua* infected larvae**
***Gutiérrez, Oscar G.[1]**; Medina, Pilar M.[1]; Adán, A.[1]; Garzón, A.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-10**
Identification and Genomic Analysis of a Second Species of Nucleopolyhedrovirus Isolated from *Spodoptera exigua* (Lepidoptera, Noctuidae)
Li, Changyou[1]; Zheng, Guiling[1]; Zhou, Hongxu[1]; Tan Xiumei[1]; Deng Fei[2]; Chen, Yingjian[1]; Qi, Benxiang[1]

POSTER SESSION. Wednesday, 16:30 **PV-11**
Arboviruses associated with *R.microplus* ticks in Yunnan China
*Shi, Junming[1,2]; Shen, Shu[1]; Wu, Hui[3]; Zhang, Yunzhi[3]; **Deng, Fei[1]***

POSTER SESSION. Wednesday, 16:30 **PV-12**
Comparison of vertical transmission of high and low virulent nucleopolyhedrovirus strains after *Lymantria dispar* L. infection
***Akhanaev, Yuri B.**; Belousova, Irina A.; Lebedeva, Darya A.; Martemyanov, Vyacheslav V.*

POSTER SESSION. Wednesday, 16:30 **PV-13**
Comparative genome analysis of related Lymantria dispar nucleopolyhedrovirus isolates differing in virulence.
***Martemyanov, V.[1]**; Lunev, E.[2]; Toshchakov, S.[2,3]; Podgwaite, J.[4]; Ilinsky, Y.[2,5,6]*

POSTER SESSION. Wednesday, 16:30 **PV-14**
The role of parasitic larvae and their symbiotic viruses as hidden players in plant-insect interactions
CUSUMANO, A.[1,3]; URBACH, S.[2]; **VOLKOFF, A.-N.[1]**

POSTER SESSION. Wednesday, 16:30 **PV-15 STU**
Reduced AcMNPV budded virus production facilitates generation of persistent infections *in vitro*
***Arinto-Garcia, R.[1]**; Bannach, C.[1]; Hawes, C.[1]; King, L.A.[1]; Possee, R.D.[1,2]*

POSTER SESSION. Wednesday, 16:30 **PV-16 STU**
Constructing a model of the larval *Spodoptera exigua* brain to study baculoviral entry and localization *Gasque, S.N.[1]; van Oers, M.M.[1]; Smid, H.M.[2]; and Ros, V.I.D.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-17**
Rhabdovirus-free clones from a *Spodoptera frugiperda* (Sf21) cell population?

Sevak, D. *[1]; King, L.A.[2]; Possee, R.D.[1,2]*

POSTER SESSION. Wednesday, 16:30 **PV-18 STU**

Usage of highly specific indel mutations for distinguishing Cydia pomonella granulovirus isolates

Yang, S.*; Fan, J.; Wennmann, J.T.; Jehle, J.A.*

POSTER SESSION. Wednesday, 16:30 **PV-19**

Quantification of *Erinnyis ello* granulovirus in a biopesticide formulation.

Araque, G.; *Gómez, J.;Barrera, G.*

POSTER SESSION. Wednesday, 16:30 **PV-20**

A novel member of *Cypovirus 2* found in *Erinnyis ello ello* larvae co-infeted with a baculovirus reveals a possible horizontal gene transfer between these two different viruses

Silva, L.A.[1]; Ardisson-Araújo, D.M.P.[2]; Melo, F.L.[1]; Souza, M.L.[3]; ***Ribeiro, B.M.****[1]*

POSTER SESSION. Wednesday, 16:30 **PV-21**

A draft of the encapsidated genome of the Cotesia flavipes Bracovirus

Morgado, F.S.; ***Ribeiro, B.M.***

POSTER SESSION. Wednesday, 16:30 **PV-22**

Baculovirus hyper expression system for virus like particles and vaccines production in insect cells

Lee, J.H.*[1]; Choi, J.B.[1]; Han, B.K.[1]; Kim, H.[1]; Gwak, W.S.[2]; Woo, S.D.[2]*

POSTER SESSION. Wednesday, 16:30 **PV-23**

AcMNPV *p48* (*ac103*) is required for the efficient scission of inner nuclear membrane invagination structures

Wang, Y.; Cai, Q.; Chen, J.; Huang, Z.; Wu, W.; ***Yuan, M.****; Yang, K.*

POSTER SESSION. Wednesday, 16:30 **PV-24**

A Study about Chimeric OBs Based on d-POLH

Xiang, Y; ***Wang, J***

POSTER SESSION. Wednesday, 16:30 **PV-25**

BacMam technologies in cells and animals; Advances towards the transport of DNA in mammals

Simonin, J.A.[1]; Giménez, C.S.[2]; Nuñez, C.; Olea, D.F. [2]; Crottogini, A.[2]; Ghiringhelli, P.D.[1]; **Belaich, M.N.**[1]

POSTER SESSION. Wednesday, 16:30 **PV-26 STU**

Evaluation of CRISPR/Cas9 based procedures for the edition of baculoviral genomes

Nugnes, M.V..*[1]; Ghiringhelli, P.D.[1]; Belaich, M.N.[1]*

POSTER SESSION. Wednesday, 16:30 **PV-27**

The persistent infection of PnV (Perina nuda iflavirus) to its heterologous cell line, NTU-LY cell line (*Lymantria xylina* cell line)

Nai, Y.-S.[1]; Lo, C.-M.[2]; Wang, C.-H.[1]

POSTER SESSION. Wednesday, 16:30 **PV-28 STU**

Improvement of baculovirus as protein expression vector and as biopesticide by CRISPR/Cas9 editing

Pazmiño-Ibarra, V.*[1], Mengual, A.[1], Targovnik, A.[1,2,3], Herrero, .S[1]*

POSTER SESSION. Wednesday, 16:30 **PV-29 STU**

Biotype and nudivirus prevalence of *Oryctes rhinoceros* in Palau Archipelago

Tanaka, S.*[1]; Kitalong, C.[2]; Inoue, N.M.[1]; Nakai, M.[1]*

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

Identification of Dendrolimus punctatus cypovirus (DpCPV) viral attachment proteins and its ligands in the host midguts

Su, L.*[1,2]; Xu, C.[2]; Cheng, C.[2]; Lei, C.[2]; Sun, X.[2].*

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 californica multiple nucleopolyhedrovirus

Yan Qing*[1], Chen Guoqing[1], Li Pei[1], Feng Guozhong[1]*

POSTER SESSION. Wednesday, 16:30 **PV-32**

Interaction between Autographa californica nucleopolyhedrovirus (AcMNPV) ME53 and VP80

Özşahin*, Emine[1]; Nagy, Éva[2]; Doucet, Daniel[3]; and* ***Krell, Peter J.****[1]*

POSTER SESSION	Wednesday, 16:30-18:00
MISCELLANEOUS	Foyer

POSTER SESSION. Wednesday, 16:30 **PMI-1**

***Galleria mellonella* larvae fat body disruption (Lepidoptera: Pyralidae) caused by venom of the *Habrobracon brevicornis* (Hymenoptera: Braconidae)**

Kryukova, N.A.; *Mozhaytseva, K.A.; Rotskaya, U.N.; Polenogova, O.V.; Glupov, V.V.*

POSTER SESSION. Wednesday, 16:30 **PMI-2**

Parasitoid envenomation changes the *Galleria mellonella* midgut microbiota and immunity, thereby promoting fungal infection

Polenogova, O.V.*[1];* *Kabilov, M.R.[2]; Tyurin, M.V.[1]; Rotskaya, U.N. [1]; Krivopalov, A.V.; Morozova, V.V.[2]; Mozhaytseva, K. A. [1]*; *Kryukova, N.A.[1]; Alikina, T.[2]; Kryukov, V.Y.[1]*; *Glupov, V.V.[1]*

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.[3]; Neuberger, K.[3]; Tilcher, R.[4]; ***Patel, A.V.****[1]*

POSTER SESSION. Wednesday, 16:30 **PMI-4 STU**

Slow release of semiochemicals in push-pull-kill strategies for biological psyllid pest control

Muskat, L*.[1]; Humbert, P.[1]; Gross, J.[2]; Görg, L.M.[2]; Dippel, C.[3]; Schulke, J.[3]; Patel, A.V.[1]*

POSTER SESSION. Wednesday, 16:30 **PMI-5**

Ectoparasites of some passerine birds

Ouarab, S.

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

Sundh, I.*[1]; Scheepmaker, J.W.A.[2]; Busschers, M.[3]; Eilenberg, J.[4]; Butt, T.M.[5]*

POSTER SESSION. Wednesday, 16:30 **PMI-7**

Bioactive compounds of *Trichoderma* spp.: A multifunctional tool for pest management.

Ramirez, S.[1]; ***Aragón, S.****[1]*

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)

Hamade, A.*[1]; Sbaity, Z.[1]; Eid, J.[1]; Kfoury, L.[2]; Rizk, F.[1]*

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]

ORAL PRESENTATIONS CONTINUED

CONTRIBUTED PAPERS — Wednesday, 18:00-20:00
MICROBIAL CONTROL 4 — Auditorium 3

Formulation and field efficacy

Chairs: Surandra Dara / Linda Muskat

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.*[2]*; Marsberg, T.*[1]*; Hill, M.P.*[1]*; Dealtry, G.B.*[2]*; Lee, M.E.*[3]*;* ***Moore, S.D.***[1,4]

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.*[3]*; Reuscher, S.*[4]*; Pelz, 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.*[2]*; Maehara, N.*[3]*; 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.[3]; Patel, A. V.[1]

CONTRIBUTED PAPERS. Wednesday, 19:15 **MC-30**

A microbial Integrated Pest Management strategy for climbing cutworm in wine grapes

Franklin, M.; ***Henderson, D.E***[1]*; Tahrir 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, 18:00-20:00
VIRUS 6 — Multispace AB

Virus-host Interactions

Chairs: Deng Fei / Jorg Wenmann

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, W*[1]*; 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, A[1]*; Parenti, S*[1]*; Han, Y*[2]*; Ros, V.I.D*[2]*; Herrero, S*[1]

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, Delphine[1,2]*; Paxton, Robert J.*[1,2]*; Bigot, Diane*[1]

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, Yu[1]*; Wennmann, Jörg T.*[2]*; Fan, Jiangbin*[2]*; Xing, Longsheng*[1]*; Jehle, Johannes A.*[2]*; Wan, Fanghao*[1,3]

CONTRIBUTED PAPERS. Wednesday, 19:30 **V-47**

Ecological implications of covert infections with RNA viruses in the beet armyworm, *Spodoptera exigua*

Mengual-Martí, A.; Martínez-Solís, M.; Jakubowska, A.; ***Herrero, S.***

CONTRIBUTED PAPERS. Wednesday, 19:45 **V-48**

The major hurdle for effective baculovirus transduction into mammalian cells is early endosomes

Hu, Liangbo[1,2]*; Li, Yimeng*[1]*; Ning, Yunjia*[1]*; Deng, Fei*[1]*; Vlak, Just M.*[3]*; Hu, Zhihong*[1]*;*
Wang, Hualin[1#]*; Wang, Manli*[1#]

CONTRIBUTED PAPERS — Wednesday, 18:00-20:00
SLUGS & SNAILS 2 — Multispace CD

IPM Toolkit - Barriers, Monitoring and Molluscicides

Chairs: Gordon Port / Nadine Sydow

CONTRIBUTED PAPERS. Wednesday, 18:00 **SS-9**
Development and use of a barrier system for reducing invertebrate damage in crops
***Evans, K. A.**[1]; Inglis, M.[2]*

CONTRIBUTED PAPERS. Wednesday, 18:15 **SS-10 STU**
Sluxagon: A pesticide-free, paintable slug fence
***Sydow, N.**[1,2]; Riedl, M.[1]*

CONTRIBUTED PAPERS. Wednesday, 18:30 **SS-11**
LIMACAPT: a self-powered connected sensor for monitoring slugs
***Benne, F.**, Crebassa, X. and Pabis, R.*

CONTRIBUTED PAPERS. Wednesday, 18:45 **SS-12**
Optimising snail and slug management in Australian crops: linking mollusc activity with climate using time-lapse cameras
***Perry, K. D.**[1]; Brodie, H. E.[1]; Muirhead, K. A.[1]; Baker, G. J. [1]*

CONTRIBUTED PAPERS. Wednesday, 19:00 **SS-13**
The development of iron-III-phosphate as an integrated slug control method in UK agriculture
***Benson, M.**[1], Baxter, I.[1], Butt T.[2]*

CONTRIBUTED PAPERS. Wednesday, 19:15 **SS-14 STU**
Can we make changes to slug pellets to improve their efficiency?
***de Silva, S. M.**[1]; Port, G.[1]; Sanderson, A. R.[1]; Rushton, P. S.[1]; Audsley, N.[2]*

CONTRIBUTED PAPERS. Wednesday, 19:30 **SS-15 STU**
Do molluscicidal control measures allowed in Lithuania kill invasive slugs?
***Adomaitis, M.**[1]; Skujienė, G.[1]; Račinskas, P.[1]*

CONTRIBUTED PAPERS. Wednesday, 19:45 **SS-16 STU**
Changes to the foraging behaviour of the grey field slug (*Deroceras reticulatum*) in the presence of molluscicide and implications for control.
***Campbell, A.[1];** Port, G.[1]; Sanderson R. A.[1]; Rushton, S. P.[1]; Audsley, N.[2]*

CONTRIBUTED PAPERS — Wednesday, 18:00-20:00
DBI 2 — Commission R8

Diseases of managed and wild bees

Chairs: Annette Bruun Jensen / Carrie Hauxwell

CONTRIBUTED PAPERS. Wednesday, 18:00 **DBI-9**
Nosema ceranae* affects peritrophic matrix structure in honey bees, *Apis mellifera
***Webster, TC.;** Kamminga, KL.; Matisoff, M.A.*

CONTRIBUTED PAPERS. Wednesday, 18:15 **DBI-10 STU**
Elucidating the honey bee immune response at the Varroa mite feeding site.
***Cooper, AL.[1],** Forward, K.[1], Freeman, T.[2], Campbell, EM.[1], Bowman, AS.[1]*

CONTRIBUTED PAPERS. Wednesday, 18:30 **DBI-11**
Prevalence and diversity of ssRNA+ honey bee-infecting viruses in wild hymenoptera
*Bigot D.[1]; **Gayral, P.[1]**; López-Vaamonde C.[1,2]; Herniou EA.[1]*

CONTRIBUTED PAPERS. Wednesday, 18:45 **DBI-12 STU**
Bacterial diversity of the *Tetragonula carbonaria* microbiome within and between hives
***Tarlinton, B.**[1,2]; McGree, J.[1]; Gloag, R.[3]; Hauxwell, C.[1]*

Microbial control — Wednesday, 20:15-22:00
DIVISION BUSINES MEETING — Auditorium 3

Microsporidia — Wednesday, 20:15-22:00
DIVISION BUSINES MEETING — Multispace AB

DBI — Wednesday, 20:15-22:00
DIVISION BUSINES MEETING — Commission R8

SLUGS & SNAILS — Wednesday, 20:15-21:15
WORKSHOP — Multispace CD

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.**[1], Nermut, J.[2]; Ross., J. [3, 4, 5]*

WORKSHOP. Wednesday, 20:45 **SSW-2**
Identification of molluscs
***Hatteland, B. A.**[1, 2]*

THURSDAY - 1st August

CONTRIBUTED PAPERS — Thursday, 8:30-10:30
MICROBIAL CONTROL 5 — Auditorium 3

Microbial control

Chairs: Nguya Maniania / Mary Barbachek

CONTRIBUTED PAPERS. Thursday, 8:30 **MC-32**
Unspecialised endophytic fungi protect herbaceous plants against insects, but experimental design is critical – a meta analysis
Gange, A.C. [1]; Koricheva, J.; Currie, A.F. [1]; Jaber, L.R.[2]; **Vidal, S.**[3]

CONTRIBUTED PAPERS. Thursday, 8:45 **MC-33**
Microbial cues to induce grooming in *Drosophila melanogaster* to resist their infection
***Yanagawa, A.**[1]; Neyen, C.[2]; Chabaud, M.A.[3]; Hata, T.[1]; Yoshimura, T.[1]; Lemaitre, B.[2]; Marion-Poll, F.[4]*

CONTRIBUTED PAPERS. Thursday, 9:00 **MC-34**
Is there a trade-off between virulence and endophytic behaviour of the entomopathogenic fungus *Beauveria bassiana?*
***Quesada-Moraga, E.**; Valverde-García, R.; González-Mas, N.*

CONTRIBUTED PAPERS. Thursday, 9:15 **MC-35**
***Purpureocilium lilacinum* is also a mycopathogen, although highly specific**
***Folgarait, P.J.**; Goffré, D.; Lucero, N.; Osorio, A.*

CONTRIBUTED PAPERS. Thursday, 9:30 **MC-36**
Roles of peritrophic matrix in Cry1Ac resistance of cotton bollworm *Helicoverpa armigera*
Minghui, J.[1, 2]; Yutao, X.[1]

CONTRIBUTED PAPERS. Thursday, 10:00 **MC-37**
NoVil: The hunt for weevil control-Update
***Maniania, N.K. ;** Amanulla, M.F. ; Demarse, A. ; Darie,A. ; Rao, I.M.*

CONTRIBUTED PAPERS. Thursday, 10:15 **MC-38**
IDH-α-mediated metabolic disorders disrupted active immunization in eusocial termites

Long Liu[1], Changcao Wang[2], Xinying Zhao[1], Junxia Guan[1], Chaoliang Lei[1], ***Qiuying Huang[1*]***

CONTRIBUTED PAPERS Thursday, 8:30-10:30
VIRUS 7 Multispace AB

Biological control

Chairs: Caroline Knox / Miguel lópez-Ferber

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, T[1]; Jun, T[1,2]; Maki, N.I[1]; Yasuhisa, K[1]; ***Madoka, N[1]***

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, 09:15 **V-52**

Insecticidal activity of granulovirus and nucleopolyhedrovirus isolated from a natural co-infection in *Spodoptera ornithogalli* larvae

Gustavo Araque[1], *Juliana Gómez[1], Judith Guevara[1], Gloria Barrera[1]*

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

Harrison, Robert L.[1]*; Rowley, Daniel L.[1]; Popham, Holly J. R.[2]*

CONTRIBUTED PAPERS. Thursday, 09:45 **V-54**

The effect of nucleopolyhedrovirus inoculum purity on the microbial load of *Helicoverpa armigera* larval cadavers

Bouwer, Gustav*; Grant, Michelle*

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.

Jukes, Michael D.[1,2]*; Knox, Caroline M.[1]; Hill, Martin P.[2]; Moore, Sean D.[2,3]*

CONTRIBUTED PAPERS Thursday, 8:30-10:30
NEMATODES 4 Multispace CD

Novel approaches in the basic and applied research on EPN

Chairs: Duarte Toubarro / Carlos Molina

CONTRIBUTED PAPERS. Thursday, 8:30 **N-25**

Antiprotozoal activity of *Xenorhabdus* and *Photorhabdus* bacteria mutualistically 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.[2]; Bode, H.B.[3]; Hazir, C.[4]; Bilecenoglu, D.K.[5]*

CONTRIBUTED PAPERS. Thursday, 8:45 **N-26**

***Steinernema carpocapsae* secrete/excreted proteins modulate insect immune responses**

Toubarro, D.[1]*; Kenney, E.,[2;] Eleftherianos, I.[2]; Simões, N.[1]*

CONTRIBUTED PAPERS. Thursday, 9:00 **N-27**

Cancelled

CONTRIBUTED PAPERS. Thursday, 9:15 **N-28**

Extending the survival of *Heterorhabditis bacteriophora* dauer juveniles through phenotypic selection and marker-assisted breeding

Molina, C.; *Nellas Sumaya, N.H.; Godina, G.; Kirsch, C.[1]; Vandenbossche, B.; Dörfler, V. ; Barg, M.; Ehlers, R.-U.*

CONTRIBUTED PAPERS. Thursday, 9:30 **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, 9:45 **N-30**

Identification of molluscs and their associated nematode parasites

Hatteland, B. A.[1, 2]

CONTRIBUTED PAPERS Thursday, 8:30-10:30
MICROSPORIDIA Commission R8

Microsporidia-host interactions: from organism to molecular levels

Chairs: Ronny Larsson / Yuliya Sokolova

CONTRIBUTED PAPERS. Thursday, 8:30 **MS-1**

Co-infection of paramyxid and microsporidian parasites in feminised amphipod crustaceans

Ironside J.*; James Pickup J.*

CONTRIBUTED PAPERS. Thursday, 8:45 **MS-2**

Firing of the harpoon-like polar tube in microsporidian parasites

Jaroenlak, P.[1]*; Cammer, M.[2]; Becnel, J.J.[3]; Ekiert, D.C.[1]; Bhabha, G.[1]*

CONTRIBUTED PAPERS. Thursday, 9:00 **MS-3**

Cancelled

CONTRIBUTED PAPERS. Thursday, 9:15 **MS-4**

PtdIns(3)P-binding Protein NbSWP12 is Significant for microsporidia proliferation in insect cells

Chen J.[1,2]*; Huang Y.[1,2]; Li Z.[3]; Mengxian L.[1,2]; Pan G.[1,2]; Zhou Z.[1,2,3]*

CONTRIBUTED PAPERS. Thursday, 9:30 **MS-5**

Pathological analysis of silkworm infected by two microsporidia *Nosema bombycis* CQ1 and *Vairimorpha necatrix* BM

***Meng, X.**[1]; He, Q.[1]; Wang, C.[1]; Pan, G.[1]; Li, T.[1]; Zhou, Z[1,2]*

Coffee Break	Thursday, 10:30-11:00 Foyer
SIP BUSINES MEETING	Thursday, 11:00-13:00 Auditorium 3
Lunch	Thursday, 13:00-14:30 Foyer
IOBC BUSINES MEETING	Thursday, 13:00-14:30 Auditorium 3
JURY STUDENT COMPETITION	Thursday, 13:00-14:30 Multispace AB

CONTRIBUTED PAPERS — Thursday, 14:30-16:30
BACTERIA 4 — Auditorium 3

Bacterial symbionts of invertebrates

Chairs: Luca Ruiu / Patricia Hernández

CONTRIBUTED PAPERS. Thursday, 14:30 **B-25**

Constraints on the evolution of increased parasitism in a caterpillar symbiont

***Raymond, B.,** Matthews, A.*

CONTRIBUTED PAPERS. Thursday, 14:45 **B-26**

Microevolutionary alterations in midgut bacterial community of *Galleria mellonella* resistant to *Bacillus thuringiensis*

***Grizanova, E.V.[1];** Mukherjee, K.[3]; Kalmykova, G.V.[2]; Akulova, N.I.[2]; Vilcinskas, A.[4]; Dubovskiy, I.M.[1,2]*

CONTRIBUTED PAPERS. Thursday, 15:00 **B-27**

Effect of symbionts on gene expression in the kissing bug *Rhodnius prolixus*

*Gilliland, C.A.[1]; **Vogel, K.J.**[1]*

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*

***Rouzé, R.**[1]; Monné, A.[1]; Delbac, F.[1]; Belsunces, L.[2]; Blot, N.[1]*

CONTRIBUTED PAPERS. Thursday, 15:30 **B-29**

Gut microbiota of *Aedes aegypti* shift in response to host blood meal source

***Ephantus J. Muturi**[1], Christopher Dunlap[1], Jose L. Ramirez[1], Alejandro P. Rooney[1], Chang-Hyun Kim[2]*

CONTRIBUTED PAPERS. Thursday, 15:45 **B-30**

Gut bacteria activate hypoxia-inducible transcription factors that impact growth and metabolism of mosquito larvae

***Strand, M.R.**; Valzania, L.; Harrison, R.; Kang, Z.; Brown, M.R.*

CONTRIBUTED PAPERS. Thursday, 16:00 **B-31**

Baculovirus and Bacillus thuringiensis based biopesticides in Brazil: Challenges and opportunities

***Valicente, F.H.**[1]; Carvalho, K.S. de[2]; Nunes, Gabriel H.F.[2]; Machado, D.H.B.[2]; Lana, U.G., de P.[1]; Aguiar, F.M.[1]; Modesto, F.; Geraldo, L.[2]; Pinho, J.M.R.[1]*

CONTRIBUTED PAPERS — Thursday, 14:30-16:30
FUNGI 5 — Multispace CD

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*

Thapa, S.[1]; Cotes, B.[1]; **Meyling, N. V.**[1]

CONTRIBUTED PAPERS. Thursday, 14:45 **F-34**

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

***Westerman, P. R.**[1]; Helsen, H.M.[2]; Wiegers, G. L.[1]; van der Sluis, B. J.[2]; van Tol, R. W.H.M.[1]*

CONTRIBUTED PAPERS. Thursday, 14:45 **F-35**

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.*

CONTRIBUTED PAPERS. Thursday, 15:15 **F-36**

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

*Nowak, M.; **Różalska, S.***

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

***Tinsley, Matt C.**[1]; Mangan, R.[1]; Polanczyk, R. A.[2]; Bussière, L.. F.[1]*

CROOSS-DIVISIONAL SYMPOSIUM — Thursday, 14:30-16:30
DBI-MICROSPORIDIA — Commission R8

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

***Sokolova Y.**[1,2]; Overstreet R.[3]; Heard R.[3]*

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

Cytology and Development of Metchnikovellideans 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 metchnikovellids

***Nassonova, E.**[1,2]; Paskerova, G.[2]; Frolova, E.[2]; Galindo, L.J.[3]; Torruella, G.[3]; Moreira, D.[3]; López-García, P.[3]; Smirnov, A.[2]*

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

Intranuclear parasites of free-living amoebae: The Rozellomycota and the origins of the Microsporidia

Corsaro D.[1]; Walochnik J.[2]; Wylezich C.[3]; Hauröder B.[4]; Müller K.-D.[5]; ***Sokolova Y.**[6]; Michel R.[4]*

SLUGS & SNAILS SYMPOSIUM — Thursday, 14:30-16:30, Multispace AB

Future of Integrated Pest Management for Mollusc Control

Organisers / Chairs: Jenna Ross / Jirka Nermut

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
Mc Donnell, 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 ,Lv.[1], Peng,Q [1], Zhang, J[1]., **Song,F**[1]
Candela,T.[2],Gilois, N.[2], **Nielsen-Leroux, C.[2]** , Lereclus, D.[2] , Gohar.M.[2]

SYMPOSIUM. Thursday, 18:40 **BS-5**

Bacillus thuringiensis: 50 years of safety to vertebrates
***Raymond, B.[1],** Federici, B.[2]*

ICTV STUDY GROUP — Thursday, 17:00-19:00, Multispace AB

Organizer: Robert Harrison

CONTRIBUTED PAPERS MICROBIAL CONTROL 6 — Thursday, 17:00-19:00, Multispace CD

Entomopathogenic fungi

Chairs: Todd Kabaluk / Edith Ladunder

CONTRIBUTED PAPERS. 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.*

CONTRIBUTED PAPERS. Thursday, 17:15 **MC-40**

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

CONTRIBUTED PAPERS. Thursday, 17:30 **MC-41**

Multitrophic interactions among endophytic *Beauveria bassiana,* aphid prey and its natural enemies on melon
***González-Mas, N.;** Cuenca-Medina, M.; Gutiérrez-Sánchez, F.; Quesada-Moraga, E.*

CONTRIBUTED PAPERS. 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]

CONTRIBUTED PAPERS. 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]*

CONTRIBUTED PAPERS. 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.*

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.; Kleespies, R.G.*

CONTRIBUTED PAPERS. 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[1]

SYMPOSIUM. Thursday, 18:30 **DBIS-4**

The contribution of extension services to the monitoring of crop pests and to the uptake of augementative biocontrols in selected low to lower-middle income countries

Edgington, S

BANQUET	Thursday, 20:00-3:00 La Cartuja

ABSTRACTS 2019

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STU indicates papers being judged for graduate student presentation awards.

MONDAY - 29th July

Opening Ceremony **Awards Presentations** **Founders Lecture**	Monday, 08:30-10:30 Auditorium 2
Coffee Break	Monday, 10:30-11:30 Foyer
PLENARY SYMPUSIUM	Monday, 11:00-13:00 Auditorium 2

Resistance to microbial control agents

Organisers / Chairs: Raquel Campos-Herrera / Joel González-Cabrera

PLENARY SYMPOSIUM. Monday, 11:00 **PL-1**

Can insects develop resistance to fungal biocontrol agents?

Butt, T.M.[1]*; Dubovskiy*[2]*, I.; Grizanova*[2]*, E.; Coates*[1]*, C.,*

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With the increasing use of insect pathogenic fungi in pest management programmes there is a concern that the target pests will, in time, develop resistance to the pathogen. No one has any idea how and when the insects could develop resistance. Both insects and fungi are in a continuous arms race. For every barrier the insect creates the fungus has countermeasures. Repeated exposure to a single strain can result in tolerance. However, the development of resistance in pest populations is unlikely. This is because end users will have at their disposal a wide range of strategies to prevent pests developing resistance. For example, use of fungal biocontrol agents (BCAs) with efficacy enhancing agents, choice of formulations, and availability of disparate virulent strains. Besides examining the role of the disparate physical-chemical barriers to infection, some of the strategies to mitigate development of resistance are discussed.

PLENARY SYMPOSIUM. Monday, 11:30 **PL-2**

Mechanisms of practical resistance to commercially relevant entomopathogenic bacteria

Jurat-Fuentes, J.L.[1]

[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee, USA;

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Bacteria are the most commercially successful entomopathogenic microbial group, namely gram-positive bacteria in the Bacillaceae family such as *Bacillus thuringiensis* (Bt) and *Lysinibacillus sphaericus*. Insecticidal proteins produced by these bacteria are the main virulence factor responsible for activity of pesticidal formulations and confer resistance to insect attack when produced by transgenic crops. Evolution of resistance in target pests is the most serious threat to the sustainable use of these bacterial pesticides and transgenic crops, and information on these mechanisms and the genes involved is vital to develop effective management practices in reducing the risk of resistance evolution. In this presentation I will provide an integrative summary of the available experimental evidence on resistance mechanisms to commercialized entomopathogenic bacteria and transgenic crops producing insecticidal proteins from *B. thuringiensis* (Bt crops). Empirical data from laboratory selection and cases of practical field-evolved resistance are in agreement and support alterations in binding of insecticidal proteins to receptors in the host as the main mechanism for high levels of resistance to entomopathogenic bacteria and Bt crops. Strategies to reduce the risk of evolution of this mechanism will be discussed.

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.

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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.

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.***

Department of Nematology, University of California, Riverside

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The entomopathogenic nematode (EPN) *Steinernema scapterisci* 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. scapterisci* 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. scapterisci* to crickets is dependent on the nematode rather than the bacterial symbiont it carries and we speculate that *S. scapterisci* may be evolving independence from *X. innexi*.

JIP and BC editorial meeting Monday, 13:00-14:30
Press room

STUDENT WORKSHOP Monday, 13:00-14:30
Multispace AB

The nuts and bolts of grant writing
Organizer: Patricia Stock

Lunch Monday, 13:00-14:30
Multispace 2

CROSS-DIVISIONAL SYMPOSIUM BACTERIA-VIRUS Monday, 14:30-16:30
Auditorium 2

The multiple layers of host-pathogen interactions
Organisers / Chairs: Umut Toprak / Salva Herrero

SYMPOSIUM. Monday, 14:30 **BVCS-1**

Effect of the peritrophic membrane on toxicity of Bt Cry toxins in insects

Wang, Ping
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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.

SYMPOSIUM. Monday, 14:50 **BVCS-2**

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

Erlandson, Martin A.
Saskatoon Research and Developmental Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada
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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.

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.[1]
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Many cases of insect resistance to toxins from *Bacillus thuringiensis* (Bt) are known, and these have been useful in identifying crucial steps in the toxin modes of action. However the agricultural relevance of these findings has tended to shift the focus away from the host-pathogen interaction. I will argue that this interspecific interaction has a long coevolutionary history and that understanding this interaction is important to the sustainability of transgenic Bt-expressing plants. At one level, there is a coevolutionary pattern between the first attack weapons, the three-domain Cry proteins, and their targets in the insect (cadherins and ABC transporters) that is beginning to be understood. How does that differ from the coevolution of the second line of attack, the VIP proteins, and their still mostly unknown targets? Another issue is what sort of pathogen Bt is in nature. Statements (mostly from virologists) that Bt is not really a pathogen come to mind from past SIP meetings. The question of what actually kills the insect host (Bt or other bacteria?) is emerging as an area of controversy. Older work on the immune response of the insect host is being confirmed by more recent studies that have identified specific molecular agents in this level of the interaction. I will evaluate the need for more information on these different levels with respect to the relevance of sustainable use of Bt toxins in pest control, and what it can contribute to our general knowledge of host-pathogen interactions.

SYMPOSIUM. Monday, 15:30 **BVCS-4**

Insect immunity as affected by stress factors

Pennacchio, F.
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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.

SYMPOSIUM. Monday, 15:50 **BVCS-5**

Pathogens associated with invasive or introduced insects threaten the health and diversity of native species

Vilcinskas, Andreas[1,2]

[1]Institute for Insect Biotechnology, Justus Liebig University of Giessen, Giessen, Germany

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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. Intensive agriculture (associated with the loss of habitat and the overuse of pesticides and fertilizers) and urbanization (associated with more traffic and artificial light at night) are considered key factors. The talk considers whether the factors driving the loss of insect diversity include also invasive 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.

Vilcinskas, A. 2019: Current Opinion in Insect Science 33, 43-48.

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[1], Simone Gasque[1], Hans M. Smid[2], Monique M. van Oers[1], ***Vera I.D. Ros***[1]

[1]Laboratory of Virology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; [2]Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands

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.

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

Insolation, diversity and ecology

Chairs: Inmaculada Garrido-Jurado / Claudia López-Lastra

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, College of Agriculture and Life Sciences, Chonbuk National University, Jeonju, Republic of Korea

Corresponding autho : jskim10@jbnu.ac.krBeauveria 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.

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

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*, *Rachiplusia nu*), 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 identification 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

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.

CONTRIBUTED PAPERS. Monday, 15:00 **F-3**

First Entomophtoral pathogen of leaf cutter ants: a possible new species of *Conidiobolus*

Goffré, D. [1]*; Jensen, A. B.*[2]*;Lopez Lastra, C.*[3]*; Valencia Carrasco, C.*[1]*;* ***Folgarait, P. J.***[1]

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Entomophtoromycotina fungi with entomopathogenic abilities have been rarely isolated from ants. Here we describe taxonomic characteristics (morphological and molecular) and preliminary pathogenic peculiarities of an isolation made from leaf cutter ants that belongs to the Entomophtoromycetes Class, Entomophtorales Order and Ancylistaceae Family. Morphologically our sample has spherical to subspherical primary conidia, variable in size, with papillae parallel sided and with a rounded tip, borne from unbranched conidiophores; these forcibly discharged replicative secondary -subglobose to slightly pinned- conidia; the isolate can be cultured on PDA, corresponding to the genus *Conidiobolus*. Because it produces microconidia and villous resting spores, it seems to be *C. coronatus*. However, phylogenies obtained from the amplified sequences of three regions (SSU, LSU and ITS), using a *Basidiobolus* strain as outgroup, placed our isolate within the *C. coronatus* clade, but not as the same species. To evaluate its virulence we placed fungi of different ages (from 1 to 6 days old) on top of an inverted agar plate changing every 24hs the ants exposed to the discharged conidia for 6 days. Ants died for all ages of the fungi, however they died much faster and greater number of cadavers exhibited our strain, when the fungi was 2 and 5 days old. It seems that a greater discharge of infective conidia or most effective conidia were discharged in particular at those two times. Our data highlights interesting aspects of this possible new species and a great potential as a biological control agent of leaf cutter ants.

CONTRIBUTED PAPERS. Monday, 15:15 **F-4 STU**

Fungal Epizootiology in Red mite, *Dermanyssus gallinae* of Chicken

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Red mite, *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae) is one of serious insect, damaging to the egg production in chickens and causing serious economic losses. Moreover, the overuse of chemical insecticides caused pest resistance and environmental residual toxicity to chickens and eggs. Therefore, this work provides a screening method to select entomopathogenic fungi as a candidate of environmentally safe control agent, having high miticidal activity against *D. gallinae*. The virulence test was conducted by spraying method with a conidial suspension (1×10^7 conidia/ml). Among several species, *Beauveria* species showed high virulence and mycosis were observed in 7-10 days. Based on these results, we selected 51 isolates having high virulence against *D. gallinae*. This fungal library has information on the identification and conidia color of entomopathogenic fungi and virulence against the red mite. Some of the selected isolates were produced on eight cereal grains for 14 days in Petri dish conditions. Of the eight granular substrates, millet, perilla seed and barley showed the high conidia production. Isolates produced on millet and rice showed high thermal stability, when exposed to 45°C for 30, 60, 90 and 120 minutes. Based on these results, this work suggests that entomopathogenic fungi can be used control the *D. gallinae.*

CONTRIBUTED PAPERS. Monday, 15:30 **F-5**

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. robertsii*, 4 *M. brunneum*, 1 *M. guizhouense*, 1 *M. lepidiotae*) 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. lepidiotae* revealed lowest efficacy (35% and 34%, respectively) of all tested isolates, while one isolate of *M. brunneum* and two of *M. robertsii* almost reached or exceeded 90%.

CONTRIBUTED PAPERS. Monday, 15:45 **F-6**

Diversity of the arthropod pathogenic 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. robertsii* 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 polypaghous 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. pinghaense*. Bioassay studies with purified conidial suspension of the fungus indicated the fungus was able to cause more than 86% mortality at the highest dose tested (1×10^8 spores/ml) against late instar larvae. This is the first report of *M. pingshaense* naturally infecting *C. punctiferalis*. Isolation of a highly virulent isolate of this fungus holds promise towards development of a 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 infects 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 *Batkoa* sp., *Conidiobolus coronatus*, *C. obscurus*, *Entomophaga grylli*, *E. planchoniana*, *E. ferdinandii*, *Neozygites* sp., *N. fresenii, Pandora* sp., P. *delphacis*, *P. dipterigena*, *P. gammae*, *P. neoaphidis*, *P. nouryi, 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 Argentinean Entomophthoralean fungal species with new records that expand their insect host range and their geographical distribution.

CONTRIBUTED PAPERS — Monday, 14:30-16:30
MICROBIAL CONTROL 1 — Multispace CD

Bacteria and proteins

Chairs: Travis Glare / Monika Maurhofer

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* (Yersiniaceae) can cause disease specific to the New Zealand pasture pest, *Costelytra giveni* (Scarabaeidae). Isolates of *S. proteamaculans* have been found to cause a variety of pathotypes (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 *Serratia* 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 adaption ability 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 Bt biofilm would certainly laid a foundation for constructing highly UV-resistant engineering biofilms based on Bt 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 used 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 Cry1Ia?

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Bacillus thuringiensis synthetizes structurally similar three-domain (3-D) crystal proteins (Cry) that accumulate in crystal inclusions, with the exception of Cry1I 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 Cry1I proteins, a clear understanding of their mode of action is critical for enhancing and sustaining their efficacy. Nevertheless, the Cry1Ia oligomer formation has not been yet experimentally addressed. In this study, for the first time, the formation of Cry1Ia oligomers has been investigated, using Cry1Ab as a control. The insecticidal activity of Cry1Ia 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 Cry1Ia toxicity, the oligomer formation has been studied after incubation of Cry1Ia with brush border membrane vesicles (BBMV) of susceptible insects and non-susceptible cultured insect cells. Based on the bioassay experiments, Cry1Ia 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, Cry1Ia 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* CHA0 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* CHA0 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* CHA0 colonizing wheat-roots, *Plutella xylostella* after oral infection, and *Galleria mellonella* hemolymph after hemocoel injection. Our results showed expression of different gene-sets under each of the examined conditions. Hence, *P. protegens* CHA0 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

Keyser, C.[1]***;*** *Davis, J.*[2]*; Hernowo, K.*[2]*; O. Anyanga, M.*[3]*; Pepe-Ranney, C.*[1]*; Bissinger, B.*[1]
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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 these sweetpotato weevil-active microbes and evaluate their performance in greenhouse and field experiments, both in the USA and in Uganda. Affordable and effective control options that can easily be implemented by African sweetpotato farmers are desperately needed. An efficacious microbial control agent has the potential to play a key role in increasing sweetpotato productivity by protecting crops from the sweetpotato weevil.

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

Double agents in the plant's service: root-colonizing pseudomonads with anti-fungal and anti-insect activities

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Fluorescent pseudomonads possess manifold plant-beneficial activities such as plant-growth promotion and disease suppression. They are commonly found in soil, and the plant rhizo- and phyllosphere. Our discovery that the two species *Pseudomonas protegens* (Pp) and *P. chlororaphis* (Pc) can additionally colonize and even kill lepidopteran insect larvae after oral uptake raises new questions about the ecological significance of *Pseudomonas*-insect interactions and the potential use of these bacteria for biological insect control. A recent survey revealed that soil insects collected from natural habitats frequently harbor Pp and Pc. Interestingly, root and insect isolates express similar root colonizing, insect colonizing, antifungal and insect killing capacities. These fascinating bacteria thus have the skills for easily switching between very different life styles. Using a comprehensive approach combining comparative genomics, transcriptomics, mutational analyses and bioassays we have identified several traits involved in insect pathogenicity of Pp and Pc and sets of genes specifically expressed in insect or roots habitats. The degree of Pp/Pc pathogenicity is strongly dependent on the insect species. Lepidoptera are generally highly, Diptera and Coleptera less sensitive. However, monitoring Pp throughout insect life cycles showed that Pp can persist inside the insect also in non-pathogenic interactions from the larval to the adult stage. We are currently exploring the potential of Pp/Pc applied alone or in combination with other biocontrol agents such as entomopathogenic nematodes and entomopathogenic fungi for the biological control of root-attacking insect pests e.g. the cabbage root fly *Delia radicum*, a pest causing severe losses in the production of brassicacean crops.

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

Interaction between novel insecticidal proteins from plants and lepidopteran pests

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Numerous lepidopteran species cause economic damage to multiple food and fiber crops worldwide. The losses can surpass billions of US dollars annually because of insect management costs and reduced yield. Today, there is widespread adoption of transgenic crops expressing one or more insecticidal toxins derived from *Bacillus thuringiensis* (Bt) that provide effective protection against plant pests. In some instances, pest resistance to Bt proteins has evolved, leading to the need to discover new actives to overcome such resistance. We have previously reported the discovery of a new family of potent insecticidal proteins from ferns. 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.

CONTRIBUTED PAPERS — Monday, 14:30-16:30
NEMATODES 1 — Commission R8

EPN ecology and behaviour

Chairs: Selcuk Hazir / Christine Griffin

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

Do *Photorhabdus temperata* and *Photorhabdus cinerea*, symbionts of *Heterorhabditis downesi*, co-exist at the same site by niche separation?

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Entomopathogenic nematodes (*Steinernema* spp., *Heterorhabditis* spp.) are mutualistically associated with bacterial symbionts (*Xenorhabdus* spp and *Photorhabdus* spp., respectively). While traditionally, the relationship has been seen as one symbiont per nematode species, this paradigm has weakened especially in regards to the *Heterorhabditis-Photorhabdus* relationship. *Heterorhabditis downesi* can associate with either *Photorhabdus temperata* or *Photorhabdus cinerea*. We have investigated the co-occurrence of these two symbionts with *H. downesi* at the same site (a coastal dune system in Ireland). We wish to understand how the niches of the two symbionts might differ to allow long-term persistence of these species at the same site. To this end, we used laboratory assays to quantify competition between the symbionts and the value of the services they provide to the nematode (including virulence for insects, ability to support nematode reproduction, ability to associate with nematode infective juveniles, anti-fungal, anti-bacterial and anti-scavenger properties). We also characterise the secondary metabolites produced by the two symbionts using HPLC/MS. The two symbiont species can grow together *in vivo* and *in vitro*, with no evidence of mutual inhibition by allelochemicals. Both symbionts support nematode reproduction equally, and have similar virulence for a range of insect species. Wherever differences were detected between the two species, *P. cinerea* appeared to have an advantage (e.g. faster speed of kill, superior protection of cadaver from desiccation and competing microbes), with the exception that *P. temperata* gave marginally better protection of the cadaver from

scavengers. We discuss the results in the context of ecological theory and secondary metabolite production by the two species.

CONTRIBUTED 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 "*insectivora*" 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 wouts agar plates. The same experiment was 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*.

CONTRIBUTED 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.

CONTRIBUTED 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 *Steinernema 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 neuromodulators 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.

CONTRIBUTED PAPERS. Monday, 15:30 **N-5 STU**

The lure of hidden death: Attractive volatile organic compounds to attract 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-caryophyllene, 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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The Scavenger Deterrent Factor (SDF) activity of 30 *Xenorhabdus* isolates (26 species with one species having two strains and another species having three strains) and six *Photorhabdus* isolates (four subspecies of *P. luminescens*, one subspecies of *P. temperarta*, and one subspecies of *P. asymbiotica*) was tested against the insect scavengers, *Gryllus bimaculatus* (cricket) and *Tapinoma madeirense* (ant) using agar plugs containing 3-day-old bacterial supernatants and 3-day-old *Xenorhabdus*- or *Photorhabdus*-killed insect larvae (*Galleria mellonella*). Results indicated that all *Photorhabdus* isolates tested had high SDF activity against both scavengers, whereas some of the *Xenorhabdus* species had deterrent activity against both scavengers, but others did not. For example, both the ants and crickets consumed the agar plugs with supernatants containing *X. beddingii*, *X. poinarii*, *X. ehlersii*, *X. japonica*, *X. kozodoi*, *X. doucetiae*, *X. romanii*, *X. eapokensis*, *X.* sp. TS4, *X. koppenhoeferi*, and *X. bovienii* SS-2004 indicating no detectable SDF activity against the tested cricket and ant. Interestingly, the ants consumed agar plugs with *X. hominickii* and *X. ishibashii*, but the crickets did not. Thus, SDF plays a significant role in the survival of some EPNs and their bacterial symbionts in the insect cadaver in nature. However, it is not produced by all *Xenorhabdus* species, and the reasons for this lack of SDF activity against the tested cricket and ant species are not known.

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

Characteization of some entomopathogemic nematodes and fungi from the soil of Afghanistan

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A study was carried out to isolate and identify entomopathogenic nematodes and fungi from northern soil habitats of Badakhshan province, Afghanistan through 2017-2018. Here five isolates of nematodes and two isolates of fungi are reported. Two nematode isolates were from *Diploscapter* (Nematoda: Diploscapteridae) and also two species were from *Oscheius* (Nematoda: Rhabditida). Morphological studies with light microscopy and scanning electron microscopy, as well as molecular analyzes using full-length small subunit rDNA gene of D2/D3, 18S and ITS genes has done. From fungi, the isolates were from *Metarhazium* (Ascomycota: Clavicipitaceae). The molecular characterization as well class identification showed the isolates as *M. robertsii*. This is the first insight into diversity of those insect pathogens from Afghanistan.

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

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

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Entomopathogenic nematodes (EPNs) which belong to the families Steinernematidae and Heterorhabditidae and have a mutualistic association with bacteria that kill the host insect by septicemia and make the cadaver suitable for the development of the EPNs. The objective of this study is to identify EPNs isolated from agricultural fields of the Central Anatolia. The identification of EPNs which are adapted to climatic conditions of these areas is important for integrated pest management (IPM) programs in Turkey. Totally, 200 soil samples were collected and stored in 350 ml plastic containers. The nematodes were isolated from these samples with *Galleria mellonella* bait traps. Infective juveniles were collected with White traps and identified by sequencing of the ITS and mtCOI regions. EPNs identified in agricultural fields in Central Anatolia were *Heterorhabditis bacteriophora, H. marelatus*, *Steinernema* sp., *S. affine*, *S. bicornotum*, *S. carpocapse*, *S. feltiae*, *S. litorale* and *S. weiseri*. This study provide both detailed information about EPN species and also potential biopesticide candidates for sustainable IPM programs of Turkey. In order to explore the diversity of EPNs in Turkish soils much more study should be required.

Coffee Break — Monday, 16:30-17:00, Foyer

CONTRIBUTED PAPERS — Monday, 17:00-19:00
VIRUS 1 — Auditorium 2

Virus Discovery and taxonomy

Chairs: Robert Harrison / Elisabeth Herniou

CONTRIBUTED PAPERS. Monday, 17:00 **V-1**

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

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Some lineages of parasitoid wasps have evolved a remarkable strategy in their parasitism arsenal: they utilize symbiotic viruses to breach host defenses. The most well-known examples are Polydnaviruses (PDVs), which are found in three diverse clades of parasitoid wasps. Despite their independent evolution, PDV genomes share some key characteristics including wasp genome-integration and dispersal of viral genes into two separate components: proviral segments (containing virulence genes) and replication genes. The replication machinery for these viruses is not packaged into virions, thus the viruses and parasitoids are reliant on each other for reproduction. Non-PDV symbiotic viruses have been documented in other parasitoid wasp species, and their recent genomic characterization has shown that they diverge from the PDV paradigm. The genome

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 *Fopius arisanus* that produces virus-like particles that do not contain nucleic acids. Despite a very young association with wasps in the genus *Fopius*, 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.

CONTRIBUTED PAPERS. Monday, 17:30 **V-2**

The variable landscape of nonretroviral RNA virus integrations in worldwide samples of the arboviral vector *Aedes aegypti*

***Crava, Cristina M.**[1]; Pischedda, Elisa[1]; Di Mattia, Annamaria; Tancredi[1]; Alessandra, Scolari, Francesca[1]; Afrane, Yaw[2]; Ayala, Diego[3]; Carballar-Lejarazú, Rebeca[5]; Bonizzoni, Mariangela[1].*

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Besides being the primary vector of arboviruses such as Dengue and Zika viruses, the mosquito *Aedes aegypti* may also be infected with insect-specific viruses (ISVs). Arboviruses and ISVs are nonretroviral RNA viruses. Surprisingly, we and others recently identified that the interplay among ISVs, arboviruses and mosquitoes involves the integration of sequences of viral origin within the mosquito genome. These Non-retroviral Integrated RNA Virus Sequences (NIRVS) are not distributed randomly in the mosquito genome, they are statistically-significantly enriched in piRNA clusters and produce piRNAs.

We used geographical samplings of wild *Ae. aegypti* mosquitoes to study the diversity and the population patterns of NIRVS. Our hypothesis is that differential viral spreads and gene flow among populations should model the NIRVS landscape of wild populations. We developed bioinformatics tools to rigorously identify novel NIRVS in wild-caught mosquitoes and to study the genetic variability of the annotated NIRVS in order to identify evolutionary patterns that could contribute to adaptation. Our results provide evidences of the recent integration of portions of currently circulating ISVs. We also demonstrate that the landscape of NIRVS is not stable across geographical populations but show a consistent population-specific behavior that we can leverage to formulate hypothesis on NIRVS phenotypic role. Our results support the role of NIRVS in adaptation to new environments and thus provide new avenues to disentangle mosquito interaction with nonretroviral RNA viruses.

CONTRIBUTED PAPERS. Monday, 17:45 **V-3 STU**

***Drosophila suzukii* viruses as potential tool for biological control**

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The invasive insect pest *Drosophila suzukii* infests ripening fruits and causes extensive damage to crops in the northern hemisphere. Novel, environmentally friendly strategies to control the spread of this species are urgently needed, and one promising approach is the use of entomopathogenic 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 28ºC 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*.

CONTRIBUTED PAPERS. Monday, 18:00 **V-4**

The invasive hornet *Vespa velutina* carries both honey bee viruses and new viruses.

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From its first introduction in 2004 in France the Asian yellow-legged hornet *Vespa velutina nigrithorax* is now largely spreading into Europe, by lack of efficient control methods. In this context, looking for natural hornet pathogens could be useful to develop biological control agents against this invasive species. Therefore, we investigated all RNA viral sequences detected from hornets samples caught in Southern France, in order to characterize any virus from asymptomatic or symptomatic hornets differentiating various dissected parts (brain, muscle, abdomen). Among almost twenty viruses detected in three RNA sequenced hornets, *Deformed wing virus B* was shown to be predominant in all samples, and much higher in the muscle from symptomatic individual, suggesting a putative cause of the deformed wings symptom. Interestingly, two new viruses closely related to *Acyrthosiphon pisum virus* and *Himetobi P virus* were detected in brain and muscle that may correspond to the circulation and multiplication forms of those viruses in the hornet. From a multiplex PCR method, two bee viruses, *Acute bee paralysis virus* and *Black queen cell virus* were detected in brain, muscle or other dissected parts. Other viruses known to infect the honey bee were also identified from the hornet intestine transcriptome such as *Aphid lethal paralysis virus, Bee Macula-like virus,* and *Mokuvirus.* Our study underlines the urgent need to study the host range of these original new viruses we described in hornets to evaluate if they can represent a new threat for the honey bees or a hope for the biocontrol of *V. velutina.*

CONTRIBUTED PAPERS. Monday, 18:15 **V-5 STU**

Characterisation of the RNA virosphere in Australian tephritid fruit flies

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The Queensland fruit fly (*Bactrocera tryoni*) is Australia's most significant horticultural pest, estimated to cause annually AU$300 million in damage, control and market loss. Control has relied on broad-spectrum insecticides; however several have been banned because of human health and environmental impacts. Sterile Insect Technique (SIT) is an effective alternative that relies on the release of mass-produced high-performing sterile males. However, little is known about virus diversity, impact and epidemiology in tephritids targeted by SIT. We analysed available transcriptome data and identified eleven viruses in multiple Australian tephritid species. Complete viral genomes were detected in most species. Phylogenetic classification revealed that the viruses predominantly belonged to the Picornavirales. RT-PCR tests confirmed the presence of three Iflaviridae and Dicistroviridae species in laboratory fly colonies. *Bactrocera tryoni* Dicistrovirus 1 (BtDV1), was found across laboratory colonies of this host and three other tephritid species, while other viruses were only detected in individual species. However, not all *B. tryoni* field individuals were BtDV1 positive. Our results suggest that BtDV1 is active and both vertically and horizontally transmitted. Further studies on transmission ecology, host fitness impacts and prevalence across host distribution are needed to better understand its role in fruit fly biology and history.

CONTRIBUTED PAPERS. Monday, 18:30 **V-6 STU**

Identification of new viruses specific of the honey bee mite *Varroa destructor*.

Millán-Leiva, A.; *Herrero, S.; Coll, S.; González-Martínez, R. M.; Parenti, S.; González-Cabrera, J.*
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Recent large-scale colony losses among managed Western honey bees have become a serious threat to the beekeeping industry. There are multiple factors contributing to these losses but the impact of *Varroa destructor* parasitism is by far the most important, along with the contribution of some pathogenic viruses vectored by the mite. So far, more than 20 viruses have been identified infecting the honey bee, most of them RNA viruses, which may be maintained either as covert infections or causing severe symptomatic infections, compromising the viability of the colony. Analysis of transcriptomic data obtained from mites collected in the USA and Europe revealed the presence of at least three different RNA viruses, not described before. In the course of this investigation, an independent study described similar variants of two of these viruses infecting also mite populations in Israel. However, the third virus has been detected and described here for the first time. In addition, we have obtained evidences of the replication capacity of the three viruses in the mite, but not in the bee, suggesting that they are selectively infecting the mite.

Currently, there is only a handful of miticides available to control the mite and there are already spots of resistance to some of them. A new control approach based on the use of these pathogenic viruses might help to reach a long-term success controlling the mite through an IPM strategy that reduces the input of acaricides to the hives.

CONTRIBUTED PAPERS. Monday, 18:45 **V-7**

Metatranscriptomics reveals novel RNA viruses of important rice invertebrates in China

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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.

COSS-DIVISIONAL SYMPOSIUM Monday, 17:00-19:00
MICROBIAL CONTROL-FUNGI Multispace AB

Microbial control of wireworms

Organisers / Chairs: Dietrich Stephan / Stefan Jaronski

SYMPOSIUM. Monday, 17:00 **MFCS-1**

Wireworm biology in Middle Europe –what are we facing?

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The well-known pest wireworms are soil-dwelling click beetle larvae (Coleoptera, Elateridae). The situation in Middle Europe is complicated already in conventional cropping due the reduction or even lack of suitable chemical control. In organic farming people are down to cultural control options. Therefore, in recent years there is a marked increase in the interest in biological control of wireworms. Field trial results vary widely, while the reasons for this variability are not always clear. The biological parameters that make wireworm control so difficult are diverse. While the three species *Agriotes obscurus*, *Agriotes lineatus* and *Agriotes sputator* are common throughout middle Europe, there are other wireworms that are nearly as important or even regionally more important. An overview is given over important species and what we know of their biology and behavior. Results of a 10 year-monitoring of *Agriotes* click beetles and of wireworms are presented together with laboratory, field and semifield experiments shedding some new lights on wireworm behavior. Additionally, to the important *Agriotes* species, also wireworms of other genera are considered.

SYMPOSIUM. Monday, 17:30 **MFCS-2**

Attract & kill: an effective control strategy targeting wireworms in potato,
but why are results not consistent?

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Wireworms, the larvae of click beetles, are one of the most important insect pests of agricultural and horticultural crops worldwide. In recent decades, wireworm damage has increased and regularly reached economically severe levels in particular for potato growers. Efficient and sus-

tainable 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 CO_2 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 days and weeks after capsule application as two crucial factors positively correlating with the efficiency of this 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.

CONTRIBUTED PAPERS Monday, 17:00-19:00
BACTERIA 1 Multispace CD

Crystal proteins mode of action

Chairs: Marianne Carey / Juan Luis Juart-Fuentes

CONTRIBUTED PAPERS. Monday, 17:00 **B-1**

Insights into the *in vivo* crystallization pathway and mechanism of toxicity of Cyt1Aa, a naturally crystalline mosquitocidal toxin

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Bacillus thuringiensis subsp. *israelensis* (Bti) is the most commonly used biological insecticide worldwide for mosquito control. Upon sporulation, Bti produces a combination of four protoxin proteins as crystals, three Cry proteins and one Cyt protein – Cyt1Aa. Once activated, Cyt1Aa exhibits low toxicity, meanwhile it is a powerful synergist of Cry toxins, increasing their toxicity by a few orders of magnitude. This is attributed to Cyt1Aa's ability to directly bind to microvillar membrane lipids, acting as a receptor for Cry toxins. As a result, combination of Cyt1Aa with Cry and other protein toxins, such as BinAB, was able to revert resistance phenotypes, notably by bypassing receptor-based toxin resistance. Therefore, Cyt1Aa is considered the key element of Bti toxicity and the reason for the absence of Bti resistance in the field.

Most research on Cyt proteins has focused on understanding their mode of action, resulting in a lack of knowledge regarding the structure of the native protoxin formed *in vivo*. In the present study, we used X-ray Free Electron Laser (XFEL) sources to determine the structure of unactivated protoxin directly from *in vivo* grown *Bt* nanocrystals. The combination of proteomic [SDS-PAGE, MALDI-TOF, SEC-MALS, Native MS] and electrophysiological studies [black lipid membrane (BLM)], with functional analyses through rationale design of Cyt1Aa point-mutants, and various microscopy techniques [transmission electron microscopy (TEM), cryo-EM, surface EM (SEM), atomic force microscopy (AFM)] enabled a com-

prehensive analysis of Cyt1Aa properties, from crystal 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 Cyt1Aa work together to bring about its toxicity. These results open new paths of research, allowing rationale 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 ortohologs in the beetle system serve as receptors also against Bt *tenebrionis* toxin.

CONTRIBUTED PAPERS. Monday, 17:45 **B-4 STU**

Receptor analysis of Cry1Ca toxin expressed on Sf9 cells

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Spodoptera frugiperda is an important pest of maize crops. Cry1Ca toxin, one of the insecticidal proteins produced by *Bacillus thuringiensis*, is highly toxic to several different *Spodoptera* species. Recent studies showed that Cry toxins extensively use insect ATP binding cassette transporter (ABC transporter) family molecules as main receptors in intoxication. Cry1Ca is also toxic to *Bombyx mori*, but Cry1Ca does not use *Bm*ABC transporter family C2 and C3 molecules as receptors, so receptors of Cry1Ca are unknown. Since Sf9 cells, which are ovary-derived cultured cells of *S. frugiperda*, are sensitive to Cry1Ca, we aimed to identify the receptor of Cry1Ca from ABC transporter family molecule expressed on Sf9 cells. The genetic analysis of *S. frugiperda* is delayed and registration of annotated sequences to NCBI has not progressed. In this study, we used a software “PRICE”, which performs *de novo* assembly of RNA-Seq data using the guide sequence information as an initial contig. We firstly obtained 40 kinds of *Sf*ABC transporter-like genes through performing BLAST search against *S. frugiperda* draft genome assembly data, based on 49 kinds of *Bm*ABC transporter expressed in the midgut. Each region of each sequence with high similarity to the *S. litura* ABC transporter was used as a guide sequence for performing PRICE against RNA-Seq data of Sf9 cell. As a result, 18 genes of *Sf*ABC transporter which seemed to express on Sf9 cells were predicted. Using ectopic expression system in HEK293T cells, we are analysing whether these *Sf*ABC transporters can act as functional receptors of Cry1Ca.

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*

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Sweetpotato weevil, *Cylas puncticollis* (Boheman) 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 acrystalliferous 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 (LC_{50}) 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 ABC 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.

CONTRIBUTED PAPERS. Monday, 18:45 **B-8 STU**

Novel method for determining the insecticidal crystal protein composition of *Bacillus thuringiensis* insecticides

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Bacillus thuringiensis (Bt) is the most used active ingredient of biological insecticides. Specific mixtures of d-endotoxins (Cry and Cyt proteins) determine the toxicity profile of each Bt strain. However, a reliable method for their identification and quantification is not available, as some crystal toxins share high sequence identity. We developed an accurate and reproducible mass spectrometry-based method (LC-MS/MS-MRM), using isotopically-labelled proteotypic peptides for each protein in a particular mixture, to determine the relative proportion of each d-endotoxin within the crystal. To validate the method two different artificial mixtures containing Cry1Aa, Cry2Aa and Cry6Aa were analysed. Mixture 1 contained equal amounts of each protein and mixture 2 contained the proteins in a molar ratio of 14:3:14, respectively. LC-MS/MS-MRM determination of the relative abundance of proteins in mixture 1 was: 32-35% Cry1Aa, 24-26% Cry2Aa and 39-44% Cry6Aa; and 43-46% Cry1Aa, 6% Cry2Aa, 48-51% Cry6Aa in mixture 2, respectively. Four Bt-based products were then analysed following this method: DiPel®DF (13-22% Cry1Aa, 16-29% Cry1Ab, 6-12% Cry1Ac and 40-64% Cry2Aa), Xentari®GD (26-33% Cry1Aa, 57-60% Cry1Ab, 7-11% Cry1Ca and 3-4% Cry1Da), Vectobac®(2-4% Cry4Aa, 10-28% Cry4Ba, 10-27% Cry11Aa, 2-4% Cry60Aa, 5-12% Cry60Ba and 38-61% Cyt1Aa) and Novodor® (70-75% Cry3Aa, 14-16% Cry23Aa and 10-14% Cry37Aa). This unique method can be applied to the characterization and design of Bt-based products providing valuable information in terms of host range toxicity, industrial crystal production and quality control.

CONTRIBUTED PAPERS Monday, 17:00-18:45
NEMATODES 2 Commission R8

EPN Biocontrol

Chairs: Ralf-Udo Ehlers / Annika Pieterse

CONTRIBUTED PAPERS. Monday, 17:00 **N-9**

Diabrotica v. virgifera management using genetically improved strains of *Heterorhabditis bacteriophora*

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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 $2x10^9$ 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 trails. 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 $1x10^9$ ha^{-1} and bringing application costs into the range of synthetic chemicals.

CONTRIBUTED PAPERS. Monday, 17:15 **N-10 STU**

Potential microbial control of xylophagous pests with entomopathogenic nematodes and fungi

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The effects of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and fungi strains (*Beauveria bassiana*) were evaluated in laboratory assays against larvae of four xylophagous pests: the Asparagus moth *Parahypopta caestrum*, the European goat moth *Cossus cossus*, the pine longhorn *Arhopalus syriacus* and the black Buprestid *Capnodis tenebrionis*. 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 EPNs suspensions to reach and infect the larvae inside the wood galleries can be a combined sustainable control system.

CONTRIBUTED PAPERS. Monday, 17:30 **N-11**

The potential use of native entomopathogenic nematodes isolated in the Italian areas infested by *Popillia japonica* (coleoptera: scarabaeidae)

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Entomopathogenic nematodes (EPNs) are one of the most important means of controlling white grubs in many infested fields around the world and their natural occurrence in several soils plays a fundamental role for their potential use in biological control programs. *Popillia japonica* (PJ) is an alien invasive insect native to Japan and reported for the first time in Northern Italy in 2014. This pest is considered one of the most harmful organisms for the agricultural crops. This white grub spends most of its biological cycle in the first layers of the soil and is susceptible to the colonization of EPNs. With this scope, a total of 155 sites were sampled in the infested areas in order to find indigenous EPNs potentially able to control the PJ spreading.

EPNs belonging to *Steinernema carpocapse, Steinernema feltiae*, *Heterohorabditis bacteriophora* and *Oscheius* sp. were isolated from 39 out of the total soil samples (25.16%). *Steinernema carpocapsae* is the most abundant (47.6%) and in three samples a mix of EPNs were recovered. Each EPN strain was tested using both pre-winter and post-winter PJ larvae to verify the most effective nematode to control this pest. The best results were obtained using pre-winter larvae and, in particular, an isolate composed by a mix of *S. carpocapsae* and *H. bacteriophora* that killed the total of larvae in all bioassays.

This study could improve the effectiveness of PJ control, implementing the biological control agents already available in nature thus minimizing the environmental impact.

CONTRIBUTED PAPERS. Monday, 17:45 **N-12**

Investigating the biological control potential of indigenous nematodes for the control of invasive slug pests

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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 carried nematodes inside. We conclude that the high susceptibility of *D. suzukii* adults to *S. carpocapsae* during the emergence process from pupae, indicate that nematode soil treatment could be a good strategy to control adult levels and reduce population of this important pest.

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 (cel04068 and cel04146), signaling (P00018; cel04150; cel04310; cel04010; and cel04020); and protein processing (cel04141 and cel03060) 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 shown that the expression profile was significantly discriminant for virulence. All together, these finds support our proposal in the use of transcriptomics and proteomicsto 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, desiccation, 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.

Slugs & Snails DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Press room
Virus DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Auditorium 2
Fungi DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Multispace AB
Bacteria DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Multispace CD
Nematodes DIVISION BUSINESS MEETING	Monday, 19:30-21:30 Commission R8

TUESDAY - 30th July

5K Race Tuesday, 07:30-09:00
Old river bed

CONTRIBUTED PAPERS Tuesday, 9:30-11:30
MICROBIAL CONTROL 2 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

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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 four of the strains showed significant percentages of deformities 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 10^8 conidia ml^{-1}. The LC_{50} were 1.7 x 10^7 and 1.8 x 10^7 conidia ml^{-1} and the LT_{50} 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 EAMa 01/58-Su (0.010±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.

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

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

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The number of *Diabrotica v. virgifera* beetles caught in South-East Styria has continuously increased since 2001. In 2018, up to 130 beetles m^{-2} were caught with an established emergence trap system in untreated maize fields. This represents a triplication of the number of adults caught in 2017 and an increase of almost 500 percent compared to 2016. The goal of this study was to test efficacy of *Metarhizium brunneum* (GranMet™, Agrifutur s.r.l.) and *Heterorhabditis bacteriophora* (Dianem™, e-nema) used alone or in combination with chemical insecticides (i.e. Poncho, Bayer) to control D. v. virgifera larvae in the field. All treatments led to a significant reduction of the number of *Diabrotica* larvae compared to untreated control fields. Results indicate presence of positive synergistic effects which will be discussed in the presentation. The targeted abundance of 5,000 *M. brunneum* CFU g^{-1} TG soil was achieved or even excelled after the first application of the fungal barley product GranMet™. Genotyping with SSR markers confirmed that the *Metarhizium* production strain was able to establish in the treatment areas without any negative side effect on the microbiome. Due to the lack of environmentally friendly, approved chemical insecticides (neonicotinoid ban in Europe), a direct control of the adult beetles with biological active substances is essential. Using a water-dispersible *Metarhizium* formulation, we were able to demonstrate for the first time that the biological control of adult beetles appears possible. Further large-scale field trials are planned.

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

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-BI-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®.

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

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 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* und *A. sputator* to the coated millet in a feed-choice-experiment in the lab. *A. obscurus* tolerated a concentration of $5x10^3$ spores/corn, whereas *A. sputator* tolerated a higher concentration of $2x10^7$. 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 ana*nasa 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

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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 nematicidal 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 nematicidal antagonists included *Pseudomonas*, *Comamonas* and *Variovorax* and were most abundant in rhizosphere and diseased root endosphere. Fungicidal 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 CO_2 gradients established in soil by plant roots to locate potential hosts. Effective plant

protection products are currently not available. Consequently, there is a tremendous need for alternative biological control options. In previous work, a biological bead formulation for wireworm control was developed, based on an attract-and-kill approach that exploits the insect's behaviour. The calcium alginate beads contain both yeast cells that produce CO_2 as an attractant and the entomopathogenic fungus *Metarhizium brunneum* Cb15 III acting as the kill component. However, the degree of efficacy and duration of action depends to a large extent on the fungal development inside the bead and thus on the cell viability after drying. A technical scale process in a self-constructed modified bottom spray fluid dryer was developed that gently dries 0.5 kg beads in 60 min down to a_w 0.2, and additionally, coats the beads. Thus, innovative coatings containing fungal blastospores or aerial conidia were investigated, aiming to provide a high level of efficacy as well as high speed to kill. Subsequently, the coating's impact on the fungal development inside the beads was examined using microelectrodes that provide insight into oxygen- as well as pH-gradients. Oxygen measurements revealed steep gradients directly below uncoated bead surface, indicating an anaerobic environment after 500 μm when baker's yeast is added to the fungal bead. These results will pave the way to a better biological control of wireworms.

VIRUS SYMPOSIUM — Tuesday, 9:30-11:30, 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

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Understanding the ecology and evolution of the interactions of between pathogens and hosts is critically dependent 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.

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

Covert infection by iflaviruses: benefits from nucleopolyhedrovirus infection in *Spodoptera exigua*

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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.

SYMPOSIUM. Tuesday, 10:30 **VS-3**

'Dark' viruses of Drosophilidae (and other invertebrates)

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Metagenomic discovery has revolutionised our understanding of the diversity of animal viruses, with several new divergent virus lineages and thousands of new viruses. However, in the absence of overt symptoms or experimentally amenable isolates, it can be hard to demonstrate that 'virus-like sequences' represent real infections—and impossible to discover viruses that do not look like (known) viruses. I will describe how small RNAs can be used to confirm virus infections in metagenomic sequencing from Arthropods, and the limitations of this approach in other major invertebrate phyla. I will then focus on the use of small-RNAs to discover completely new 'Dark' viruses and —using *Drosophila* viruses as an example—discuss what might be required to find out whether such viruses are really 'covert', and whether they could be considered commensal or perhaps even beneficial.

SYMPOSIUM. Tuesday, 11:00 **VS-4**

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.

CONTRIBUTED PAPERS Tuesday, 9:30-11:30
FUNGI 2 Multispace CD

Mode of action

Chairs: Stefan Jaronski / Nemat Keyhani

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

Lipid processes in the infection process of insect pathogenic fungi

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The ability of fungal insect pathogens to penetrate into and out of the host involves the activities of a host of cuticle-degrading enzymes. While the importance of proteases and chitinase have long been recognized, the ability to assimilate lipid substrates has received far less attention. The genomes of both *Beauveria* and *Metarhizium* species encode for a family of secreted and cytoplasmic lipases whose functions remain obscure. Targeted gene knockouts of individual lipase genes in *B. bassiana* resulted in only minor phenotype and little effects on overall virulence. Impairment of lipid homeostasis via deletion of specific genes has been shown to lead to decreased virulence. The cuticle waxy-layer and its lipid constituents are also known to effect conidial virulence and lipid mobilization. Oleic acid acts as a potent "primer" of *B. bassiana* virulence resulting in 4-5-fold decrease in the mean lethal dose (LD_{50}) required to kill target hosts as compared to standard media. Fungal proteins involved in lipid droplet formation and turnover have been shown to be critical for appressorial turgor pressure and hence cuticle penetration. The caleosin protein appears to function in spore dispersal, and may be linked the production of secondary metabolites, including oosporein, that acts as an antimicrobial compound on the insect cadaver. Current models that take into account lipid assimilation and biosynthetic pathways suggest important lipid-mediated functioning linked to autophagy, mitophagy, overall fungal fitness, and virulence in entomopathogenic fungi that can be manipulated to increase the effectiveness of conidial spores in pest biological control applications.

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

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 infects and colonizes 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.

CONTRIBUTED PAPERS. Tuesday, 10:00 **F-11**

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

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In order for entomopathogenic fungi to colonise an insect host, they must first attach to, and penetrate, the cuticle layers of the integument. Herein, we explored the interactions between the fungal pathogen *Metarhizium brunneum* **and two immunologically distinct morphs of the greater** waxmoth *Galleria mellonella* (melanic and non-melanic). We first interrogated the cuticular compositions of both insect morphs to reveal significant differences in thickness, melanin accumulation, candidate gene expression (e.g., phenoloxidase, DOPA-decarboxylase), hydrocarbons and fatty acid contents. Topical exposure of larvae to *M. brunneum* **conidia demonstrated clearly a reduced capacity of the fungus to adhere to the integument of the melanic morph, with a 3-fold reduction in the number of germinating conidia at 12 hours post-exposure. Critically, stress-associated genes (e.g., heat-shock proteins) were up-regulated in fungi that remained on the integument. Candidate gene expression patterns between the insect** morphs indicated the melanic larvae are primed to 'switch-on' defence- and immunity-associated genes within 6-12 hours of conidia exposure. We reveal previously uncharacterised mechanisms of attack and defence in fungal-insect antibiosis. The authors gratefully acknowledge funding from the RFBR (Grant Numbers 18-316-20007 mol_a_ved and 19-016-00121 a).

CONTRIBUTED PAPERS. Tuesday, 10:15 **F-12**

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

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Membrane proteins are crucial components of membrane system and involved into a variety of physiological processes. An integral membrane protein gene, designated as *BbIMP1*, was identified in *Beauveria bassiana*, a filamentous entomopathogenic fungus. BbIMP1 was mainly associated with vacuolar membranes. Disruption of *BbIMP1* resulted in a significant decrease in sporulation. Conidial yield decreased by 32.25% compared with wild type, and blastospore yield decreased by 14.08%. The Δ*BbIMP1* mutant strains displayed the enhanced sensitivity to thermal stress. Gene loss resulted in a significant reduction in conidial germination under starvation conditions. Within 24 h on water agar plate, the germination rate of the wild-type strain was 57%, while Δ*BbIMP1* mutant only showed the rate of 19%. The virulence of the Δ*BbIMP1* mutant strain was significantly impaired. The median lethal time (LT_{50}) of gene disruption mutant was delayed by 1.02 d and 0.3 d in the topical and intra-hemocoel injection bioassays, respectively, when compared with that of the wild type. These results suggested that the BbIMP1, a vacuolar membrane protein, plays an important role in the environmental fitness and infection cycle of *B. bassiana*.

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.**

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Lipase (triacylglycerol acylhydrolase, (E.C. 3.1.1.3)) is a class of esterases that catalyze 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, conidiogenesis, 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 (Bbmsn2/BbΔmsn2). 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 Bbmsn2/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 Bbmsn2/BbΔmsn2 and incubated for 120h showed fungal 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.*

***Lovera, A.**[1,4]; Belaich, M. [2]; Villamizar, L.[3]; Patarroyo, M. A. [4,5]; Barrera, G.[1]*

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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 codifying to a chitinase (Chi37) was cloned and expressed in *E. coli*. The effect of the recombinant protein (*r*Chit37) over the mortality of *Diatraea saccharalis* larvae infected with Bv062 conidia was studied. The *r*Chit37 was produced on soluble and insoluble fractions of *E. coli* culture and both presented chitobiosidase (170 mU/μL) and endochitinase (90 mU/μL) enzymatic activities, being 45°C and pH 5,0 the optimum conditions for enzyme performance. The soluble fraction *r*Chit37 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 10^6con/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 *r*Chit37 at 200 μg/mL, however *r*Chit37 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 *r*Chit37 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*

***Cai, Q.**[1]; Ren, K.[2]; Shao, W.[2]; Ying, S.[2]; Feng, M-g.[2]*

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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 (m*H3*^{K56R} and m*H3K*^{56Q}) by replacing K56 on H3 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 m*H3*^{K56R} and m*H3K*^{56Q} showed increased sensitivities to various stresses (DNA damage, oxidation, cell wall perturbation, high osmolarity 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*.

BACTERIA WORKSHOP Tuesday, 9:30-11:10
PART 1 Commission R8

Domain-based spedificity 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
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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.

WORKSHOP. Tuesday, 09:50 **BWS-2**

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

Nelson, M.E.*[1]*; *Jiménez Juárez*[1]*, N.; Pérez Ortega*[1]*; C., Pence, H.*[2]*; Bowling, A.*[2]*; and Lu., A.*[1]

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Western corn rootworm (WCR) is a major pest of corn in North America and Europe. Damage caused by WCR has the potential to cost U.S. growers alone billions US$ in yield loss annually if not controlled. Current commercial traits based on proteins identified from *Bacillus thuringiensis* (Bt) bacteria have been an important tool available to farmers to reduce yield loss. Reports of field resistance to current commercial traits highlight the need for new traits based on new modes of action. Corteva Agriscience™ (Agriculture Division of DowDuPont) has pursued multiple strategies including screening of non-Bt sources for proteins that can be developed into insect resistance traits. These efforts identified several proteins that are highly efficacious in protecting roots from WCR feeding when expressed in transgenic plants. Here we report results of work focused on evaluating the mode of action of these proteins. These data show that these proteins represent novel modes of action compared to current commercial rootworm traits and selectively target and kill epithelial cells lining the gut resulting in larval death. Non-Bt sources represent a rich source of actives for the development of future insect resistance traits.

WORKSHOP. Tuesday, 10:10 **BWS-3**

WCR-active protein complexes from fungal genus *Pleurotus*

Sepčić, K.[1]*; Panevska, A.*[1]*; Razinger, J.*[2]*; Modic, Š.*[2]

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Aegerolysins are low molecular proteins found in several eukaryotic and bacterial taxa. Their common feature is the ability to bind different lipids and lipid derivatives, as well as biological and artificial lipid membranes. Aegerolysins from the fungal genus *Pleurotus* preferentially bind to ceramide phosphoethanolamine (CPE), which is the major membrane sphingolipid of invertebrates (particularly insects and molluscs). Moreover, the genomes of *Pleurotus* mushrooms have nucleotide sequences that encode proteins with membrane-attack complex/ perforin (MACPF) domain. In the presence of a protein with a MACPF domain, *Pleurotus* aegerolysins can function as bi-component lytic complexes for target cell membranes. Our recent experiments clearly demonstrated the potential of the *Pleurotus* aegerolysins OlyA6, PlyA2 and EryA in concert with their MACPF-protein partner PlyB to: (i) efficiently form transmembrane pores in artificial lipid vesicles that contain physiologically relevant concentrations of CPE; and (ii) selectively kill larvae and adults of two coleopteran pests of the Chrysomelidae family; the western corn rootworm (*Diabrotica v. virgifera*; WCR) and the Colorado potato beetle (*Leptinotarsa decemlineata*; CPB). The toxicities of these protein complexes against WCR and CPB are comparable to, and in the case of CPB even greater than, that of the Cry34Ab1/Cry35Ab1 complex from *Bacillus thuringiensis*.
Due to their interactions with their specific insect membrane lipid receptor, and not with pest proteins that can be prone to mutation, the chances that insect larvae can evolve resistance to these aegerolysin-based protein complexes should be significantly lower.

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.[1] *; Milligan, J.*[1] *; Bean, G.*[1] *; Slightom, R.*[1] *; Howe, A.*[1] *; Werner, B.*[1] *; Moore, R.*[1] *; Pleau, M.*[1] *; Nance, A.*[1] *; Yin, Y.*[1] ***; Bowen, D.***[1]

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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. Corn, 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 root worm 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.

WORKSHOP. Tuesday, 10:50 **BWS-5**

Mode of Action of new corn rootworm-active proteins

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The general mode of action (MOA) of traditional *Bacillus thuringiensis* (*Bt*) 3-domain Cry proteins has been extensively studied for over forty years and is relatively well-understood. More recently, non 3-domain insecticidal proteins from various genera and species have been discovered,

many of which have toxicity against corn rootworm (CRW), *Diabrotica virgifera virgifera*. Although the structures of many of these non-traditional insecticidal proteins vary, they still have relatively the same MOA as traditional 3-domain Bt Cry proteins: Ingestion, proteolysis, receptor binding, membrane insertion, pore formation leading to insect death. Using a domain-based protein characterization approach that includes bioinformatic 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.

Coffee Break Tuesday, 11:30-12:00
Foyer

BACTERIA WORKSHOP Tuesday, 12:00-14:00
PART 2 Commission R8

Domain-based spedificity 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

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The *Clostridium perfringens* epsilon toxin (ETX) is a pore-forming toxin that forms ion-conductive channels in biological membranes and is noted for causing disease in ruminant animals. The toxin belongs to the ETX_MTX2 family of proteins whose members include numerous insecticidal proteins. Despite limited amino acid sequence similarity among family members, structural conservation suggests these proteins share a similar mechanism of action. Evidence indicates that variable domains within these proteins are responsible for target specificity and differences in cytotoxic activity. Experiments exploring ETX cell specificity and receptor binding will be discussed in relation to other ETX_MTX2 family members.

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[a], Ruby H.P. Law[a], Charles Bayly-Jones[a], Stephanie Kondos[a], Siew Siew Pang[a], Hari Venugopal[a], Tom T. Caradoc-Davies[b], James C. Whisstock[a], Michelle A. Dunstone[a]

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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. Decades of structural research on the MACPF/CDC family suggested that oligomer assembly on the target membrane is mediated via a dedicated ancillary domain. This is followed by planar diffusion upon the target membrane into a ring shaped prepore. The prepore undergoes a concerted conformational change to form the final pore. This model of mechanism, however, is only consistent with Cholesterol Dependent Cytolysins (CDCs), pleurotolysin and perforin that all have dedicated membrane binding ancillary domains. It is now emerging that this canonical mechanism 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 challenge the existing dogma in MACPF/CDC pore assembly that ancillary domains 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 prepore, a pore used within the phagolysome of macrophage, sheds light on a mechanism that binds to a membrane in an "upside-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.

WORKSHOP. Tuesday, 13:20 **BWS-10**

Analysis of mammalian and invertebrate-active structural homologs

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Invertebrate-active pesticidal proteins belong to a number of distinct structural classes. The demonstrated activity of some of these classes is restricted 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 mammalian cells. 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.

WORKSHOP. Tuesday, 13:40 **BWS-11**

Structural studies of the Vip3A proteins by site-directed mutagenesis

***Ferré, J.**[1]; Quan, Y.[1]; Banyuls, N.[1]; Van Rie, J.[2]*

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From an alanine scanning on Vip3Af, we found several mutations that decreased or abolished the insecticidal activity. The residue substitutions covered 92% of the part of the protein starting from amino acid residue 166. Some substitutions had a strong deleterious effect on the toxicity of the protein and became very informative on the role of the residues position on the function/stability of the protein. These mutations were distributed into two clusters (aa167-272 and aa689-741), as well as residues Glu483 and Trp552. Based on *in silico* models and on protease digestion patterns, we have defined five domains of the Vip3Af protein. Domain I ranges from aa12-198, domain II from aa199-313, domain III from aa314-526, domain IV from aa527-668 and domain V from aa669-

788. Mutations in the first cluster are distributed from near the end of domain I up to the end of domain II; some of them affect the stability of the first three domains of the protein. Mutations in the second cluster fall all within domain V and affect the stability of the C-terminal domains (IV and V). More extensive residue changes were done at positions 483, 552 and 689, which revealed the importance of these residues for the stability of the protein. For example, the change Glu483Ala prevented tetramer formation. In addition, the change of Glu to Ala or His made the protein highly unstable to trypsin, but the change to Asp did not have any effect on both stability and toxicity.

CONTRIBUTED PAPERS Tuesday, 12:00-14:00
MICROBIAL CONTROL 3 Auditorium 3

Entomopathogenic fungi allone or in combination

Chairs: Giselher Grabenweger / Waqas Wakil

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, *Metarhizium brunneum* (lab isolate) and *Beauveria bassiana* (the commercial product Botanigard), for preventative control of the false codling moth (*Thaumatotibia leucotreta*; 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 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 the control treatments. The third assay evaluated the level of contamination of fruit by FCM that treated with either type of fungi versus control. Ori 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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Pollen beetles (*Brassicogethes* 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, 12:30 **MC-19 STU**

Compatibility of the parasitoid *Hyposoter didymator* and the entomopathogenic fungus *Metarhizium brunneum* for the control of *Spodoptera littoralis*

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Combined use of multiple micro- and macro-BC agents may enhance the effectiveness of any IPM programme. The cotton leafworm, *Spodoptera littoralis*, is one of the most destructive pests in the Mediterranean basin; it is predominantly controlled using synthetic chemical pesticides. Strain EAMa 01/58-Su of the fungus *Metarhizium brunneum* and the parasitoid *Hyposoter didymator* are promising biological control (BC) agents for IPM of cotton leafworm. In this study, we assessed the compatibility between these two BC agents to control *S. littoralis* under joint attack scenarios. In a first series of bioassays, the possible direct and indirect effects of the fungus towards parasitoid adults, the worst case scenario, were studied. The fungus decreased life expectancy of the parasitoid (LC_{50}=1.85×10^6 conidia ml^{-1}; AST=92.2h) when applied at relatively high concentrations of conidia (1×10^8 conidia ml^{-1}), whereas it did not affect the reproductive potential of the parasitoid females during the three days after treatment. In a second series of bioassays, the combinations between the two BC agents to control *S. littoralis* under different simultaneous use scenarios were investigated, with high compatibility and additive effect in all cases. Nonetheless, parasitoid time releasing-dependent mortality of *S. littoralis* larvae was detected when parasitoid was released after fungal inoculation, with *S. littoralis* mortality value of 77% (when parasitism occurred 48 h after fungal inoculation). Minor effects on fitness of the F1 parasitoid generation were detected. It was observed that parasitization significantly reduced the total haemocytes in *S. littoralis* haemolymph compared with the control, promoting fungal infection. Finally, parasitoids showed a significant preference for non-inoculated *S. littoralis* larvae.

CONTRIBUTED PAPERS. Tuesday, 12:045 **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 *Heterorhabitis 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. bassisna* (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. bassisna* × *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. bassisna* 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, sublethal impact on development

CONTRIBUTED PAPERS. Tuesday, 13:15 **MC-22 STU**

Ecological control of Japanese pine sawyer beetle, *Monochamus alternatus* vectoring pine wilt nematode using *Metarhizium anisopliae*

Kim, Jong Cheol; *Shin, Tae Young; Kim, Sihyeon; Lee, Mi Rong; Park, So Eun; Li, Dong Wei; Beak, Sehyeon; Jo, Min Sung; Gasmi, Laila; Kim, Jae Su*
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Japanese pine sawyer beetle, *Monochamus alternatus*, is the main pest that mediates pine wilt nematode, *Bursaphelenchus xylophilus*, that causes serious damage to the pine forests. So, in order to prevent the spread of *B. xylophilus*, *M. alternatus* have been actively managed, mainly depending on chemical insecticides. However, the use of chemicals for long time induced environmental toxicity and insect resistance. In this work, we developed a strategy to control *M. alternatus* using entomopathogenic fungi. The fungi were collected from mountain soils by an insect-baiting method and two fungal isolates (*Metarhizium anisopliae* JEF-197 and JEF-279) showed high virulence against *M. alternatus*. Strategically the *Metarhizium* isolates were applied to live pine trees to control emerged adults or to damaged trees as overwintering place of larval stage. The JEF-197 showed high insecticidal activity in the two different application methods. In addition, the interaction between JEF-197 and *M. alternatus* was investigated in transcription level to more deeply elucidate the fungal mode of action and insect response. This work could contribute to the development of entomopathogenic fungi-based biological control agents, considering ecological aspect of target insect.

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 robertsii* and avermectins on mosquito larvae: survival and immune responses

Noskov, Yu.A.[1,2], *Polenogova, O.V.*[2], *Yaroslavtseva, O.N.*[2], *Belevich, O.E.*[2], *Yurchenko, Yu.A.*[2], *Chertkova, E.A.*[2], *Kryukov, V.Yu.*[2], *Glupov V.V.*[2]
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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. robertsii*. 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. robertsii* 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.

CONTRIBUTED PAPERS Tuesday, 12:00-14:00
VIRUS 2 Multispace AB

Population genetics and ecology

Chairs: Adly Abd-Alla / Gealan Burke

CONTRIBUTED PAPERS. Tuesday, 12:00 **V-9**

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 space after this paragraph. Keep the length of the abstract's 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) Enping 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.

CONTRIBUTED PAPERS. Tuesday, 12:15 **V-10 STU**

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 the efficacy of control is higher. It is important to determine both the success of replication in larvae taken from treated orchards, and the relative proportions of each virus genotype, to assess the resistance levels of the insect population.

The HRM method takes advantage of 24 bps size difference between the *pe38* gene alleles present in CpGV-R5 and CpGV-M. The allele present in CpGV-R5 is shorter compared with that in CpGV-M. The method has been applied to the detection of genetically pure and mixed viral populations. It has been tested on mixtures of Occlusion Bodies, and also on OBs recovered from larvae dying from mixed infections. Last, tests were done using hemolymph -that contains Budded Virus- from infected larva. Samples containing 90% of one genotype and 10% of the second are reliably quantified.

CONTRIBUTED PAPERS. Tuesday, 12:30 **V-11**

Canonical 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

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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 baculoviruses 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 collinear genomes, they bear unique genomic features, such as insertion/deletion mutations and most stingingly 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.

CONTRIBUTED PAPERS. Tuesday, 12:45 **V-12 STU**

Genetic variability in Chrysodeixis includens nucleopolyhedrovirus

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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 Chrysodeixis 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 colony tested. Restriction endonuclease analysis of individual larvae revealed that "ChinNPV-K" was the most prevalent in larvae inoculated with a higher concentration (LC_{90}) of occlusion bodies (OBs) of Mex-1. Surprisingly, a higher diversity of genotypes was detected in larvae inoculated with lower concentration (LC_{20}) of OBs. The "ChinNPV-K" genotype was not observed in larvae that consumed the LC_{20} inoculum. These findings highlight the dose-dependent nature of genotype transmission and its role in the maintenance of within-host diversity in nucleopolyhedroviruses.

CONTRIBUTED PAPERS. Tuesday, 13:00 **V-13**

A fish perspective of a shrimp disease: yellow head virus in two polyculture ponds

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Simultaneous farming of different aquatic species in the same system (polyculture) is an alternative to the common practice of farming one aquatic species per system (monoculture). The former is used to increase the sustainability and profitability of aquaculture by, for example, rising water quality parameters, reducing the prevalence of pests and diseases, and targeting different commercial markets. In contrast to monoculture, direct in-field observations of the Asian aquaculture hint to a reduction of diseases incidence of shrimp key pathogens (*e.g.* yellow head virus - YHV) when adopting polycultures of tilapia (*Oreochromis* spp.) and shrimps (*Penaeus* spp.). The hypothesis behind the reduction of disease incidence is that the secondary farmed species captures and/or inactivates the pathogen, thus reducing the pathogen load in the system. While attention to multi-trophic aquaculture is an increasingly developing theme, the connection between farmed species, removal/reduction of pathogens, and disease incidence is not jet fully understood. The proposed mechanisms of reduction in polyculture involving tilapia, shrimps, and shrimp pathogens is that the pathogens (e.g. YHV particles) are physically captured by the mucous coating the fish, the gills, and the intestine, and are therefore unavailable to infect the shrimps. To test this hypothesis, we collected and analysed biospecimens from two YHV infected polyculture systems in Thailand and analysed the samples by standard molecular methods (PCR) and by next generation sequencing.

CONTRIBUTED PAPERS. Tuesday, 13:15 **V-14 STU**

Discovery, full-genome sequencing and phylogenetic analysis of a novel Deformed Wing Virus variant found in the dwarf honeybee, *Apis florea*

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Positive sense RNA viruses infect a wide variety of beneficial insect species, including honeybees (*Apis mellifera* and *A. ceranae*), with a broad spectrum of outcomes including asymptomatic infection, acute systemic disease. The most important +ve sense RNA virus affecting honey bees is Deformed wing Virus (DWV) which is causing colony losses worldwide and is transmitted by, and exacerbarted by, the parasitic mite, *Varroa destructor*. In this study, we discovered a novel virus designated as AF_DWV from the wild dwarf honeybee *Apis florea* in Saudi Arabia that in initial studies appeared different from classic Deformed Wing Virus (DWV). The complete genome was amplified by reverse transcription–PCR from *Apis florea.* The AF_DWV has a single-stranded RNA genome of 10.131 nucleotides, including the poly (A) tail. It has 95% identity at the amino acid level with the genus *Ifavirus*. Evolutionary distances of the complete coding sequence showed that AF_DWV represented a distinct lineage within *Iflaviridae*. AF_DWV is a phylogenetically distinct "species" of *Iflaviridae* but closely related to Deformed Wing Virus and VDV-1. AF-DWV infection in *Apis florea* was at a high viremia but was also detected in nearby managed Arabian honeybee (*Apis mellifera jementica*) hives, suggesting that AF_DWV could infect other hymenopterans. Thus, *Apis florea* or their parasites may play a role in the spread of this virus in nature.

CONTRIBUTED PAPERS. Tuesday, 13:30 **V-15**

Seasonal pattern of viral load in colonies of *Apis mellifera* from Italian and French apiaries

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Among the pathogens of the honey bee *Apis mellifera*, viruses are receiving increasing attention. Indeed, immunosuppressing agents, such as the reduction of pollen variety, the agrochemical pollution, and the parasitic mite *Varroa destructor*, which also plays a role in virus transmission, may turn latent viral infections into overt ones. We present here the results of a two-year survey carried out in several apiaries located in Piemonte (Italy) and Provence-Alpes-Côte d'Azur (France). The work was carried out in the frameshift of the Interreg-Alcotra project "Innov'api". Viral loads of the most prevalent viruses (DWV, ABPV complex, CBPV, BQCV, SBV) were estimated in adult populations by Real-time qPCR. Data were collected at the colony level, from a total of 150 hives, both in the production season (summer) and in the pre- and post-wintering periods. At the same dates, the gene expression of three markers of the physiological status of the colonies (vitellogenin, insulin-receptor 1, adipokinetic hormone receptor) was recorded. The same colonies were monitored for varroa infestation, by counting the phoretic mites in the hives all over the sampling period. Besides the plurality of environmental and climatic conditions, this survey embraced different management practices: stationary and nomadic beekeeping, as well as chemical or biotechnical control (coupled with organic treatments) of *V. destructor*. With this experimental plan, we could depict the sanitary status of the apiaries under different conditions. In particular, we gained insights into the seasonal variations of the titre of the main viruses infecting *A. mellifera*.

CONTRIBUTED PAPERS. Tuesday, 13:45 **V-16**

Occurrence and molecular phylogeny of honeybee viruses in hornets

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Since the discovery of correlation of colony decline and the presence of honey bee viruses, major breakthroughs have been achieved on viral pathology and infection processes in honey bee viruses. Transmission patterns and the study of virus vectors such as *Varroa destructor*, had drawn great concern to manage honey-bee health issues. However, little is known about the occurrence and prevalence of honeybee virus in bee predators like wasps and hornets. In the present study, we investigated the occurrence of 13 honeybee virus species in five wasp species from 4 provinces of China and 2 hornet species from 4 regions of France. The results showed that all wasp species from 9 regions of China were infected by different types of honeybee viruses and particularly most of them carried *Apis mellifera filamentous virus* (AmFV), *Deformed wing virus* (DWV) and *Israeli acute paralysis virus* (IAPV), even some were infected by more than 4 viruses simultaneously. DWV was found to be the highest prevalent in France and only two and one sample was found infected by BQCV and SBV, respectively. Phylogenetic analysis on BQCV and IAPV indicated that most of IAPV strains belonged to a single phylogenetic group, while BQCV strains from China and France belonged to several groups. KV from hornet was totally different from those of *Apis mellifera*. This work is also the first report of detection of LSV in hornet in China. These results could serve as a basis for further investigations of transmission and origin of honeybee pathogens in Vespidae species, especially their potential role as carrying viral vectors for bees.

FUNGI SYMPOSIUM — Tuesday, 12:00-14:00, Multispace CD

Managing ticks populations with fungi: Accomplishments and challenges

Organisers / Chairs: Jae Su Kim / Stefan Jaronski

SYMPOSIUM. Tuesday, 12:00 **FS-1**

Managing Tick Populations with Fungi: The Korean Experience

Lee, M. R.[1]; Kim, J. C.[1]; Kim, S.[1]; Park, S. E.[1]; Li D.[1]; Jo, M.[1]; Shin, T. Y.[1]; Lee, D. H.[2]; ***Kim, J. S.***[1]

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Longhorned tick (bush tick), *Haemaphysalis longicornis* (Ixodida: Ixodidae), is a serious pest in Korea as it transmits SFTS virus to humans and has a wide distribution in this peninsula. The tick has been monitored by a governmental research institute in Korea. Still chemical pesticides have not been registred in Korea. Some miticides and synthetic pyrethroids could be used, but the use of chemical controls is not favored for environmental and health reasons, so more environmentally sound management methods need to be developed. Herein, we describe use of an entomopathogenic fungal library to build a fungus-mediated tick management system. Field-collected nymphs were assayed to investigate the virulence of conidia from entomopathogenic fungi including species in the genera *Beauveria, Metarhizium, Isaria*, and *Lecanicillium*. Three *M. anisopliae* isolates showed high virulence against the tick under spray assay conditions in a dosage-dependent manner. Given that the longhorned tick dwells on the soil surface except for blood-feeding periods, the soil surface was sprayed with a fungal spray. In the test, monitoring of longhorned ticks on soil is not always feasible due to their very small size and similar color to soil. To make the monitoring of infected ticks much better, fluorescent red dye was makred on the dorsal part of ticks and placed on the soil. Application of the fungal conidial suspensions on the soil surface where the longhorned ticks were previously released resulted in 60 - 80% mortality 30 days after application. Our results suggest that *M. anisopliae* is virulent against the longhorned tick and that soil surface spraying could be an effective biological control strategy to reduce the population density of this tick.

SYMPOSIUM. Tuesday, 12:24 **FS-2**

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 Ixodidae ticks, *Rhipicephalus annulatus, R. sanguineus* and *Hyalomma 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

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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 chronicle 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[1]
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No abstract could be submitted by the deadline

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

WEDNESDAY - 31st July

CONTRIBUTED PAPERS Wednesday, 08:30-10:30
BACTERIA 2 Auditorium 3

Molecular insights into Bt toxicity
Chairs: OP Pereia / Colin Berry

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

Determination of critical regions for toxicity of the Vip3A protein in European, American, African and Asian pests
Gomis-Cebolla, J.[1]*; Bel, Y.*[1]*, Ferreira Dos Santos, R.*[2]*; Wang, Y.*[3]*; Caballero, J.*[4,5]*; Caballero, P.*[4,5]*; He, K.*[3]*; Jurat-Fuentes, J.L*[2]*; Ferré, J.*[1]
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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 familes 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 acticity of the parental (Vip3Aa and Vıp3Ca) 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 their *vip3* 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. The phylogenetic analysis indicated that the Vip3Aa65 protein fell in the same cluster of Vip3Aa47, Vip3Aa35 and Vip3Aa59 by one amino acid difference. The gel filtration chromatography of the protoxin and trypsin-activated toxin demonstrated that the Vip3Aa65 protein adopted a tetrameric conformation in solution. Regarding the insecticidal spectrum, Vip3Aa65 was tested against five lepidopteran species (*Spodoptera exigua, Spodoptera littoralis, Spodoptera frugiperda, Grapholita molesta* and *Helicoverpa armigera*). The Vip3Aa65 was highly toxic against *G. molesta* (LC_{50} = 49 ng/cm^2) and moderately toxic to the rest of the insect species (LC_{50} = 496–2660 ng/cm^2). In addition, Vip3Aa65 and Vip3Aa16 (control of toxicity) exhibited similar toxicity 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 Bt 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 bacteria 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: spore-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 **B**iofilm **I**ntegration **P**rotein). 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

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 and a signal peptide, suggesting that Bip might be exported and anchored on the cell wall. By using immunofluorescence methods we confirmed that Bip is located at the bacterial surface. We therefore expect Bip to interacts with molecular components of the biofilm, which we are currently looking for. We expect that this study will shed new light on how incoming pathogenic bacteria integrate host resident biofilms.

CONTRIBUTED PAPERS. Wednesday, 10:00 **B-15**

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

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

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Midgut is the primary site of infection for *per os* infecting agents such as *Bacillus thuringiensis (Bt)*. However, insects develop various defence strategies to overcome bacterial infections in their midgut, which basically includes humoral immunity reactions by midgut epithelial cells. It is important to understand the midgut defence biochemistry in order to develop more effective *Bt* isolates. Such defence reactions would also be important in detecting the target midgut receptors binding into *Bt* toxins; as well as understanding the resistance developed by insects against *Bt*. However, most of the knowledge on these topics have been derived from lepidopteran hosts. In the current study, we examined the expression of larval midgut genes in a beetle model, the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera:Chrysomelidae), in response to *Bacillus thuringiensis* ssp. *tenebrionis* at different time points using an Illumina-based sequencing strategy. Three midgut libraries (biological reps) have been generated at 0 h (control-no infection), and 4 h.p.i. (infected) and 12 h.p.i. (infected) from the 4th instar *L. decemlineata* larvae inoculated with *Bt* ssp. *tenebrionis*. There were no significantly differentially expressed genes between the 0 h and 4 h time points, while there were only 12 significantly differentially expressed genes between the 0 and 12 h time points. Interestingly, the 4 hr vs 12 hr revealed 32 differentially expressed genes. Overall, up- or down-regulation of specific midgut genes provide unique hints in understanding how *L. decemlineata* larvae activate their humoral defence and try to overcome the *Bt* infections by down-regulating putative midgut receptors.

CONTRIBUTED PAPERS. Wednesday, 10:15 **B-16**

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

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Populations of the fall armyworm (*Spodoptera frugiperda*) in Puerto Rico, Florida, North Carolina, Brazil and Argentina have developed practical field-evolved resistance to transgenic corn producing the Cry1F insecticidal protein from the bacterium *Bacillus thuringiensis* (Bt). Recent identification of this insect as a devastating invasive pest in Africa, India and China highlights the importance of understanding the mechanisms and the frequency of the alleles responsible for resistance in *S. frugiperda* populations. We previously reported that resistance in *S. frugiperda* from Puerto Rico is genetically linked to a mutation (PR1 allele) in an ATP Binding Cassette subfamily C2 gene (*SfABCC2*) resulting in a truncated, non-functional Cry1F toxin receptor SfABCC2 protein. Using a genotyping test developed to detect this PR1 allele, we present data on its frequency in *S. frugiperda* from the USA, Brazil, Togo and Kenya. Moreover, we present data supporting that resistance to Cry1F in a population from Florida is genetically linked to a different allele from PR1, and provide data on its frequency in field populations of *S. frugiperda* in Florida and neighbouring states.

Considering high levels of resistance to Cry1F are frequent in *S. frugiperda* from diverse locations, interest is growing on evolution of resistance to corn producing the Vip3A toxin from Bt. While Vip3A-resistance alleles have been isolated from *S. frugiperda* field collections, there is no available data on the mechanisms involved. In closing, we will present results from testing for the mechanism responsible for resistance to Vip3A in a strain of *S. frugiperda* originally isolated from Louisiana.

CONTRIBUTED PAPERS — Wednesday, 08:30-10:30
VIRUS 3 — Multispace AB

Pathogenicity and Virulence

Chairs: Cristina del Rincón / Mariano Belaich

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

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

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A crucial step in the pathogenesis of orally infectious viruses is to overcome the gut barrier of their host. In Insects, the gut barrier is composed by a single-layered epithelium lined by a protective chitinous matrix, the peritrophic matrix (PM) that displays functions similar to the mucous secretions of the vertebrate digestive tract. The PM thus constitutes a first-line defense that viruses must cross to reach the epithelium and initiate infection. This process is poorly known for most insect viruses, whether they are pathogenic for or transmitted by insects. Here we address this issue by investigating the lectin-like activity of an insect parvovirus on the digestive tract of a caterpillar host. The *Junonia coenia densovirus* (JcDV) is orally infectious and pathogenic for caterpillars of the pest *Spodoptera frugiperda*. Upon ingestion, the naked capsids of JcDV rapidly concentrate on the PM suggesting their strong affinity for glycans. Our results showed that JcDV interaction with the PM is mediated through its affinity for carbohydrates including GlcNAc, GalNAc, mannose and fucose. Moreover, we discovered that, in addition to a lectin-like activity, capsids likely carry a yet to be determined activity which triggers a localized disorganization of the PM ultrastructure. Such disruption of the PM, may aid viral particles to go through and reach the epithelium. In conclusion, this study has revealed that JcDV earliest stage of pathogenesis triggers the dysfunction of the gut barrier, which may help building biocontrol strategies using densoviruses.

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 *polh* 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 repetitions 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 ectoparasitic *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

***Vlak, Justinus M.**[1], Ali, Ghulam[1], van Strien E.A.[3], van der Werf, Wopke[2], Schijlen, Elio G.W.M.[4] and Zwart, Mark P.[3]*
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Two genetically distinct Spodoptera litura nucleopolyhedrovirus (SpltNPVs) 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 (dN/dS and dI/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 the enhanced virulence of the SpltNPV-Pak-TAX1. This information could help to understand the enhanced activity of SpltNPV-Pak-TAX1 and to select better SpltNPV isolates for the control of *S. litura* in Pakistan and 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*

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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 iflavirus* (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
FUNGI 3 — 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 underlaying 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 entomopoathogenic 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 robertsii* (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 robertsii* 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. robertsii* 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. robertsii* 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. robertsii* 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, 09:15 **F-20 STU**

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

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Charcoal rot, also known as Macrophomina crown rot, caused by *Macrophomina phaseolina* is an important disease of strawberries in California. While conventional strawberry fields are usually fumigated, complete control of the disease is sometimes difficult and the threat is greater in organic strawberries. A potted plant study was conducted to evaluate the potential of two California isolates of *Beauveria bassiana* (ARSEF 8318) and *Metarhizium anisopliae* s.l. (ARSEF 8319) in antagonizing *M. phaseolina* and improving plant health. Entomopathogenic fungal (EPF) inocula at $1X10^{10}$ viable conidia per pot were applied 1 week prior to, at the time of, and 1 week after the application of *M. phaseolina* to the potting medium, and the disease progression was monitored for the next few weeks. EPF appeared to have a positive impact on preventing the deterioration of plant health, although the time of application did not seem to have an effect. These preliminary data show some promise for using EPF in protecting strawberry against plant pathogens.

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 competion 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.

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

Effect of endophytically-colonized tomato and nightshade host plants on life-history parameters of *Tuta absoluta* (Lepidoptera: Gelechidae)

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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* ICIPE20 was identified as a potent alternative to synthetic insecticides to control adult moths through inundative application. However, endophytes that induce systemic resistance are best-suited to target the cryptic stages of *T. absoluta* such as 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. *Hypocrea 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.

NEMATODES SYMPOSIUM — Wednesday, 08:30-10:30, Commission R8

Nematode application, what, when and how?

Organisers / Chairs: David Shapiro-Ilan / Raquel Campos-Herrera

SYMPOSIUM. Wednesday, 08:30 **NS-1**

Scaling up EPN production for sugarcane pest management in 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 levis* (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 m^3 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).

SYMPOSIUM. Wednesday, 08:54 **NS-2**

Entomopathogenic nematode application: tools left in the box

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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 expend their field of application explored.

SYMPOSIUM. Wednesday, 09:18 **NS-3**

Enhancing the aboveground efficacy of entomopathogenic nematodes

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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.

SYMPOSIUM. Wednesday, 09:42 **NS-4**

Entomopathogenic nematode application against root-damaging *Diabrotica* larvae in maize: what, when, and how?

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Due to restrictions of neonicotinoid seed coatings as well as the toxicity of tefluthrin soil insecticides, biological control solutions have been

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-Wuerttemberg, 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

MICROBIAL CONTROL SYMPOSIUM Wednesday, 11:00-13:00 Auditorium 3

Biopesticides IV. Realising the potencial: Ecological benefits of microbial biocontrol

Organisers / Chairs: Roma Gwynn / Mike Brownbridge

SYMPOSIUM. Wednesday, 11:00 **MCS-1**

Realising 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.

SYMPOSIUM. Wednesday, 12:00 **MCS-3**

Integrating different strategies: Can we predict the outcome of multiple agents at different scales?

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Strategies for using entomopathogenic fungi as biocontrol agents for pest control should be designed with consideration of the biology and ecology of the fungus, the target pest and the other organisms and conditions influencing their interaction, i.e. crop stage and species/cultivar, substrate, other control methods, abiotic conditions, etc. If applied under field conditions, surrounding vegetation and indigenous natural enemies must also be taken into account. Here, I will give examples of how the effects of entomopathogenic fungi can depend on context and how the fungi could interact in different ways with the crop and other organisms. The impact of these interactions will be discussed in relation to application strategies and potential compatibilities with other methods for improvement of plant health.

SYMPOSIUM. Wednesday, 12:30 **MCS-4**

***Quo Vadis*, Commercial Microbial Control? Where have we been, where are we going, and how do we get there?**

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Although they still represent a very small part of the global crop protection market, biopesticides in general, and commercial microbial control products in particular, have experienced double digit growth in market value over the past two decades, as the introduction of new chemical active ingredients has slowed to a trickle. Significant improvements in production efficiencies, advances in formulation quality, and discovery of new microbial modes of action are an important component in the rise of biologicals. However, just as important has been a shift in the motivations for adoption, how these products are sold and used, and perception of their value among end users, their customers, and the distribution channels supplying them. This presentation will explore those factors, compare the present commercial microbial landscape with the not-so-distant past, and examine how recent trends in technology, regulation, and economics might impact the future path of commercial microbial control.

CONTRIBUTED PAPERS — Wednesday, 11:00-13:00
VIRUS 4 — Multispace AB

Infection cycle and morphogenesis

Chairs: David Thielman / Manli Wang

CONTRIBUTED PAPERS. Wednesday, 11:00 **V-25**

Autographa californica multiple nucleopolyhedrovirus *ie2* is critical for virus replication

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Autographa californica 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 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 Sf21, TN-368 and Hz-AM1. Viruses containing partial deletions of IE2 had reduced viral DNA synthesis, gene expression, budded virus (BV) production and occlusion body formation in Sf21 cells but not Tn-5B1-4 cells suggesting it may play a role in virus host range. Plutella xylostella nucleopolyhedrovirus-CL3 (PlxyNPV-CL3) is essentially identical to AcMNPV except for IE2, which retains only 37% amino acid identity PlxyNPV-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 PlxyNPV-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 analysis that the Ac108 protein is a constituent of the ODV entry complex, which formation was abolished in absence of this protein. Based on these results, we conclude that *ac108* encodes a *per os* infectivity factor (PIF9) that is also an essential constituent of the ODV entry complex.

CONTRIBUTED PAPERS. Wednesday, 11:30 **V-27**

Interaction of Autographa californica multicapsid nucleopolyhedrovirus GP41 and two host Proteins

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Baculovirus GP41 is the only recognized tegument (O-glycosylated) protein of the occlusion-derived virion (ODV) phenotype. It was shown to be required for egress of budded virion (BV) from the nucleus. However, the precise function of GP41 in the baculovirus replication cycle remains unclear. In this study, GP41 of Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) was found to interact with the putative coil-coil-helix-coil-coil-helix domain-containing protein 2 (CHCH2) and the glycyl-

tRNA synthetase (GARS) of the fall armyworm *Spodoptera frugiperda.* The loci of GP41 the CHCH2 and GARS interacted were localized to the regions of AA162-209 and AA82-329 respectively. Point mutations at a few key residues within these regions resulted in reduced virus production or abolished virus replication completely, in cell cultures. Knock-down of the gars gene expression of Sf9 cells, by RNA interference, caused a five folds reduction in budded virus productivity in infected cell culture. These data suggested that the interaction between GP41 and these two host proteins play important roles in in virus replication.

CONTRIBUTED PAPERS. Wednesday, 11:45 **V-28 STU**

Decoding morphogenesis of Ichnovirus associated to the parasitic wasp *H. didymator* by RNA interference

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Polydnaviruses (PDVs) are a group of mutualist DNA viruses associated to several endoparasitic wasps. They are integrated in the wasp genome and they are produced in a specialized region of the ovaries called calyx. PDVs are injected during wasp oviposition in lepidopteran hosts, causing immune and developmental dysfunctions that permit the wasp larva to develop. PDVs are classified into two taxa Bracoviruses (BVs) and Ichnoviruses (IVs) associated to Braconidae and Ichneumonidae wasps respectively. BVs originates from the integration of a nudivirus in the genome of a wasp ancestor whereas IVs originates from the integration of a virus yet uncharacterized. The function of several genes involved in the formation of BV virions have been characterized, thanks to their sequence homology with core genes shared between Nudivirus and Baculovirus. Recently, 54 genes that may be involved in IV morphogenesis were identified in six clusters in the wasp *Hyposoter didymator* genome. To date, IVs are related to no virus taxa, so we are unable to predict functions associated with these genes. In this work, we tried to determine if some of these genes were actually involved in IVs morphogenesis, using RNA interference technique. Our results show the feasibility of this experimental approach and confirm the involvement of some of these genes in IV morphogenesis.

CONTRIBUTED PAPERS. Wednesday, 12:00 **V-29**

The cysteine-rich region of a baculovirus VP91 protein contributes to the morphogenesis of occlusion bodies

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The core proteins of baculoviruses play an important role(s) in different processes of virus infection. In this study, we dissect the function of a core protein VP91 (HA76) in Helicoverpa armigera nucleopolyhedrovius (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.

CONTRIBUTED PAPERS. Wednesday, 12:15 **V-30**

NSP2 forms viroplasms during Dendrolimus punctatus cypovirus infection

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Reoviruses are thought to replicate and assemble in special cytoplasmic structures called 'viroplasms', while little is known about the viroplasms of the insect reoviruses, the cypoviruses. To investigate the viroplasm of Dendrolimus punctatus cypovirus (DpCPV), all proteins encoded by the 10 genomic segments of DpCPV were expressed in Sf9 cells. It was found that the viral nonstructural protein NSP2 encoded by genomic segment 8 formed viroplasm-like aggregates. This characteristic structure colocalized with the endoplasmic reticulum and was surrounded by intracellular membranes. Colocalization and coimmunoprecipitation assays showed that NSP2 interacts with most of the structural proteins, such as VP1, RdRp, VP3 and VP4, and also the nonstructural proteins NSP1 and NSP3. Immunoelectron microscopy revealed that NSP2 were nearby the endoplasmic reticulum and surrounded by intracellular membranes, and the viral particles were present in the viroplasm-like electron-dense inclusions formed by NSP2. We conclude that NSP2 forms viroplasms during DpCPV infection.

CONTRIBUTED PAPERS. Wednesday, 12:30 **V-31**

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

Marzachì, Cristina[1]*; Ottati, Sara[1]; Persico , Alberto[1,2]; Abbà, Simona[1]; Rossi, Marika[1]; Vallino, Marta[1]; Turina, Massimo[1]; Galetto, Luciana[1]*

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Euscelidius variegatus 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. variegatus*, and named *E. variegatus* virus 1 (EVV1). EVV1 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. variegatus* populations from France and US were found to be virus free (EVV1-), and one virus free population was therefore established in our lab starting from the French one. EVV1 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. EVV1 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 EVV1-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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Various methods have been developed to follow virus entry and replication in cells. They are based on the labelling of the virus particle, or on the modification of the virus genome by insertion of marker genes, the expression of which will identify the infected cell. These approaches do not allow the course of infection (successful or abortive) to be followed over time for each virus particle and its lineage. Baculovirus genomes have been previously modified by various authors by insertion of marker genes such as *lacZ* or fluorescent proteins, either standalone or fused to virus proteins.

The ANCHOR™ technology allows high intensity fluorescent labelling of a genome. When applied to a virus genome, it is possible to follow both individual particles, and the overall course of infection in living cells. This technology has been adapted to the model baculovirus, AcMNPV, as a first step to its generalisation to other baculoviruses. Accordingly, AcMNPV was modified by insertion of the ANCHOR™ system. The virus obtained replicated correctly in Sf9 cells and *in vivo*, and both budded viruses and occlusion bodies were clearly distinguished. Infecting cells or larvae with these virus particles, respectively, allowed the infection process to be monitored. Various ANCHOR™ variants, differing in their fluorescence characteristics, exist. Two AcMNPV viruses carrying ANCHOR™ 1 and ANCHOR™ 3 (red and green fluorescence, respectively) were constructed, which allowed recording of double infections in living cells over time by microscopy. In the future, other baculovirus species will be labelled in such ways in order to study mixed infections.

CONTRIBUTED PAPERS — Wednesday, 11:00-13:00
FUNGI 4 — Multispace CD

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 longhornef tick, *Haemaphysalis longicornis*

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The longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae), is widely distributed 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 (1×10^7conidia/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. anisopliea* and genetic diversity with other *M. anisopliae* isolates. This work suggests that entomopathogenic fungi, particularly *Metarhizium* anisopliae could be used to control longhorend 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 cull 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 (1×107conidia/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

within 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 CO_2 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 CO_2 released by encapsulated baker's yeast was investigated and for the first time distinct attractive concentration ranges were identified. Beside CO_2, 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 biotechnical 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 $1.6x10^7$ 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, 12:00 **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 1×10^8 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 BBCNK2 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 m^2 of moss by counting them and calculating their average number in 6 m^2 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.

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, Parastagonospora nodorum*) demonstrated high potential to produce insecticidal metabolites when tested against the cereal aphid, *Schizaphis graminum*. 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 fungi to produce insecticidal metabolites.

The research was supported by Russian Fund of Basic Research (project # 17-04-01445).

CONTRIBUTED PAPERS Wednesday, 11:00-13:00
NEMATODES 3 Commission R8

EPN infection process and bioprocessing

Chairs: Li Xingyue / 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 beicherriana*)

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Entomopathogenic nematodes (EPN), as microbial bio-control pesticides, have both characteristics of insect natural enemies and insect pathogens, to which pest resistance rarely develops. However, as biological living material, EPNs are more sensitive to the external environment, and its pest-control efficiency is significantly lower than chemical pesticides in the field. This study tries to take advantages of "Biorational pesticide mechanism", and combining thiourea as polyphenol oxidase (PO) inhibitors with low toxicity and high activity with EPNs. To investigate the acceleration effect of thiourea on PO activity inhibition and EPN's insect lethal processing, we studied the infectivity of *H. beicherriana* to *Galleria mellonella* and the activity changes of insect's humoral PO activity which were injected with *H. beicherriana* suspension at different dosages (0, 20, 40, 80 and 160 infective juveniles per larvae) in thiourea solution (1 mmol·L^{-1}) for 72 h. We found that thiourea could suppress *G. mellonella* larvae's humoral immune by inhibiting the PO activity by 15%, and increase the infectivity of *H. beicherriana* to *G. mellonella* by 20%. Moreover, the motility of *G. mellonella* larvae infected by *H. beicherriana* was significantly increased by 10% at all times by thiourea. In addition, thiourea solution (1 mmol·L^{-1}) had no negative effect on the survival rate of *H. beicherriana* infective juveniles. Thus, thiourea can improve the biological control efficiency of EPN and can be used widely as EPN environmentally friendly auxiliaries.

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

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Entomopathogenic nematodes (EPN) are commonly used as biocontrol agents. Infective juveniles (IJs) 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' behaviours 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 dauers/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.

CONTRIBUTED PAPERS. Wednesday, 11:45 **N-20 STU**

***In vitro* liquid culture and optimisation of the entomopathogenic nematode, *Steinernema jeffreyense,* using shake flasks**

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Entomopathogenic nematodes (EPNs) are effective biological control agents against a variety of economically important insect pests and are an environmentally friendly alternative to chemical insecticides. To compete with these chemical insecticides currently in the market, optimisation of the current culture techniques is required. The aim of this study was to develop a basic protocol for the *in vitro* liquid mass culture of the South African isolated EPN, *Steinernema jeffreyense*, in 250 ml Erlenmeyer flasks, to optimise this protocol and finally to provide inoculum for upscaling to large fermenters or bioreactors. Optimisation of the protocol was tested on three fronts, namely volume of liquid media, two different shaking platforms and initial IJ inoculum density. Results show that a lower media volume is preferred over a higher volume, the two different orbital shakers did not differ significantly, and a lower initial IJ inoculum is preferred over medium and high inoculum density. This is the first successful report of the *in vitro* liquid culture of *Steinernema jeffreyense.*

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

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Life history traits (LHTs) 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 × 10^9 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 nematicidal activity of secondary metabolites produced by the entomopathogenic bacterium *Photorhabdus luminescens sonorensis* (Enterobacteriaceae) against the root knot nematode, *Meloidogyne incognita* (Tylenchidae)

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The banning 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, antifungal, insecticidal, and nematicidal activities. In this study, we evaluated three metabolites that were isolated and purified from culture filtrates of *Photorhabdus luminescens sonorensis* (strain Caborca). 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 nematicidal 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. sonorensis*. These compounds revealed different inhibitory activity ranging from a transient quiescence to death. AK1 and AK2 exhibited nematicidal activity to *M. incognita*. The LC_{50} 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 nematodes species. All entomopathogenic species tested were resistant to AK3. This work sheds light on ascertaining the potency of the *Photorhabdus*-derived SMs as nematicides.

CONTRIBUTED PAPERS. Wednesday, 12:30 **N-23**

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

Vandenbossche, B.[1]; *Molina, C.[1]; Barg, M.[1]; Dörfler, V.[1]; Consoli, E.[2]; Centurion Carrera, A.[2]; Ayral, S.[2]; Strauch, O.[1]; Ehlers, R.-U.[1]*
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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*. LD_{50} 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 analysed 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.

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 COMUNICATION — Wednesday, 13:00-14:30, Multispace CD

Science Comunication

Organizer: A. Lorena Passarelli

SCIENCE COMUNICATION. Wednesday, 13:00

Science communication: How does it help the public, science education, research, and the scientist?

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Science communication is an important responsibility that scientists perform in addition to research. Research studies are presented at scientific meetings or during seminar presentations and, when completed, published in journals. Thus, scientists share scientific information and communicate findings for other scientists to build on, ask additional questions, or predict new hypotheses. Scientists are accustomed to disseminating information to other scientists but often have difficulty communicating with the public. There is increasing awareness that the public is being misin-

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.

Lunch Wednesday, 13:00-14:30
Multispace 2

CONTRIBUTED PAPERS Wednesday, 14:30-16:30
BACTERIA 3 Auditorium 3

Entomopathogenic bacteria diversity
Organisers / Chairs: Shuyuan Guo / Christina Nielsen-Leroux

CONTRIBUTED PAPERS. Wednesday, 14:30 **B-17**

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 stablish 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 analyse the serological diversity of Bt and on this basis predict the presence of Bt strains carrying new insecticidal genes.

CONTRIBUTED PAPERS. Wednesday, 14:45 **B-18**

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 probed 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.

CONTRIBUTED PAPERS. Wednesday, 15:00 **B-19**

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

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Bacillus thuringiensis subsp. *israelensis* (Bti) has been widely used as microbial control agent against larvae of many mosquitoes and blackflies. *Aedes aegypti*, together with some other species of the genera *Aedes*, Anopheles, and *Culex*, are vectors of many tropical diseases such as yellow fever, dengue, chikungunya and Zika. The larvicidal activity of Bti resides at least in four Cry (4A, 4B, 10A, and 11Aa) and two Cyt (1Aa and 2Ba) proteins. The insecticidal activity of the whole crystal is high in comparison to individual toxins due to the synergism among them. Synergic processes are starting to be understood, but requires additional research. New sequencing technologies have demonstrated the existence of new *cry* genes in Bti strains, such as *cry60Aa* and *cry60Ba*. These two proteins have been previously described in Bt subsp. *jagathesan* (Btj). They share more than 95% identity with Bti genes and have insecticidal activity against *Culex quinquefasciatus*.

We performed a molecular and functional analysis of the less-studied genes from Bti, such as *cry60Aa, cry60Ba* and *cyt2Ba*. For this, the genes of interest were cloned in the pSTAB plasmid and expressed in the acrystalliferous Bt BMB171 strain. The *cry60Aa* and *cry60Ba* genes were cloned together because they are physically close to one another forming an operon. Both proteins expressed at the same time in Bt were able to form crystal inclusions and were visualized in an SDS-PAGE gel. The toxic activity of Cry60B-Cry60A or Cyt2Ba-containing crystals has been determined against larvae of *Aedes aegypti* and also their capacity to synergize other Bti toxins. The toxicity of the proteins in other insect orders, such as coleopteran or lepidopteran larvae has also been studied.

CONTRIBUTED PAPERS. Wednesday, 15:15 **B-20 STU**

Identification and functional analysis of two novel Cry proteins from *Paenibacillus popillaie* 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 propagates 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 sporulation, 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.

CONTRIBUTED PAPERS. Wednesday, 15:30 **B-21**

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.

CONTRIBUTED PAPERS. Wednesday, 15:45 **B-22 STU**

***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.

CONTRIBUTED PAPERS. Wednesday, 16:00 **B-23**

Histopathology of *Anticarsia gemmatalis* strains susceptible and resistant to Cry1Ac protein and their susceptibility to bionsecticides 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* ssp. *kurstaki* HD-73, a Cry1Ac producer strain. Insects were exposed to the commercial products Agree®, 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 Agree® 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.

CONTRIBUTED PAPERS. Wednesday, 16:15 **B-24**

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

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Biological systems use regulatory pathways to transmit signals and coordinate multiple processes and to efficiently respond to internal and external stimuli. Gene transcription networks exhibit an approximately scale-free distribution, signifying the potential of transcription factors to regulate a multitude of target genes. The signaling networks in many insects including *H. zea* have not been studied previously. In the present

study, third instar larvae of a Cry1Ac tolerant strain of *H. zea* was challenged with a discriminating dole 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
VIRUS 5 Multispace AB

Inmunity and host response

Chairs: 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

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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 in 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 (SfAV-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 synthase 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 gloverin 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. SfAV-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

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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 *Snellenius manila* injects 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

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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 nucleopolyhedorvirus (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.

CONTRIBUTED PAPERS. Wednesday, 15:15 **V-36**

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

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Basal lamina is a thin and flexible extracellular matrix that surrounds insect tissues. This extracellular layer also works as a barrier for pathogens to infiltrate into host tissues. Baculoviruses are well-known insect pathogens that can establish systemic infection by overcoming the basal lamina. Engelhard et al. (1994) reported that Autographa californica multicapsid nucleopolyhedrovirus circumvents the basal lamina by infecting host tracheal system, especially tracheal terminal cells (tracheoblasts), which has been considered as the major infection route for the systemic spread of baculoviruses. In this study, we discovered a novel infection route other than tracheoblasts. We hemocoelically injected GFP-expressing recombinant Bombyx mori nucleopolyhedroviruses (BmNPV) into *B. mori* larvae and followed the progression of infection in tissues. Surprisingly, GFP-positive cells (i.e. virus-infected cells) were observed not only at tracheoblasts but also at thick trunks and branching points of the tracheal system at the initial stage of infection (20-24 hpi). At these non-canonical infection loci, virus-infected hemocytes were frequently attached to the tracheal surface. In addition, a knockout mutant of *actin rearrangement-inducing factor 1* gene (*arif-1*), which we previously reported as an enhancer of systemic infection, did not show such non-canonical infection although this virus successfully established infection via tracheoblasts. These results suggest that BmNPV enhances systemic infection by attaching *arif-1*-expressing hemocytes to the basal lamina of tracheal trunks and branching points and infiltrating it by an unknown mechanism.

CONTRIBUTED PAPERS. Wednesday, 15:30 **V-37**

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 during starvation effect. We confirm that starvation is successful trigger for activation of covert baculovirus infection. Moreover, we show that higher level of phenoloxidases activity in haemolymph of starved larvae did not prevent the activation of baculovirus form covert to overt infection. At the same time, the high population density showed vice versa effect. It was found that the population density of gypsy moth larvae did not affect the mortality induced by the activation of the covert virus infection or the total mortality rate. We demonstrated that an increase in the population density of larvae *per se* facilitates some changes in fitness and innate immunity traits but is not related to the activation of covert baculovirus infection. We suggest that an increase in population density does not increase the risk of epizootics triggered by the activation of covert baculovirus infection and that researchers should pay more attention to studying density-associated factors, such as starvation. This study was supported by Russian scientific foundation (grant # 17-46-07002).

CONTRIBUTED PAPERS. Wednesday, 15:45 **V-38**

Deep sequencing of microRNAs analysis in SeMNPV persistently infected Se301 cells

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Abstract: Baculovirus latent infection has been frequently observed in insect populations, however, the molecular mechanism has not been clarified. In our previous work, *Spodoptera exigua* nucleopolyhedrovirus (SeMNPV) persistently infected Se301 cells were established. Then a cell strain, named P8-Se301-C1 cell, was cloned. The cells do not produce viral progeny but contain some SeMNPV gene transcripts and show the trait of superinfection exclusion after infection with homologous SeMNPV. MiRNA plays an important role in viral infection and host immune regulation. Using small RNA sequencing technology and by comparing the miRNA expression profiles of P8-Se301-C1 cells and Se301 cells, a total of 1370 host miRNAs were detected, of which 1166 miRNAs were known and 204 miRNAs were newly predicted. Moreover, compared to Se301 cells, there were 645 differentially expressed host miRNAs in P8-Se301-C1 cells, among which 313 were up-regulated and 332 were down-regulated. The predicted target genes of these miRNAs are associated with immune defense, spliceosome, peroxidase, mTOR, Notch, Jak-stat and other life processes.

In addition, 16 SeMNPV encoded miRNAs were detected in P8-Se301-C1 cells. Target gene prediction showed that some viral miRNAs could target immediate-early and early genes of the virus itself, as well as insect host genes related to cell cycle, immune regulation and energy metabolism. Quantitative reverse transcription PCR analysis showed that 5 viral miRNAs had different responses to superinfection of SeMNPV and *Autographa californica* MNPV, with significant differences in miRNA expression. The results provide helpful information to reveal the molecular mechanism of baculoviral persistent infection and superinfection exclusion.

CONTRIBUTED PAPERS. Wednesday, 16:00 **V-39**

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

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The endosymbiotic bacterium *Wolbachia pipientis* manipulates the reproductive success in infected arthropods through cytoplasmic incompatibility and also has been demonstrated to induce RNA-virus refractoriness in the dipteran models, *Drosophila melanogaster* and the yellow fever mosquito *Aedes aegypti*. While the molecular mechanism of the cytoplasmic incompatibility has been resolved, questions still remain about the *Wolbachia*-mediated virus-restriction phenotype and how extensive this phenomenon may be within other arthropods. To explore this, we set out to examine broad patterns of *Wolbachia*-mediated virus interference in lepidopteran cell lines. To achieve this, we generated two lepidopteran cell lines stably transinfected with two *Wolbachia* supergroup A and B strains for *Spodoptera frugiperda* cells (Sf9.*w*AlbB and Sf9.*w*MelPop-CLA). Production of the cell lines has been successful with stable *Wolbachia* infection now for over forty passages and with the next stage to challenge cell lines with a diverse range of viral species: a dsDNA virus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) (Family: *Baculoviridae*), a negative sense ssRNA virus Spodoptera frugiperda rhabdovirus (Sf-RV)

(Family: *Rhabdoviridae*), and also a positive sense virus Flock House virus (FHV) (family: *Nodaviridae*). Preliminary virus inoculation trials with Sf9 cells suggest that *Wolbachia* infection has no effect on AcMNPV and Sf-RV, while FHV trials are ongoing. This work improves our understanding of *Wolbachia*-mediated pathogen interference and may provide potential biocontrol strategies for virus infections of agriculturally beneficial arthropods.

CONTRIBUTED PAPERS Wednesday, 14:30-16:30
SLUGS & SNAILS 1 Multispace CD

IPM Toolkit - Biological Control, Mollusc Behaviour and Mollusc Biology

Chairs: R Rae / Solveig Haukeland

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

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

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To date (March 2019), there are eleven described species belonging to the genus *Phasmarhabditis*. They have a worldwide distribution, and are soil-dwelling facultative parasites of slug and snails that parasitize many mollusc families e.g. Limacidae, Agriolimacidae, Arionidae, Milacidae, Vaginulidae, 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 *Phasmarhabditis´* 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 (*P. bohemica*, *P. bonaquaense* and *P. apuliae*), isolated in the Czech Republic and Italy from cadavers of *Deroceras reticulatum*, *Malacolimax tenellus* and *Milax gagates* or *M. sowerbyi* respectively, are effective agents capable of infecting and killing the grey garden slug, or impacting on feeding, even in natural polyxenic cultures. These promising preliminary results open the way for subsequent research targeting monoxenization and mass cultivation of these slug-parasitic nematodes.

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*

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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; *Alloionema appendiculatum*, *Angiostoma margaretae, Phasmarhabditis hermaphrodita*, *Entomelas* sp., two trematode species; *Brachylaima mesostoma, Eurytrema* sp., and one tapeworm species; *Skrjabinia* sp. *Alloionema appendiculatum* was the most common nematode 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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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 *Phasmarhabditis 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

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Slugs are important pests in agriculture and pose an increasing problem in wet periods causing considerable damages to winter wheat and oilseed rape in autumn. The most damage is done by the grey field slug, *Deroceras reticulatum*. Parameters such as no-tillage and catch crops, along with climate change, favour population growth of slugs. Consequently, approaches to sustainable slug control is needed.

Aarhus University, Danish Technological Institute, the global company BASF, the Danish company FieldSense A/S and SEGES have joined forces in a big four-year project with funding from the Danish Agricultural Agency.

The project aims at developing a knowledge- and data-driven smart-farming tool for an area-specific prediction of slug damage in arable crops. The tool will generate pest control-strategy maps, which identify risk areas in a field and provide the farmer with suggestions for optimal strategies (mechanical, pesticide, nematodes). In addition, the use and application of the nematode biocontrol agent Nemaslug® will be adjusted and optimized for application to Danish fields. The project cover: Study of *D. reticulatum* and Nemaslug® in laboratory experiments, slug distribution and ecology in fields, field experiments of control strategies, optimization the application technology of Nemaslug® and development of a slug infestation incidence forecast model.

CONTRIBUTED PAPERS. Wednesday, 15:30 **SS-5**

Winter survival of the invasive slug *Arion vulgaris* in agricultural fields in Norway

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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*

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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 chimiotherapeutic 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 Biomphalysin, 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 Biomphalysins 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 Biomphalysins. The function of these toxins, named Glabralysins, 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*

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A wild population of *Cornu aspersum* (Leioa, Biscay, 43º19´N, 2º58´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 (Z532 = -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

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This study deals with the adaptation of two invasive mussel species *D. polymorpha* and *D. bugensis* to hypoxia. The mussels were exposed to hypoxia (0.56-1.1 O2 мг/л) 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 of ROS elevated and oxidative stress in *D. polymorpha*. Herewith, this species *D. polymorpha* prefers solid substrates and it is more adapted to active hydrodynamic conditions.

CONTRIBUTED PAPERS Wednesday, 14:30-16:30
DBI 1 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 rearing 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 grylli*. 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.

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

Addressing the health of *Macrobrachium rosenbergii* in Bangladesh aquaculture

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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*

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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 *Ameson* genus.
We have discovered nemertean worms in both the gill lamellae of male and female lobsters, and in the broods of berried lobsters. DNA was extracted and the cytochrome oxidase gene was amplified. Molecular sequencing has demonstrated that there are two nemertean egg-predators in lobsters from Saint Kitts, one of which is closely related to *Carcinone-*

mertes conanobrieni, 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.

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

Discovery of Marteilia parasites in UK common cockle (*Cerastoderma edule*) fisheries and comparison with *Marteilia cochillia*

Ilze S.*[1]*; *Bass D.[2]; Villalba García, A.[3]; Carballal Durán, M.[3]; Cao Hermida, A.[3]; Iglesias Estepa, D.[3]; Macarie, A.[1], Shaw, P.[1]; Feist, S.[2]; Hooper, Ch.[2]; Kerr, R.[2]; Ironside, J.[1]*

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Marteiliosis, caused by paramyxid parasites of the genus *Marteilia*, is 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 in the British Isles appeared to be free from *Marteilia* sp. Molecular screening of cockles from ten sites on the Welsh coast indicates that a *Marteilia* parasite is widespread in Welsh *C. 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 sinus 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.

CONTRIBUTED 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).*

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Acute hepatopancreas 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 recently indicates the genome appears to be highly plastic, and deletion insertions often occur surrounding the toxin gene loci. Our data suggest that detection of *pirA* and *pirB* genes by DNA-PCR, as approved by OIE protocol, may not be enough for detecting pathogenic *Vibrio parahaemolyticus* causing AHPND and additional assays such as either detecting the binary toxins or bioassay are need as confirmatory test(s) for AHPND screening.

CONTRIBUTED PAPERS. Wednesday, 15:45 **DBI-6**

The Aquatic *Nudiviridae*

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Few viruses from marine invertebrates have been taxonomically assigned to a virus family and crustacean viruses have been tentatively assigned to families based upon morphological and developmental characteristics and where they replicate within the host cell. This was largely due to the lack of crustacean cell lines for culturing viral infections, but with the recent development and availability of high-throughput sequencing technologies comprehensive descriptions are occurring, facilitating full classifications and placement of novel viruses into families. Multiple rod-shaped viruses have been described infecting the epithelial cell nuclei within the hepatopancreas tubules of crustaceans and they all share the ultrastructural characteristics of rod-shaped enveloped virions that do not form occlusion bodies, resembling Nudiviruses, viruses frequently associated with arthropod (mainly insect) hosts. The *Nudiviridae* family currently contains 2 genera that have been approved by the ICTV (*Alphanudivirus* and *Betanudivirus).* Recently a third genus (*Gammanudivirus*) was proposed that comprises Penaeus monodon nudivirus (PmNV), a virus isolated from *Penaeus monodon* that was found to be different from the other (terrestrial) nudiviruses. Using a combination of histology, electron microscopy and high-throughput sequencing we have characterised three novel nudiviruses derived from European lobster (*Homarus gammarus*), brown shrimp (*Crangon crangon*) and shore crab (*Carcinus maenas*), respectively. We assembled the full genome sequences of these viruses (ranging from ~107 kb-132 kb in length) and comparative genomics and phylogenetic analyses confirmed that Homarus gammarus nudivirus (HgNV), Crangon crangon nudivirus (CcNV) and Carcinus maenas nudivirus (CmNV) are related to PmNV and therefore belong to the genus *Gammanudivirus* within the *Nudiviridae*.

CONTRIBUTED PAPERS. Wednesday, 16:00 **DBI-7 STU**

Understanding the molecular basis of susceptibility to white spot syndrome virus infection in shrimp *Penaeus vannamei*

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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

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.

Al Arimi, WSM.[1,2*].; *Bass, D.[1,3]; Stentiford, GD.[1]; Wilfert, L.[4]; Longdon, B.[2]; Tschirren, B.[2]; van Aerle, R.[1]; Bateman, KS.[1]*

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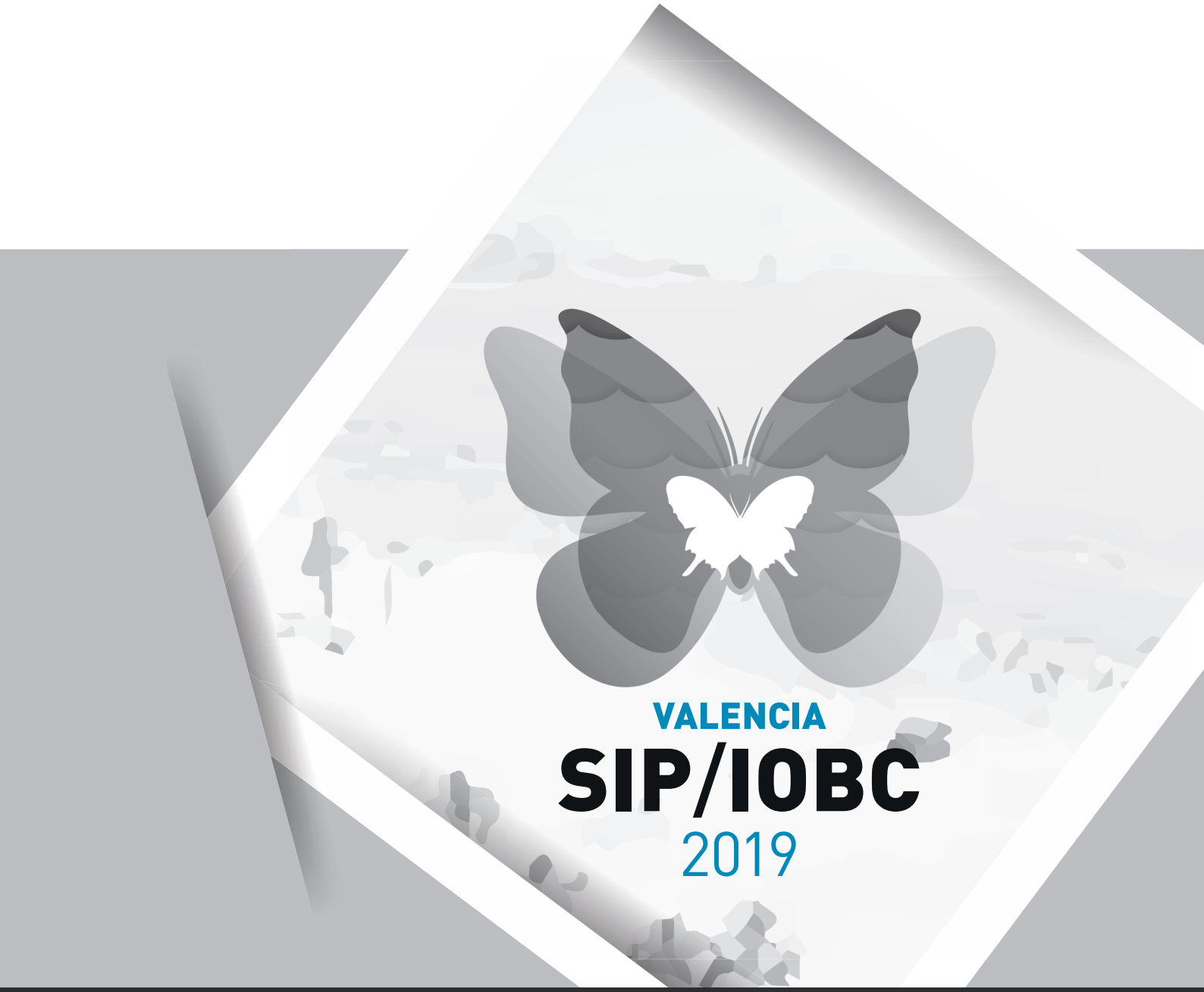
White Spot Syndrome Virus (WSSV; the sole member of the *Nimaviridae* family within the genus *Whispovirus*) was first discovered in farmed Japanese tiger prawn (*Penaeus japonicus*) in Taiwan in the 1990s. The virus has spread globally throughout shrimp farming regions and has had a devastating impact 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.

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



ABSTRACTS - POSTER SESSION 2019

POSTER SESSION Wednesday, 16:30-18:00
BACTERIA Foyer

POSTER SESSION. Wednesday, 16:30 **PB-1 STU**

Effect of a new nematicidal Bacillus thuringiensis strain on Meloidogyne incognita in tomato plants

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Crystal proteins from the soil bacterium Bacillus thuringiensis (Bt) are globally used in agriculture as biological control agents against insect pest, but its use as a nematicidal control agent is still under development. Previously, a total of 310 Bt strains were screened for activity against the free-living nematode Caenorhabditis elegans. LBIT-107 (serotype neoleonensis) showed significant toxicity levels. The discovery of endophytic Bt strains opened new perspectives for studies aimed at the control of sap-sucking insects and plant parasitic nematodes. In this study, penetration and translocation of nematicidal LBIT-107 strain in tomato plants (Licopersicon esculentum) inoculated with spore-crystal suspension delivered by soil-drench and their pathogenicity to Meloidogyne incognita were investigated. Tomato seedlings were transplanted in pots with infested soil of M. incognita. Weekly inoculations were performed with 200 μg of spore-crystal complex of LBIT-107, HD73 and distilled water. The counting of the galls was done after 60 days of treatment. 37% less galls were observed in plants inoculated with LBIT-107. The soil previously treated with the strain LBIT-107 was used to transplant new tomato seedlings and was treated as previously described for 90 days. Interestingly, up to 90% less galls were observed in plants inoculated with LBIT-107. Finally, LBIT-107 was identified in the sap of the plants treated with this strain. These results indicate the penetration and distribution of LBIT-107 in the plant as well as an effect in the reduction of the number of galls of M. incognita.

POSTER SESSION. Wednesday, 16:30 **PB-2 STU**

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

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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. In recent times, some new *Bt* potentials have been explored. These new features include the presence of *Bt* as an endophytic bacterium in plants. Therefore, the search for *Bt* as endophyte is a promising technique. In this work, leaves of 30 plant species, between trees and herbaceous, 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* (lavanda) plant and bipyramidal structure in the sap of *Euphorbia pulcherrima* (noche buena) plant were identified. Interestingly, lavanda strain was toxic to *Aedes aegypti* and noche buena strain was toxic to *Manduca sexta*. Quantitative bioassay with spore-crystal complex and pure crystals show that both strains are highly toxic. Flagellin gene sequencing was carried out and sequence analysis indicated that lavanda strain is 99% identical to the israelensis serotype and noche buena strain is 89% identical to the HD-1 strain.

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*]*
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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 cytochrome 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-4**

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 bacillaceous 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**

Mosquitocidal activity of a Cry1C toxin of *Bacillus thuringiensis* and its synergy with Cyt1A

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The Cry1C protein family of *Bacillus thuringiensis* form bipyramidal 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 bipyramidal 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 Cyt1A 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.

Kim, J. H. *[1]; Choi, J. Y.[1]; Park, D. H.[1]; Park, M. G.[1]; Kim, J. Y.[1]; Wang, M. H.[1]; Cho, H. Y.[1]; Kim, C. J.[2]; Je, Y. H.[1]*
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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 *Plutella 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 *Streptomyce*s sp. AN120537 could be useful resources for development of eco-friendly insecticidal agents.

POSTER SESSION. Wednesday, 16:30 **PB-9 STU**

Mosquitocidal 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 screened for their IGR activities and mosquitocidal 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 *Cyt1*Aa sporulation-dependent promoter with STAB-SD sequence was constructed. This vector was introduced into Bt strain NT0423 expressing Cry1-types toxins. 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**

***Tomita, Y.**[1]; Yamazak, K.[1]; Aiuchi, D.[2]; Asano, S.[3]; Koike, M.[1]*
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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 JackpotR 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, immunoevasion 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

catalysed by phenoloxidase; this increase was not observed when we used the lectin pre-treated with saccharides selected according to its specificity. Although, *P. laumondii* is not considered to be 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).

POSTER SESSION. Wednesday, 16:30 **PB-14**

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* larvae 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.

POSTER SESSION. Wednesday, 16:30 **PB-15 STU**

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.

POSTER SESSION. Wednesday, 16:30 **PB-16 STU**

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.

POSTER SESSION. Wednesday, 16:30 **PB-17**

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.

POSTER SESSION. Wednesday, 16:30 **PB-18**

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

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 stains. 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 innate immune homeostasis in Drosophila, 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 (*cry*1Ab, *cry*1Bd, *cry*1Cb, *cry*1Db, *cry*1Fb, *cry*1Ga, *cry*1Gb, *cry*1Ib, *cry*2Ac, *cry*9Aa) and three vip genes (*vip*1Ac, *vip*2Ae, *vip*3Aa). 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* gene 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 glycerol 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 multilocus 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 lepidoptera and coleoptera 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, enhancins, chitinases, among others. The two *cry8*-like genes, homologous to the sequences Cry8Ac1 and Cry8Qa1 with 73,4 % and 88,9 % identity respectively, were cloned and expressed into the 4Q7/pSTAB system and the larvicidal activity were 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.

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POSTER SESSION. Wednesday, 16:30 **PB-24**

The impact of the absence of the *Bacillus cereus* siderophore Bacillibactin and the FeuA siderophore binding protein 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 haemocoel 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* infection (injection into the hemocoel of *G. mellonella)* shows that the *feuA* mutant and particularly the double mutant ΔfeuAΔentA were as virulent as the willdtype 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 Coleoptera 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. Singularly, 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^{0}79'N; 87^{0}30'E), Chany Lake (54^{0}36'N, 78^{0}12'E), and the Kolyma River (68^{0}27′N, 160^{0}48′E). The collected invertebrates belonged to different orders of Insecta (Coleoptera, Diptera, Hemiptera, and Trichoptera), Crustacea (Amphipoda, Arguloida, 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%). *Rickettsia* were 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 Comamonadacea. 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 grew 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, demandin 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 *Brevibacillus* 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 84% 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* isolates being over 97% similar to reference *B. thuringiensis* strains in the GenBank further confirming the presence of Bt. The determination of the *cry* genes associated with the local *B. thuringiens*is isolates was carried out with six general primers. Most isolates had a multiple *cry* gene profile which included *cry*2, *cry*4, and *cry*9. This suggests that the Jamaican *B. thuringiensis* isolates may be toxic to Lepidopteran and Dipteran insects.

POSTER SESSION. Wednesday, 16:30 **PB-30 STU**

Critical structural and functional amino acids of Vip3Af 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 Vip3Af, several residues have been found to be critical regarding the stability and function of Vip3Af. In

this study we have focused on these Ala-mutants. The insecticidal activity against *Spodoptera frugiperda* has been compared between the protoxin and activated toxin forms. Mutants T167A, E168A, F229A, W552A and G727A totally lost insecticidal activity, both as protoxin or activated toxin. However, E483 was moderately toxic as protoxin, 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 E483A protoxins 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 Vip3Af 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 bipyramidal 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, Acaloleptin 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* Acaloleptin 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. Transcriptom 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 maura* (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 maura* (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. maura* adults in the field resulted in eight novel AMPs in BLAST analysis. These denoted *E. maura* hemiptericins 1-8 (EmHEM1-8). The full length *E. maura* 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. maura 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. maura* defensin 1-3 (EmDEF1-3) have been identified as well. The *E. maura* 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 (Kuhner 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 carbondioxide-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 carbondioxide 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.

POSTER SESSION - DESEASES OF BENEFICIAL INVERTEBRATES	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PBDI-1**

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 antiserum. 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 mortality upon WSSV infection. These results indeed provide a direction to develop efficient antiviral strategies or therapeutic methods by using polyamine CQD.

POSTER SESSION. Wednesday, 16:30 **PBDI-2**

Decrease of NOS/NO is involved in BH4 deficiency-dependent lethality of the *Bombyx mori* mutant *lem*^{l}

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Tetrahydrobiopterin (BH4) is an essential coenzyme for aromatic amino acid hydroxylases and nitric oxide synthase (NOS). NOSs 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* (*lem*^{l}) 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 *lem*^{l} 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. Whatsmore, addition of L-NAME, 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 *lem*^{l} 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 *lem*^{l} larvae.

POSTER SESSION FUNGI	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PF-1**

New Isolates of Beauveria bassiana from Kyrgyzstan and their Entomopathogenic Potential

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Beauveria bassiana is the best-known entomopathogenic fungus causing insect disease worldwide, and it remains a poorly studied biocontrol agent in Kyrgyzstan In this study, we aim to obtain new isolates of this fungus from natural sources in Kyrgyzstan, to study their biological characteristics, to optimize the media for growth of *B. bassiana* and to undertake bioassays of different formulations for their efficacy, in order to select the more active entomopathogenic species for biological control of hazardous soil and ground pests.
Using simple biochemical tests, the ability of natural *B. bassiana* isolates to produce enzymes such as amylase, protease and lipase has been identified. Based on morphology and the ITS sequence and partial sequencing of the 18S (SSU rDNA) and EF1-α genes, the isolates were identified as *Beauveria bassiana.*
These formulations based on *B. bassiana* were bioassayed against white grub larvae *(Phyllophaga fullo*) (*Coleoptera*, *Scarabaeidae*) and whiteflies *Trialeurodes vaporariorum* (*Hemiptera*, *Aleyrodidae*) for nymph and adult populations. The efficiencies of tested *B. bassiana* strains for the third stage of *P. fullo* larvae varied. Only two strains showed entomopathogenic activity, with mortality reaching 73.2% for Bav.5-Gal treatment and 74.8 for Bav.1-Lep treatment. These same strains have showed a significant effect on *T. vaporariorum* (whiteflies) for nymphl and adult populations. In six days, the mortality of the experimental insects was 65.7-79.2% for a triplicate.

POSTER SESSION. Wednesday, 16:30 **PF-2**

***First record of entomopathogenic fungus Entomophaga aulicae* in the populations of browntail moth in Bosnia and Herzegovina**

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Browntail moth, *Euproctis chrysorrhoea* (L.) (Lepidotera: 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. Browntail moth occurs periodically in high numbers (outbreak). Entomopathogenic fungus *Entomophaga aulicae* (Reichardt and Bail) Humber (Zygomycotina: Entomophtorales, Entomophtoraceae) is widespread Holarctic species, with many host insects from order Lepidoptera, where are some of the most economically harmful, outbreaking species of forest defoliators

In sessile oak forests of eastern Bosnia and Herzegovina, the population density of browntail moth was determined by using route measurement during the growing season in the period 2015-2016. Browntail 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 Sjemeć). 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 browntail 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 browntail 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 × 10^5 conidia/ml of *B. bassiana* CNMN-FE-01 strain caused 100% mortality of *S. lineatus* adults on the day five after treatment. LC_{50} value was equal to 1.127 × 10^4 conidia/ml. The mortality and LC_{50} 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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Stenoma cecropia Meyrick (Lepidoptera: Elachistidae) is an important defoliator pest in oil palm plantations. Searching for alternatives to its control, two strains of *Cordyceps cateniannulata* (CPIsp1201 and IPIsp1201) and three strains of *Beauveria bassiana* (CPBb0502; CPBb0411; CPBb0404) were evaluated against *S. cecropia* larvae. The pathogenicity tests were evaluated under laboratory conditions (27.8 ± 1 °C; 72.9 ± 13.1%). The bioassays were performed using oil palm leaflets infested with one larva per leaflet. Each larva was inoculated with 5 µl of a conidial suspension that contained 1 x 10^7 conidia/ml. The five strains were pathogenic against *S. cecropia*. However, CPIsp1201 and IPIsp1201 strains of *C. cateniannulata* were selected to virulence evaluation because caused more than 80% of mortality under laboratory conditions. The virulence evaluation was performed under a sunshade that allows 70% of sunlight (27.1 ± 3.6 °C; 87.5 ± 16.1%), using nursery palm infested with 15 larvae per leaf. Each leaf was sprayed using 45 ml of a conidia suspension containing 4.37 x 10^8 conidia/ml per leaf. No significant differences were found between two strains of *C. cateniannulata* according to Tukey's test (p=0.05). The mortality caused by IPIsp1201 and CPI1201 strains was 93.3% and 84.0%, respectively. These strains were selected for dosage evaluation made under same conditions of virulence assay. The dosages evaluated were 5 x 10^{12}, 1 x 10^{13} and 1.5 x 10^{13} conidia/ha and no differences were found. The strains CPIsp1201 and IPIsp1201 were selected for further field evaluation against *S. cecropia* under oil palm plantation.

POSTER SESSION. Wednesday, 16:30 **PF-5 STU**

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, conidiogenic 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 gluthatione 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.

POSTER SESSION. Wednesday, 16:30 **PF-6**

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 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-7**

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

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Some insect pathogenic fungi can produce resistance structures called microsclerotia (MS), which are potential candidates for use in biological control programs. The main goals of this study were to compare the microsclerotial growth in *Beauveria bassiana* strain GHA and *Metarhizium robertsii* strain ARSEF 2575 and to characterize the expression pattern of genes and activity of enzymes involved in oxidative stress and peroxisomal biogenesis. Fungi were cultured in agitated (250 rpm) complete liquid medium with optimal carbon/nitrogen ratio for MS production. Daily aliquots were collected and examined by both optical and transmission electron microscopy (TEM) after staining with the peroxidase activity marker 3,3-diaminobenzidine (DAB). Samples were also used for qPCR analysis to study the expression pattern of superoxide dismutases, catalases, and peroxin genes involved in peroxisome biogenesis. DAB staining revealed high peroxidase activity in MS for both fungi, with lower staining in hyphae close to the borders of the structure. TEM images also showed high peroxidase activity in mitochondria and peroxisomes. Although peroxin genes were induced in both fungi, *Bbpex7* was more induced in *B. bassiana*, whereas *Mrpex19* showed higher expression levels in *M. robertsii*. At least one of each oxidative stress marker family was also induced in both strains. We conclude that an oxidative stress scenario is triggered during MS formation, including proliferation of peroxisomes and high peroxidase activity. Additional in-depth studies are needed to elucidate the relationship between MS formation, oxidative stress and peroxisomal biogenesis to better understand the similarities and differences found in microsclerotial development by both fungi.

POSTER SESSION. Wednesday, 16:30 **PF-8**

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 alanysis on ITS and β-tubulin and morphogical 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 $1x10^8$ conidia/ml by spray application. *S. invicta* chitin synthase 1 gene (SiCHS-1) obtained by NGS analysis on *S. invicta*. Feeding dsRNA, which is specific to SiCHS-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-9 STU**

Differential susceptibility towards UV-B radiation among *Metarhizium* spp. isolates from different ecosystem compartments

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Metarhizium spp. is a diverse group of entomopathogenic fungi. Evidences indicate that a *Metarhizium anisopliae* subclade (Mani 2) is predominantly adapted to explore insects as a resource, while *M. robertsii* and *M. brunneum* are more adapted to interact with plants. 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 robertsii* (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: $1x10^6$ 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. robertsii* isolate was the most tolerant, with viability higher than 50%. There was considerable within-species variability for *M. robertsii* and *M. anisopliae*. Tolerance to UV radiation therefore varies intra- 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.

POSTER SESSION. Wednesday, 16:30 **PF-10**

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 fugus 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-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. vaporarioum*. The eggs were exposed to a concentration of 1×10^8 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 haemocoel 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 *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. muscarium* 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 whiteflies egg 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-14**

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

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 of *A. apis*. There were carried out 3 experiments to better evaluation of suitable conditions for 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 Neubauer 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.

POSTER SESSION. Wednesday, 16:30 **PF-15**

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

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Blastospores are yeast-like cells produced by entomopathogenic fungi that are infective to arthropods. The aim of this study was to increase blastospore production of two *Metarhizium* isolates in liquid cultures. A modified Adamek's medium was supplemented with two glucose concentrations and the fermentation time was accelerated by using a blastospore pre-culture as inoculum. Air-dried blastospores were applied against larvae of the cattle tick, *Rhipicephalus microplus*. Our results revealed that blastospore production of *Metarhizium* is isolate- and species-dependent. Glucose-enriched cultures (140 g glucose/L) inoculated with pre-cultures improved yields with optimal growth conditions attained for *Metarhizium robertsii* ESALQ1426 that rendered as high as 5.9 x 10^8 blastospores/mL within 2 days. Resultant air-dried blastospores of ESALQ1426 were infective to cattle tick larvae with comparable efficiency to conidia. Altogether, we argue that both osmotic pressure, induced by high glucose concentrations, and isolate selection are critical to produce high yields of blastospores that hold promise to control cattle-tick larvae.

POSTER SESSION. Wednesday, 16:30 **PF-16**

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 Lidorr, STK & BASF or Syngenta. Another trend is to collaborate to develop novel biopesticides, such as Bayer & Forth, Corteva & Evogene, Nufam & Marrone Bio, FMC & Chr Hansen, Syngenta & DSM, BASF & Jülich or PAT, 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. liacinus*), two *B. bassiana* products of Arysta Life Science, Chongchaesak® of Farm-Hannong (*B. bassiana* ERL836), Rizotec® of Rizoflora Biotecnologia (*Pochonia chlamydosporia* PC10), and Broadband® & Velifer® of BASF (*B. bassiana* PPRI53339). 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.

POSTER SESSION. Wednesday, 16:30 **PF-17**

Single Cell Encapsulation via Pickering Emulsion for Biopesticide Applications: *Metarhizium brunneum* against foliar pests

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A new approach for single cell microencapsulation in an oil-in-water (o/w) Pickering emulsion is presented. The water/paraffin emulsions were stabilized by amine-functionalized silica nanoparticles. The droplet size of the emulsions was highly tunable, and ranged from 1 to 30 μm in diameter. The controllable droplet size along with the high colloidal stability of the Pickering emulsions was harnessed to obtain single cell microencapsulation. Successful encapsulation of the conidia entomopathogenic fungus *Metarhizium brunneum* by the studied Pickering emulsions was confirmed via confocal laser scanning microscopy. The resulting systems were implemented to develop a novel biopesticide formulation for arthropod pest control. The conidia incorporated in the emulsions were applied to *Ricinus communis* leaves by spray assay. After drying of the emulsion, a silica-based honeycomb-like structure with an ordered hierarchical porosity is formed. This structure preserves the individual cell encapsulation. The successful single cell encapsulation has led to a high distribution of conidia cells on the leaves. The Pickering emulsion-based formulation exhibited significantly higher pest control activity against *Spodoptera littoralis* larvae compared to the control systems, thus making it a promising, cost-effective, innovative approach for tackling the pest control challenge.

POSTER SESSION. Wednesday, 16:30 **PF-18**

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, 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 D*pr1C*, D*pr1H1* and D*pr1H2*, 20–30% in D*pr1B* and D*pr1G* and 5–10% in D*pr1A1*. 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.

POSTER SESSION. Wednesday, 16:30 **PF-19**

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 defoliate *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 boxvood 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.

POSTER SESSION. Wednesday, 16:30 **PF-20 STU**

Entomopathogenic fungi as biocontrol agent against the bulb mite, *Rhizoglyphus robini*

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The bulb mite, *Rhizoglyphus robini* 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 occurrence of entomopathogenic fungi (EPF) in onion and garlic fields in Israel and the Czech Republic and test selected EPF strains against *R. robini* under laboratory conditions. Strains of *Beauveria* sp., *Isaria* sp., *Lecanicilium* 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 *Lecanicillium*) *of* EPF were tested against *R. robini* 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. robini* mites was found in strains of *Metarhizium* sp. isolated from Kolence (99.3 %) and Meziříčí (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. robini*.

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

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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) Vuill. (Hypocreales: Cordycipitaceae) were evaluated for potential control of redbanded stink bug. Four concentrations ($7x10^4$, 10^5, 10^6, 10^7) of both a Mississippi Delta native strain (NI8) 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* tested were 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 (LC_{50}) and lethal sporulation (LS_{50}) 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 NI8. 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.

POSTER SESSION. Wednesday, 16:30 **PF-22**

Bacterial decomposition of insects post *Metarhizium* infection, possible influence on plant growth.

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Strains of entomopathogenic fungi may have substantional distinction at the final stages of mycoses. Insect cadavers usually overgrow with

mycelium after colonization of insect body, but in many cases bacterial decomposition of the colonized hosts is occurred. We studied two *Metarhizium robertsii* 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 (*Solanum lycopersicum*) on sand substrate in laboratory conditions. It was shown the infection did not cause significant shift in midgut 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**

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 experimenter, 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 10^5 conidia/cm^2. In this range, the automatic (R^2 = 0,946) and semi-automatic (R2 = 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**

Determining relative infectivity/virulence of selected entomopathogenic fungi against Asian Citrus Psyllid using spray exposure bioassays

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As part of an integrated pest management strategy against *Diaphorina citri,* a screening protocol was developed to determine infectivity, virulence, and overall mortality of entomopathogenic strains of fungi. Thus far, 13 strains sourced from commercial mycoinsecticide formulations as blastopore and conidiospore suspensions, as well as two newly-found strains of *Beauveria spp.* isolated from *D. citri* adults collected off residential trees of south Texas have been tested against a standardized mycoinsecticide in a controlled setting. A statistical ranking system has been established in which top performing pathogenic strains can be selected for field. Potter spray towers are utilized to deliver a range of doses of viable spores per milliliter on adult *D. citri* in a spray pattern consistent with the spores/hectare observed in real-world spray applications. After a 7 day incubation period at parameters selected to reflect the conditions in the Lower Rio Grande Valley (LRGV), mortality of the *D. citri* is assessed and the collected data is analyzed against a mycoinsecticide standard, PFR97® (*Isaria fumosorosea;* Apopka97 strain). The resulting data is analyzed in terms of infectivity and virulence, providing insight into mortality, rate of infectivity, and susceptibility to infection. Of the strains tested, 5 have shown promise when evaluated against the standard. This protocol is being adapted for both primary and secondary acquisition, and will elucidate potentially effective strains to be selected for field testing and application on citrus across the LRGV and other citrus growing areas.

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

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The Asian Citrus psyllid (*Diaphorina citri*) vectors "*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 *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 *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**

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.

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Rhipicephalus sanguineus s.l. is a tick of medical and veterinary importance. Entomopathogenic fungi, e.g., *Metarhizium anisopliae* s.l., infect ticks by adhesion of conidia and their penetration through the host cuticle. High temperatures, however, may limit the efficacy of entomopathogenic fungi to cause infection. In this study we investigated the effects of heat-exposure of *M. anisopliae* s.str. conidia to the fungal penetration through the cuticle of *R. sanguineus* s.l. Engorged females were 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 (45 ± 0.2 °C for 4 h). Plates with treated females were then incubated at 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 tick 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 RBP2 and TEF and the intergenetic 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-28**

Histological changes in the *Culex pipiens* mosquito larvae treated by the entomopathogenic fungus *Cladosporium sp.*

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The Culicidae are biting insects, the most harmful to people, they are almost all bloodsuckers, and they are responsible of the spread of many important diseases such as malaria, yellow fever, and elephantiasis. Entomopathogenic microorganisms occupy an important place among the alternative methods of fighting against pests insect. The fungus *Cladosporium sp* is an entomopathogenic agent naturally present in the ecosystems. It offers a very interesting potential for controlling populations of mosquitoes.
This study aimed to show the histological changes that occured in *Culex pipiens* larvae infected by *Cladosporium*. The 4th-instar larvae were infected with *Cladosporium sp.* using a 10^7 spore/ml dilution. In histological sections the fungi infected all the body parts specially cuticle, epidermis, fat bodies and midgut. Finally, the infected insects have a white appearance and are covered with a thick coat of hyphae. Our results show that the application of *Cladosporium sp* to the cuticle of the fourth stage larvae of *Culex pipiens* was dependent of an apparent disturbance on the structure of the cuticle or degeneration of its different parts. Infection of the fungus does not stop at the cuticle. The fungus colonizes the adipose tissue, epidermal cells and alimentary tract.

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.

POSTER SESSION. Wednesday, 16:30 **PF-30 STU**

Can nematotoxic proteins of *Coprinopsis cinerea* be used to protect plants against plant-parasitic nematodes?

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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 *Coprinopsis cinerea*. When exposed to fungivorous nematodes, *C. cinerea* 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.

POSTER SESSION. Wednesday, 16:30 **PF-31**

Effect of Entomopathogenic Fungi onPopulations of *Euschistus heros* (F.) in Soybean Crops (*Glycine max* (L.) Merril) in Paraguay

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The brown stink bugs, *Euschistus heros* (F.) (Hemiptera: Pentatomidae), it is a pest that is distributed throughout the region in the production of soybeans in Paraguay and in other countries in South America, and can result in large losses in performance. The control of this pest in the present time is performed using chemical control, using commercial products of medium and high environmental impact, speaking mostly nicotinoids insecticides pyrethroids that possess a large detrimental effect on the entomofauna beneficial mainly. At present there is a great demand for solutions to these problems, which motivates the development of handling techniques for control of these insects, which could reduce the environmental impact. The use of biopesticides achieves these goals with good values of control, which makes them safe and efficient. In the present study we evaluated the pathogenicity of isolates of entomopathogenic fungi on *Euschistus heros* as a control tool in the plan of Integrated Pest Management (IPM). Monitoring and collections were made of adult bugs in the eastern region of the District of New Toledo - Caaguazú Department, reproductive stage R5 of the crop. It was determined the pathogenicity of 3 strains of *Metarhizium* and a native strain of *Beauveria bassiana* by performing the application by immersion in fungal suspensions with $1x10^8$ conidia ml^{-1} and evaluated during 7 days. According to the results obtained significant differences were found between the strains used of *Beauveria bassiana* and the strain of *Metarhizium brunneum* with 43.84±6.92 and 81.15±8.86 % of mortality and a median survival time of 5.63 ±0.338 and 4.73 ±0.397 days for *M. brunneum* (ARSEF 4556) in relation to the control.

POSTER SESSION. Wednesday, 16:30 **PF-32 STU**

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± 51 fungal spores/mm, equivalent to 2.5 $x10^{12}$ 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.

POSTER SESSION. Wednesday, 16:30 **PF-33**

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 in a study 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 10^7 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 fungus, and of any saprobic 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 298, 300 or 305 (*Metarhizium* species) at 10^7 conidia/ml died most rapidly (LT_{50} 4.3–4.8 days and LT_{90} 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.

POSTER SESSION. Wednesday, 16:30 **PF-34**

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 LC_{50} values were calculated as $1x10^8$ conidia/ml for major worker and $2x10^6$ conidia/ml for minor worker after 120 h application by spraying. Hydramethylon 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 ($1x10^4$ conidia/ml) by spraying with 1 ppm of hydramethylon 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 synergistism 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.

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POSTER SESSION	Wednesday, 16:30-18:00
MICROBIAL CONTROL	Foyer

POSTER SESSION. Wednesday, 16:30 **PMC-1**

The oak processionary moth (*Thaumetopoea processionea*) in climate change –
bionomy, phenology and natural antagonists

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The oak processionary moth (OPM), *Thaumetopoea processionea* L. (Lepidoptera: Notodontidae), is becoming more and more threatening in Central Europe since the 1990s, possibly linked to the current climate change. The caterpillars cause fatal defoliation of host trees, and their dangerous hairs (setae) severely affect human health. Therefore, basic elements of an online early warning system comprising regional data on the development and density of OPM populations as well as on the associated hazard for forests and human health are currently developed in the course of the present project “ModEPSKlim”. It is funded by the German “Federal Ministry of Food and Agriculture” and “Federal Ministry for the Environment, Nature Conservation and Nuclear Safety” and involves five cooperation partners. Data ascertainment on OPM phenology, population dynamics (especially natural regulation factors as parasites, parasitoids, predators, and microbial antagonists including viruses), aerial spread of setae (dependent on OPM abundance, spatial distance and weather (forecast)), contribute to make current and future threat of defoliation by the caterpillars and setae pollution predictable. This aims at a timely and effective application of preventive and regulatory measures against OPM in favour of oak tree and human health protection.

POSTER SESSION. Wednesday, 16:30 **PMC-2 STU**

Endophytic effect of *Beauveria bassiana* in tomato on greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)

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The application of entomopathogenic fungi as an endophytic agent leads to improving the efficiency of biological control of pests. It has been demonstrated that the endophytic fungus, *Beauveria bassiana*, as one of the most successful entomopathogens is able to control different pests effectively. Therefore, in the present study, endophytic effect of *B. bassiana* on the mortality percentage of different developmental stages of *Trialeurodes vaporariorum* was investigated. Tomato plants (*Solanum lycopersicum* L., Falat cultivar) were inoculated with conidial suspension employing four different methods; foliar spray, rhizosphere inoculation, seed treatment and soil drench before seeding. Based on our findings, the highest fungal colonization rate was observed in the foliar spray treatment two weeks post-inoculation. Subsequently, the endophytic effect of *B. bassiana* on was assessed following spraying of fungal suspension either on whole tomato or one part of foliar. Two weeks post-inoculation, greenhouse whitefly adults were introduced into the inoculated plants and removed after 48 hours. The number of hatching eggs, living early nymphs, latal nymphs and adults were counted. The colonization of tomato plants by *B. bassiana* adversely affected the survival of different developmental stages of *T. vaporariurum*, excluding the eggs. The percentage of egg mortality on inoculated plants did not differ significantly compare to that of control. Our results clearly showed the suppression of greenhouse whiteflies on treated plants. Due to successful colonization of *B. bassiana* in tomato and the pathogenic effect of endophyte on *T. vaporariurum*, the application of this fungus in biological control of greenhouse whiteflies is highly recommended.

POSTER SESSION. Wednesday, 16:30 **PMC-3**

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. citr*. The psyllids were reared on *Myrraya exotica* in an artificial growth chamber (90%RH, D : L = 12h : 12h). At the spore concentration of 1×10^6 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. citr*. As the results indicated, the mortality increased with the spore concentration of *Beauveria bassiana*. Among the serial dilution (1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 con/ml) of the spores concentrations, the psyllids showed 100% mortality on the 8th days after the infection at the concentration of 1×10^7 and 1×10^8 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-4 STU**

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 foliar 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-5**

First report of *Conidiobolus coronatus* in Paraguay as biological control of leaf cutting ants

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Acromyrmex landolti fracticornis 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 appropriated 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. landolti fracticornis*) 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 Asuncion in Paraguay, for spore and mycelium isolation in accordance to standard decontamination procedures. Based on morphological characteristics and molecular phylogenetic analyses, the Entomophthoral 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 (gardeners, 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-6 STU**

Development of a SNP based tool for the identification and discrimination of *Melolontha melolontha* and *M. hippocastani*

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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. hippocastani* 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. rarum* 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 hold 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 containing moistened sand (15% moisture), with the holes facing down. A suspension containing 100 IJs was inoculated around each culm of sugarcane. The evaluation was performed 7 days after the starting of the experiment, based on larval mortality. The isolate PAM25 caused 75% mortality of the larvae hold within the culm, suggesting to have a higher insect search capacity. Some features may have contributed for this nematode performance, such as a cruiser behaviour as well as its smaller size compared to the other isolates, which may helped to penetrate the culm and reach the insect.

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

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 Cenipalma in Barrancabermeja (Colombia) from a fruit scraper 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 LC_{50} 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 passes 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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Beuaveria 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-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 endospores 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-*

ticillium 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* (Bt) 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 median lethal time of 7.8 days and caused significant rates of mycosis on *S. frugiperda* larvae. In the isolation of entomopathogenic fungi from artificially inoculated soils, *S. frugiperda* larvae proved the general suitability to serve as baiting insects and did not perform significantly worse in comparison to *Galleria mellonella*. PET bottles as alternative fermentation containers and insect larvae as alternative fermentation substrates were successfully implemented during solid-state fermentation experiments. Conidia harvests of 1 x 10^9 conidia per g of dry matter produced on insect larvae and fermented in PET bottles corresponded at large to the conidia harvest of 8 x 10^9 conidia per g of dry matter produced on cereals in standardised glass reactors. High loads of contaminating microorganisms in the fermented larval substrate need further evaluation due to the potential risk for human health. Further studies are needed for assessing the feasibility of the concept in praxis.

POSTER SESSION. Wednesday, 16:30 **PMC-13**

Host response of *Lymantria dispar dispar* to *Chromobacterium* spp. infections

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Two strains of entomopathogenic bacteria from the *Chromobacterium* genus—*Chromobacterium subtsugae* (PRAA4-1^T) and *Chromobacterium sphagni* (14B-1^T)—were used to independently infect 3rd-instar larvae of the European gypsy moth, *Lymantria dispar dispar*. The PRAA4-1^T strain of *C. subtsugae* exhibits oral toxicity against a broad range of important invertebrate pests (including Hemipteran, Coleopteran and Lepidopteran insects), whereas the 14B-1^T 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 midgut 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 brassicacean 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.

POSTER 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

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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.

POSTER SESSION. Wednesday, 16:30 **PMC-16**

Biological control of the Japanese beetle (*Popillia japonica*) with entomopathogenic fungi

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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 leafs, 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 42 dpi. 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.

POSTER SESSION. Wednesday, 16:30 **PMC-17**

Cancelled

POSTER SESSION. Wednesday, 16:30 **PMC-18**

Cellulase improves the endophytism of encapsulated *Metarhizium brunneum* on potato plants

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Biocontrol 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 phytopathogenic 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, cellulose 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 $2.5 \times 10^8 \pm 6.1 \times 10^7$ spores per bead. This correlated 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.

POSTER SESSION. Wednesday, 16:30 **PMC-19 STU**

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™, Agrifutur s.r.l.) is actively used or tested against different insect pests in Europe (i. e. *Phyllopertha horticola*, *Amphimallon solstitialis*, *Bothynoderes punctiventris*, *Popillia japonica*, *Otiorhynchus sulcatus, Agriotes spp., Diabrotica v.virgifera, 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 inseticide protein. Application of in silico tools can significantly improve the detection of new and diferents 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, Q79SG2, Q938Z1, Q938Z1 and I6R0W9, as expected according to the alignment performed by the ClustalW program with Vip3 characterized by MIR162 Vip3Aa (ABG20429.1) 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. robertsii* IP 146 on cuticle of *Dermacentor nitens* and *Amblyomma sculptum* 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. sculptum*. The total lipid extracts from cuticle of *A. sculptum* or *D. nitens* inhibited the growth of *M. robertsii* 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. sculptum* or *D. nitens* caused reduction in the number of germinated conidia to 59,5%. In conclusion, the cuticle of *A. sculptum* 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 robertsii*) 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

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 (*Acantholyda posticalis* (Matsumura, 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 m^2 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 *Lasioptera 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 bioassayed on aboveground pest *Lasioptera rubi* (Cecidomyiidae) (the raspberry gall midge) larvae at different nematode concentrations under laboratory conditions. The significantly highest mortality was observed in treatments with 50 and 200 infective juveniles per insect larvae within 8 days after inoculation. Progeny of *H. bacteriophora* ISO9 was abundant, and insect cadavers stayed mummified for 18 days post infection. The raspberry gall midge is widely distributed all over Europe to the far east of Russia and Japan, and farmers in Europe mostly rely on mechanical control measures against this pest, which reduce plant fitness and yields. *H. bacteriophora* ISO9 could be used to prevent pest adult emergence and possibly prevent subsequent infestations in biological and greenhouse farming systems.

POSTER SESSION. Wednesday, 16:30 **PMC-27**

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 (a_w) ≤0.1 and 25 ºC. Viability of dried samples decreased when stored under increasing moisture levels, surviving only a few days at 0.5 a_w. 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 a_w. By contrast, fungal culture (not dried) applied to solid substrate then dried to and stored at 0.5 a_w retained viability longer than the same culture dried to <0.2 a_w then stored at 0.5 a_w. These results highlight the need to fully understand the impact of moisture on the fungus as it relates to varied pest control systems.

POSTER SESSION. Wednesday, 16:30 **PMC-28**

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 worlds´ 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.

POSTER SESSION. Wednesday, 16:30 **PMC-29**

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

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Wireworms, the polyphagous soil-dwelling larvae of click beetles (Coleoptera: Elateridae), are a major insect pest of worldwide relevance causing tremendous yield losses in several crop production systems, such as potatoes. Biological control of wireworms with entomopathogenic fungi is challenging because of the sometimes low efficacy, difficult handling and limited shelf life of these organisms compared to synthetic pesticides. In the projects ATTRACT and INBIOSOIL, we developed novel mechanically stable beads containing CO_2 emitting baker's yeast as an attract component, an isolate of *M. brunneum* as a kill component and a substrate as a nutrient source and drying aid. These beads, commercialized as ATTRACAP®, can be applied in innovative attract and kill strategies based on solely biological components.
ATTRACAP® beads are produced by bioencapsulation of the micoorganisms and the substrate in alginate and dried with an innovative fluidized-bed drying process. The formulation allows a lower application dose of 30 kg/ha with an active substance of 4.8 x 10^{11} conidia/ha, thus making the product cost-effective. In 2019, ATTRACAP® obtained for the fourth time the emergency registration for 3.000 ha in Germany. One focus in the current BMEL project (Acronym: ATTRACAP) is on external factors influencing the efficacy of ATTRACAP® such as temperature, soil humidity and wireworm species. Additionally, there are new interesting results regarding the storage at different temperatures.
To conclude, this novel CO_2-releasing bead system is suitable for cost-effective delivery of low doses of fungal biological control agents to the soil and can also be transferred to other pest problems.

POSTER SESSION. Wednesday, 16:30 **PMC-30**

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, Grapholita 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 (Bl4NA, Bl5NA andBl9NA) showed high toxicity in both, spore-crystal mixtures and supernatant cultures against the three lepidopteran species.
The *Bt* isolates Bl4NA and Bl9NA express a protein of 130 kDa (with

match with the MW of Cry1 or Cry9 proteins), whereas the *Bt* isolate Bl5NA 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 Bl4NA, Bl5NA and Bl9NA. The results indicate that the *Bt* isolates (Bl4NA, Bl5NA and Bl9NA) 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 comercial 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 *Encephalitozoon* spp. 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.

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

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The Brown marmorated stink bug, *Halyomorpha halys* is an aggressive, polyphagous, invasive pest in Georgia. High infestation rates of the pest cause significant damage to agricultural crops, especially to hazelnut orchards resulting in losses for individual farmers as well as to the country's overall economy. The aim of our investigation was to find and identify entomopathogenic organisms in the Georgian populations of the stink bug as potential biocontrol agents for this pest insect. Therefore, screening of stink bug nymphs as well as adult individuals was conducted in different seasons of 2018. Stink bugs were collected in Samegrelo, Guria and Imereti regions where an outbreak of *H. halys* was reported. 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 Samegrelo region, from 9 sampling collections only 2 individuals were found infected. From Imereti 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* composes 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 screen and sequenced for part of the small subunit rRNA. Bayesian phylogeny 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 roeselum* are infecting *G. roeselii* over its range and are specific to this host. It suggests a co-diversification 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, amphipod 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, humans, 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 to *Anncaliia* 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 sporoblasts 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 slog 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 enterocytozoonids 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 parasitie 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 considerable 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 fumiferana*. 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 SLUGS AND SNAILS	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PSS-1**

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 Nemaslug® recommended dosage of 30 IJ/cm^2, and a higher dosage of 150IJ/cm^2, caused mortality to *Deroceras reticulatum* and *Lehmannia valentiana*, but not on the three earthworm species *Eisenia hortensis*, *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 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 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.

POSTER SESSION	Wednesday, 16:30-18:00
NEMATODES	Foyer

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, parasitise or paralyse 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 nematofage potential for use in biocontrol of garlic nematodes.

Native isolates of *A. oligospora* were characterized by using light microscopy and molecular markers. The effect of temperature, pH and nutrition on the growth rate and trap formation of representative isolates were determined. The rDNA internal transcribed spacer of *A. oligospora* isolates was sequenced. The optimum growth of *A. oligospora* strains is at 27-30°C on 1- 2% corn meal agar (CMA), in the pH interval from 5,6 - 8,6. The factors responsible for the trap formation of these fungi strains were identified. Trap formation was induced by contact with natural earthworm extrats. The predaceous efficacy of nemalophagous fungi was investigated against the garlic stem nematodes. Preliminary studies proved *A. oligospora* to be potentially effective biological control agents, immobilizing 80% of garlic stem nematodes.

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 physiochemical 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 *Prismatolainus*. Two-year pre-maize cultivation raised pH value up to 7.6 and *Plectus* spp. became eudominant 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-l,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 juvenile (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 (RILs) 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 Kompetitive 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 efficiently 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 (IJs) 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 opportunistic fungus (*Aspergillus flavus*). 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 saprobionts. 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 M1cog (Ma) and the nematodes *Steinernema carpocapsae* All strain (Sc) or *Steinernema glaseri* strain NJ-43 (Sg). In laboratory, dosages of 18×10^5 or 9×10^5 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 Sc 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 effectivity of the entomopathogens used against *Phyllophaga* spp.

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

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Entomopathogenic nematodes (EPN) of the genus *Heterorhabditis* are globally important and effective biological control agents against insect pests. However, due to high sensitivity of the nematodes to environmental extremes such as heat and desiccation, the use of EPN as biological control agents is limited to protected niches such as soil, against soil dwelling pests. The distribution of EPN in the soil is considered as patchy and variable, both spatially and temporally. In the present study, ten strains

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 implying they can sense the pathogen presence and avoid them intentionally. Larvae can also distinguish between EPN and nonpathogenic nematodes (*Caenorhabditis elegans*). Thanks to FIMTrack, we were able to observe the evasive behavioural patterns of *Drosophila* larvae with great resolution in real time. We also used FIMTrack for description of reversible paralysis in *Galleria mellonella* larvae after being forcefed caffeine. The peak of paralysis corresponds to peak of theophylline, one of the caffeine metabolite, in haemolymph. This study was supported by grant No. 17-03253S from the Czech Science Foundation and Carl Tryggers Foundation CST 16:474.

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*

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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.

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 agrocenosies and urban area (inducing foliage, coniferous, ornamental plants as well). At present, BMSB has become a key pest in many crop and makes grate economical loses. 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 (IJs) 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(IT) 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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The olive fruit fly *Bactrocera oleae* is one of the most serious and economically damaging insects worldwide, affecting the quality and quantity of both olive oil and table olives. Third instar larvae and pupae of several tephritid flies were reported to be susceptible to entomopathogenic nematodes (EPNs), but few studies have been carried out on the olive fruit fly. Laboratory bioassays were conducted to evaluate the susceptibility of *B. oleae* larvae and pupae to two commercial EPN species, *Steinernema feltiae* and *Heterorhabditis bacteriophora* and two indigenous Italian strains of *H. becteriophora* and *Steinernema carpocapsae*. 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.

POSTER SESSION. Wednesday, 16:30 **PN-12**

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 *Escherichia coli*, and two specific entomopathogenic nematodes (EPNs), *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*.
Our results demonstrated that larvae are more resistant than adults of either sex to bacterial challenge and they release less EPNs progeny after infection. Adult males and females did not differ in their immune response towards the unspecific bacterial pathogen, while, considering the EPNs, *S. carpocapsae* was more virulent than *H. bacteriophora* both in terms of host mortality and more abundant progeny released by hosts after death. The outcomes found with unspecific and specific pathogens could be due to the activation of different immunity pathways and, by identifying the more effective natural pathogen, provide useful information for a more efficient and sustainable management of this invasive pest.

POSTER SESSION. Wednesday, 16:30 **PN-13**

Recombinant *Bacillus thuringiensis* for intestinal nematodes

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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 nematicidal 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 nematicidal *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 nematicidal 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.

POSTER SESSION. Wednesday, 16:30 **PN-14**

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 (IJs) dispersal leading to increased efficacy. First, we determined whether pheromone pretreatment improved IJs movement/dispersal in soil columns baited with *Tenebrio molitor* larvae. We found that 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.

POSTER SESSION. Wednesday, 16:30 **PN-15**

Evaluation of potential of entomopathogenic nematodes for

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 *Steinernema 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 ageist 10 larvae in Petri diches (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%moratality 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, *Steinernema carpocapsae* and *Steinernema 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 effectivity 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 cm^2 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. *Steinernema 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 cm^2 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 symbionts 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. thiacloprid, neonicotinoid). 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 (Prlekija) and the other in northern (Gorenjska) Slovenia. The efficacy of the treatments was very similar at both locations, despite the approximately 5-fold lower WCR population in Gorenjska compared to Prlekija, 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

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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 models *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 (SfNPV-An2, SfNPV-Arg, SfNPV-Fx, SfNPV-Ho, SfNPV-Sin and SfGV-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 (SfNPV) was used for the five NPV studied and the *S. frugiperda* granulovirus (SfGV) 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 SfNPV 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 SfGV used as a reference. The SfNPV and SfGV-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 viruses in *Campopleginae* parasitoid wasps

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Nudiviruses are known to be endogenized 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 injected 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-Particules (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 RNAi interference), genomic (long-read high-throughput sequencing) and molecular evolutionary approaches undertaken to study recent viral symbiosis evolution within these *Campopleginae* parasitoid wasps.

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

Survey of CpGV mixed-genotype infection occurrence in treated orchards

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The success of biological control of the codling moth, *Cydia pomonella*, with commercial products based on Cydia pomonella granulovirus (CpGV) isolates relies both on the ingestion of a sufficient amount of occlusion bodies (OBs) between their hatching from the egg and the penetration inside the fruits, and the correct replication of the virus in the insect larvae. Larvae resistant to one isolate, CpGV-M are frequently found in European orchards. In recent years, efforts have been devoted to obtain virus isolates able to control resistant insects, among them, CpGV-R5. Ingestion of CpGV-M by a resistant larva does not allow replication of CpGV-M. Ingestion of CpGV-R5 results in virus replication and larval death. Ingestion of a mixture of CpGV-M and CpGV-R5 allows replication of both genotypes and better insect control.
Both CpGV-M and CpGV-R5 are present in the commercial product Carpovirusine Evo 2 (NPP, Arysta LifeSciences). No recent data have been collected concerning the actual amount of virus ingested by the neonate larvae. As mixed infection CpGV-M and CpGV- provide better protection, this is an important tool to assess the success of treatment in orchards.
We analysed the distribution of virus droplets in leaves on two orchards using two different dispersion techniques. We observed the mortality of larvae allowed to feed on these contaminated leaves for selected amounts of time. We then used High resolution melting (HRM) to estimate the occurrence of each genotype alone and mixed genotypes in individual larvae.

POSTER SESSION. Wednesday, 16:30 **PV-4 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 domesticus*

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In recent years, several cricket species are mass reared as an alternative source of protein to conventional sources. However, viral diseases are common in large scale cricket rearing. During the last decade, outbreaks of the Acheta domesticus densovirus (AdDV) in colonies of the house European cricket *Acheta domesticus,* have left rearing companies in financial trouble, positioning AdDV as the most common and devastating viral pathogen in commercial cricket rearing known up to date. Outbreaks occur probably as a result of the combination of stress factors during the production cycle. Limited knowledge is available on the effect of environmental conditions on the development of this and other viral diseases. Such knowledge is crucial to optimize cricket rearing conditions and to develop measures for the prevention and control of viral outbreaks. In this work, the effect of temperature and relative humidity on the activation of a covert infection with AdDV was investigated. Two population densities were tested. Mortality and individual weight resulted to be higher at 30°C and 75±5% RH compared to 25°C and 55+5% RH. Presence of AdDV was detected on the surface of the eggs (not inside) used to obtain the crickets for the experimental set up and in all symptomatic crickets. A variable large proportion of non-symptomatic individuals did also resulted positive for AdDV in all the treatments.

POSTER SESSION. Wednesday, 16:30 **PV-5**

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

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A drastic reduction in the use of chemicals and pesticides in agriculture requires new sustainable biocontrol strategies to restrain pathogen multiplication and virulence in plants. VIROPLANT is a EU-funded research project whose primary objective is to propose viruses as potential biocontrol agents of some specific plant diseases spread in Europe. As a first step, RNAseq was applied to characterize the virosphere of *Scaphoideus titanus,* vector of Flavescence dorée phytoplasma, *Frankliniella occidentalis* and *Thrips tabaci*, vectors of Tomato spotted wilt virus and Onion yellow spot virus, respectively.
Populations of *S. titanus* were collected from Italy, France, Switzerland, Hungary, and the USA. All European samples presented the same unclassified dsRNA virus and a previously uncharacterized densovirus. A new iflavirus was occasionally found in some Italian and French insects. 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.

POSTER SESSION. Wednesday, 16:30 **PV-6**

Unraveling the multiple nudiviral integration traces within insect genomes

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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 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 innovation 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 integrated 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 able to the production of 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 generation of 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 neuropteran 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 LC_{95} of SeMNPV (specific NPV of *S. exigua*) and the LC_{95} 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. LC_{95} 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*, LC_{50} and LT_{50} for the 4th instar larvae of *S. exigua* were 3.81×10^5 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 SeMNPV-QD and 17 ORFs unique to SeMNPV-US1. The pairwise distance of the nucleotide sequences of *lef-8*, *lef-9*, *polh* and concatenated *lef-8*/*lef-9*/*polh* fragments between SeMNPV-QD 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

species. The taxonomic proposal has been submitted to International Committee on Taxonomy of Viruses (ICTV).

POSTER SESSION. Wednesday, 16:30 **PV-11**

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 chu-, rhabdo-, 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.

POSTER SESSION. Wednesday, 16:30 **PV-12**

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 effectiveness of vertical transmission and following reactivation of multiple nucleopolyhedrovirus (*Ld*MNPV) 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 effected 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 were grown in the laboratory conditions. When larvae reached IV instar, they were starved to activate the vertically transmitted virus. Frequency activation of virus in the experiment was not depend on the virulence of tested strains. These results will helpful for understanding of the strategy of viruses survival in natural and for the selection of most effective strains with prolonged effect in subsequent years after pest treatment. This study was supported by the Russian science foundation (Project No. 17-46-07002).

POSTER SESSION. Wednesday, 16:30 **PV-13**

Comparative genome analysis of related Lymantria dispar nucleopolyhedrovirus isolates differing in virulence.

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Nucleopolyhedroviruses (NPVs, Baculoviridae) are specific viruses of insects that are used for pest control. High variation in virus virulence significantly depends upon genetic factors. Here we compare genomes of closely related genetic variants of Lymantria dispar multiple NPV (LdMNPV) that were isolated from the standard Gypchek strain and were differed in virulence. Five viral genotypes, differing in virulence, were isolated from the standard strain of NPV in the biopesticide “Gypchek”. Complete genomes of these five genotypes were sequenced using Illumina technology by paired end sequencing of fragment genomic libraries, and assembled by SPAdes 3.9.0 . Initial assemblies consisted of 1-3 scaffolds with a total assembly length of 159-174 kbp. Genome coverage was in range of 200x-850x. Closing of scaffold gaps was performed by Sanger sequencing of amplicons. To determine genetic relatedness among studied LdMNPV variants a phylogenetic tree was reconstructed based on a set of core loci. Comparative analysis of candidate genes was performed to reveal genetic determinates of 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).

POSTER SESSION. Wednesday, 16:30 **PV-14**

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 polydnaviruses (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 altered 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 vitro* is rare but in 2011 we generated a persistent infection in *Trichoplusia ni* (Hi5) cells with an AcMNPV *p10*-deletion mutant (AcUW1.*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* 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 *fp25k* 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 *Gasque, S.N.*[1]*; van Oers, M.M.*[1]*; Smid, H.M.*[2]*; and Ros, V.I.D.*[1]

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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 Autographa 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 pelleted 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 leafs 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 and characterized for their intra-genetic composition based on single nucleotide polymorphisms (SNPs). In the present study, the NGS data were used to identify insertion/deletion (indel) locations which are highly iso-

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 indel 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.

POSTER SESSION. Wednesday, 16:30 **PV-19**

Quantification of *Erinnyis ello* granulovirus in a biopesticide formulation.

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The hornwom, *Erinnyis ello* Linneaus 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 Erinnyis 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 using 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 10^9 OBs/g) was very close to that expected in the manufacturing procedure (1.0 x 10^9 OBs/g). This methodology could be used as a tool for quality control of the biopesticide manufacturing process.

POSTER SESSION. Wednesday, 16:30 **PV-20**

A novel member of *Cypovirus 2* found in *Erinnyis ello ello* larvae co-infeted with a baculovirus reveals a possible horizontal gene transfer between these two different viruses

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The hornworm *Erinnyis ello ello* (Lepidoptera: Sphingidae) is an important pest in Brazil. This insect has been observed in many plant species, such as *Manihot esculenta* (cassava). Outbreaks of this insect pest are common in Brazil and can beget great impact in crop production. Insect cadavers of *Erinnyis ello ello* with baculovirus infection symptoms were found in cassava plants and were collected. Two different types of occlusion bodies (OBs), granular and a polyhedral-shaped structures, were observed in extracts of insect cadavers by light and scanning electron microscopy (SEM), suggesting a mixed infection. Interestingly, the polyhedral-shaped OB's surface revealed indentation resembling a cypovirus polyhedra. After OB's nucleic acid extraction followed by deep sequencing, the results confirmed the presence of a new *cypovirus* isolate and a betabaculovirus (Erinnyis ello granulovirus, ErelGV). Phylogenetic analysis of the RdRp showed that the new cypovirus was closely related to another lepidopteran-infective cypovirus, *Inachis io cypovirus 2* (IiCPV-2) and according to these results we proposed the name Erinnyis 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.

POSTER SESSION. Wednesday, 16:30 **PV-21**

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 *Diatreia saccaralis*, 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 (*Cf*BV) 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 *Cf*BV 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 *Cf*BV genome presents a new contribution to the comparative genomics and the understanding of the complex interactions between the parasitoid wasp and its host.

POSTER SESSION. Wednesday, 16:30 **PV-22**

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**

AcMNPV *p48* (*ac103*) is required for the efficient scission of inner nuclear membrane invagination structures

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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 nucleocapsids 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**

A Study about Chimeric OBs Based on d-POLH

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Autographa californica multiple nucleopolyhedrovirus can hyper-express very late gene polyhedrin, the main component of occlusion bodies (OBs). There have been many studies about the utilization of OBs, such as incorporating foreign proteins to obtain chimeric OBs. The latest strategies, dimidiate polyhedron (d-POLH), in which POLH is divided into N-terminal 150 amino acids (phN150) and C-terminal 95 amino acids (phC95), can form normal OBs and efficiently packaging foreign protein, but the number of OBs is much less than wild type. In this study, we attempted to obtain chimeric OBs more efficiently through fusing EGFP to different positions of the d-POLH. The results suggested that only when EGFP fused to the N or C-terminal of phC95, the d-POLH can form small amounts of OBs. We constructed cis complementation recombinant viruses and found that when EGFP was fused to the C-terminal of phC95 and complemented by the wild type POLH, the recombinant viruses not only can form as much OBs as wild-type virus, but also have high embedding efficiency as the d-POLH. Meanwhile, when phC95 was fused to C or N-terminal of the EGFP can self-aggregate near the outer nuclear membrane in the cytoplasm. It could form big spherical aggregates in nucleus, when the phC95 with a nuclear localization signal (KRKK) at its N-terminus was cloned into the C-terminal of EGFP which also possessed KRKK in its N-terminus. Furthermore, we defined the least truncation of C-terminal of POLH which can form this kind of polyhedra-like particles.

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.

POSTER SESSION. Wednesday, 16:30 **PV-26 STU**

Evaluation of CRISPR/Cas9 based procedures for the edition of baculoviral genomes

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Baculoviruses are invertebrate pathogens of the orders Lepidoptera, Hymenoptera and Diptera. These viruses carry large genomes of double-stranded DNA (of 80-180 kbp) and, depending on their genetic load and their host, are classified into four genera: *Alphabaculovirus*; *Betabaculovirus*; *Gammabaculovirus*; and *Deltabaculovirus*. Their many applications, which include biological pest control, recombinant protein expression technologies and gene therapy, among other, confer these viruses a great biotechnological importance. In order to improve and facilitate these uses we aim to the optimization of their genomes through genetic engineering procedures. In recent years, a new tool originated from prokaryotic systems and known as CRISPR/Cas emerged to facilitate genome editing. CRISPR/Cas makes it possible to carry out Double Strand Breaks in any DNA sequence, which added to cellular homologous and

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 as a useful and complementary tool in the basic and applied studies on baculoviruses, to conduct functional genomic researches and to generate better baculoviral platforms for biotechnological purposes, respectively.

POSTER SESSION. Wednesday, 16:30 **PV-27**

The persistent infection of PnV (Perina nuda iflavirus) to its heterologous cell line, NTU-LY cell line (*Lymantria xylina* cell line)

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PnV (*Perina nuda* iflavirus) 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 seeding 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 exammed 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.

POSTER SESSION. Wednesday, 16:30 **PV-28 STU**

Improvement of baculovirus as protein expression vector and as biopesticide by CRISPR/Cas9 editing

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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 ribonucleoprotein (RNP) complex into Sf21 insect 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.

POSTER SESSION. Wednesday, 16:30 **PV-29 STU**

Biotype and nudivirus prevalence of *Oryctes rhinoceros* in Palau Archipelago

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Coconut rhinoceros beetle (CRB: *Oryctes rhinoceros*) has invaded the Pacific before 1940s, and damaged palm trees seriously. Microbial control using Oryctes rhinoceros nudivirus (OrNV) has been succeeded to reduce the palm damages in invaded area since it introduced in the 1960s. However, in 2007, the invasion of new OrNV-resistant CRB biotype into Guam was documented. This new biotype (G-type) showed a base substitution in COI region from that in non-Guam biotype, and low susceptibility to OrNV when the adults were administrated with a concentration to kill enough non-G-type adults. G-type has invaded not only Guam but also other Pacific islands including Palau Archipelago where nudivirus released previously. Interestingly both of G-type and non-G-type were coexist in Palau. It is possible that G-type can reinvade into other Pacific islands, but the interaction of the both biotypes was not well documented. In this study, CRB adults were captured by pheromone traps in 2018 and the biotype and virus prevalence was examined. More than 70% adult showed OrNV positive by PCR detection, though no significant difference between biotypes. OrNV virions were observed in the tissue from PCR positive samples under transmission electron microscope which suggests that Palauan population of CRB might not covertly infected with OrNV.

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

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 turret proteins of DpCPV (VP3, VP4 and VP5) were selected for investigation, in which VP3 was truncated into GTase (1-366 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 augment 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 californica multiple nucleopolyhedrovirus

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1. State 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-AcΔPol-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 SpltNPV 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-AcΔPol-HA:NlPol^{NLS}. 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 californica nucleopolyhedrovirus (AcMNPV) ME53 and VP80

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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 (NTS) suggesting a chaperone might help translocate ME53 to the nucleus. VP80 is one potential chaperone since it has two NLSs and, like ME53, is observed in the nucleus only at late times post infection.
By yeast two-hybrid (Y2H) we identified protein-protein interactions (PPIs) using ME53 as bait and two Y2H libraries as prey. VP80, essential for BV formation in the nucleus, was identified as a putative ME53 binding partner. By reciprocal Y2H studies, intact ME53 and VP80 enabled yeast growth, while ME53 lacking NTS failed to interact with VP80. ME53 and VP80 were found to colocalize in the nucleus but in the absence of VP80, ME53 failed to localize to the nucleus suggesting that VP80 facilitates ME53 nuclear translocation. ME53 and GP64 form foci which are predicted as budding zones at the cytoplasmic membrane. Microscopy showed that ME53 is not essential for BV, ODV and polyhedra production, though an ME53 knockout virus was restricted in spreading to only a few adjacent cells. Our results suggest that the ME53 and VP80 interaction is necessary for efficient budding perhaps by providing a bridge between the nucleocapsid and the infected cell membrane.
Since ME53 has two zinc finger motifs and its nuclear translocation is critical for virus production we analysed transcription of five genes, noting that ME53 deletion decreased their transcription.

POSTER SESSION MISCELLANEOUS	Wednesday, 16:30-18:00 Foyer

POSTER SESSION. Wednesday, 16:30 **PMI-1**

***Galleria mellonella* larvae fat body disruption (Lepidoptera: Pyralidae) caused by venom of the *Habrobracon brevicornis* (Hymenoptera: Braconidae)**

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Capacity of the *Habrobracon brevicornis* to elevate host's nutritional suitability for their offspring developing, was studied. Concentration of the total lipids and main sugars in the paralyzed *G. mellonella* larvae lymph were analyzed. Significant increase of the lipids level was fixed only on the second day after paralyzation. Significant increase of the total trehalose count was detecting during all three days, while glucose concentration rise was noted only on the first day and it was not significant. Possible, emission of lipids and carbohydrates into the lymph is caused either of metabolic activation or regulation, or of fat body tissues destruction. Well observed disruptions were fixed in the larvae's *G. mellonella* fat body thin and semi-fine section starting from second day after paralyzation. Significant increase both of the phospholipase A and C enzyme activity and acid proteases has been detected in the wax moth fat body after paralyzation, during all experimental time. At the same time, disbalance in the system of antioxidants: superoxide dismutase, peroxidases, catalase and glutathione-S-transpherase, were detected. The reliable increase of the expression by gene coding hsp70 was fixed both for the 24 and 48 hours after paralyzation while reliable increase of the expression by gene coding IAP was detected only after 24 hours after wax moth larvae paralyzation. Taking into account, absent by the DNA fragmentation in the fat body cells from paralyzed wax moth larvae we can hypothesize necrotic way of the fat body disruption.

POSTER SESSION. Wednesday, 16:30 **PMI-2**

Parasitoid envenomation changes the *Galleria mellonella* midgut microbiota and immunity, thereby promoting fungal infection

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Gut bacteria influence the development of different pathologies caused by bacteria, fungi and parasitoids in insects. Wax moth larvae became more susceptible to fungal infections after envenomation by the ectoparasitoid *Habrobracon hebetor*. In addition, systemic bacterioses occurred more often in envenomated larvae. We analyzed alterations in the gut bacterial communities and immunity of the wax moth after *H. hebetor*

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

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Birds cause large annual losses in agriculture because they eat or damage both freshly sown seeds and young seedlings. Since the use of synthetic defence substances is not permitted in organic agriculture, the need for biological alternatives, such as plant extracts, to protect plants from bird feeding is very high.

Plant extracts are compositions of different substance classes with different physiochemical and biological properties. Due to their complex nature and instability, a formulation of the active ingredients is essential for practical application to control their stability and release, as well as to improve shelf life and handling.

Therefore it is the aim of the formulation work in the project DevelOPAR (Development Of a Plant extract based Avian Repellent) to increase the repellent effect of plant extracts on birds when used as seed treatment, as well as to improve soil persistence and shelf life by means of a suitable formulation.

The plant extract E22 was successfully encapsulated in capsules < 300 µm in various formulations. The addition of different formulation additives increased the encapsulation efficiency, changed the loading and the release properties in water and soil.

Since the active substances in soil are exposed to a variety of negative influences, further investigations will be carried out on the protective properties of the formulations for the active substances.

POSTER SESSION. Wednesday, 16:30 **PMI-4 STU**

Slow release of semiochemicals in push-pull-kill strategies for biological psyllid pest control

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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 Passeriformes Europe Greenfinch (*Carduelis chloris*), the black robin (*Turdus merula*) and hybrid sparrow (*Passer domesticus* x *Passer hispaniolensis*) and Columbiformes rock dove (*Columba livia*) in Bouinane region (Blida).

About 701 arthropods collected, we determined 6 mite species with a dominance of *Ornithonyssus bursa* (58.5%), followed by *Dermanyssus gallinae* (11.4%), and a single species of lice *Menopon* sp.11.8 %. Note the total absence of flea and tick in these 40 nests analyzed. Mites are used as epidemiological tools to detect the pathogen by sensitive PCR molecular biology. The PCR results show that *Dermanyssus gallinae* and *Menopon* sp. are vectors of *Borrelia* sp. in our study area, with shells sparrows and Europe Greenfinch.

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 emmited 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 desease *in planta*, (3) the oviposition rate of the greenhose whithefly *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 assesed *in planta* by exposing the roots of tomato seedling to the VOCs emmited by *Trichoderma* spp in a sealed environment for 21 days. The progress of the disease and the severity were assesed. 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-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 cysteine 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.

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.

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ABSTRACTS 2019 - ORAL PRESENTATIONS (CONT.)

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STU indicates papers being judged for graduate student presentation awards.

CONTRIBUTED PAPERS Wednesday, 18:00-20:00
MICROBIAL CONTROL 4 Auditorium 3

Formulation and field efficacy

Chairs: Surandra Dara / Linda Muskat

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), *Listronotus 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), false codling 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 Cryptophlebia 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. Melocont™ was applied as positive control. In 2017 soil samples were analysed before, 35 and 196 days after application. Because of the extreme draught 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 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 transmission value score than that of fumigation treatment in the serious 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.

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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 derivates 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 derivates 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**

A microbial Integrated Pest Management strategy for climbing cutworm in wine grapes

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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 comes* and *Abagrotis orbis*. 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. Entomopathic nematodes are a second biocontrol tool with potential for managing wine 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 wine grape trials at different temperatures.

CONTRIBUTED PAPERS. Wednesday, 19:30 **MC-31**

The California experience: promoting microbial control through effective outreach

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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.

CONTRIBUTED PAPERS Wednesday, 18:00-20:00
VIRUS 6 Multispace AB

Virus-host Interactions

Chairs: Deng Fei / Jorg Wenmann

CONTRIBUTED PAPERS. Wednesday, 18:00 **V-41**

Transcriptional responses of the *Trichoplusia ni* midgut to oral infection by the baculovirus Autographa californica Multiple Nucleopolyhedrovirus

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Infections of *Trichoplusia ni* larvae by the baculovirus AcMNPV are initiated when occlusion bodies disassemble in the midgut releasing virions that enter midgut epithelial cells and establish the primary phase

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 (*fp-25k*), acceleration of systemic infection (*v-fgf*), and enhancement of viral movement (*arif-1/orf20*), 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 neuropeptidome 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, 18:30 **V-43**

***Chrysodeixes includens* NPV infection induces apoptosis in an *Anticarsia gemmatalis* cell line**

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There are two important pests of soy beans in Brazil: the larvae of *Chrysodeixes includens* and *Anticarsia gemmatalis*. Each possess distinct baculoviruses that are able to orally infect and kill these species in the field, *Chin*NPV and *Ag*MNPV respectively. These two biocontrol agents have proven efficacy to each of their natural hosts but little is known about their ability to infect the other host. The recent rise of *C. includens* as one of the major pests of soy in Brazil, together with the long-term programs of large-scale application of *Ag*MNPV in soy fields, suggests that *Chin*NPV is unable to orally infect *C. includens.* In this work we investigated the ability of an isolated *Chin*NPV strain (*Chin*NPV-UNB1) and a recombinant virus containing the fluorescence reporter *gfp* gene under the control of the constitutive *hsp70* promoter (*Chin*NPV-GFP) to infect an *A. gemmatalis* cell line (UFL-Ag-286). The infection of UFLAG-286 cells resulted in apoptosis as observed by light microscopy and caspase activity assays. Fluorescence microscopy of *Chin*NPV-GFP infected cells revealed that the virus was able to enter the cell`s nucleus and express its DNA, indicating that viral gene expression or viral DNA replication induces apoptosis. The *Chin*NPV *iap2* and *iap3* genes were cloned in a *p35* defective *Ac*MNPV and both failed to inhibit apoptosis in UFLAG-286. Our results demonstrate that *Chin*NPV lacks the genetic arsenal that would allow it to infect an *A. gemmatalis* cell line *in vitro* and suggests that apoptosis is an important antiviral defense *in vivo*.

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 silkworm, *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 BmFerHCH 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 each time point. Eight genes (*pp31/39K*, *p6.9*, *orf36L*, *fgf-3*, *dbp*, *orf72*, *orf77*, *orf132*) out of top 20 highest expressed viral genes are consistent in infected samples at 48 hpi and 96 hpi. They are mainly related to DNA binding and virus transcription in the early stage of infection. These results showed highly expressed viral genes that are likely associated with the virus replication process during CpGV infection in *C. pomonella* larvae.

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 Iflavirus 1* that could contribute to reduce the pest abundance in field conditions.

CONTRIBUTED PAPERS. Wednesday, 19:45 **V-48**

The major hurdle for effective baculovirus transduction into mammalian cells is early endosomes

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Baculoviruses, although they infect insects in nature, can transduce a wide variety of mammalian cells and are therefore promising gene therapy vectors. However, baculovirus transduction into many mammalian cells is very inefficient and the limiting stages and factors remain unknown. An important finding is that a short duration trigger with low pH can significantly enhance virus transduction efficiency, but the mechanism is poorly understood. Herein, we performed a detailed comparative study on entry mechanisms of the prototypical baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) into insect and mammalian cells. The results showed that AcMNPV can be internalized into mammalian cells efficiently, but fusion in early endosomes (EEs) appeared to be the major obstacle. Measurement of endosomal pH suggested that virus fusion might be restricted under high pH conditions in mammalian cells. Interestingly, the major viral fusion protein GP64 mutants with decreased fusogenicity did not affect virus infection of insect cells, whereas virus transduction into mammalian cells was severely impaired, suggesting a more stringent dependence on GP64 fusogenicity for AcMNPV entry into mammalian cells than into insect cells. An increase in fusogenicity of GP64 mutants by low pH triggered the rescue of fusion-deficient recombinant virus transduction efficiency. Based on the above findings, the pH of EEs was specifically reduced with a Na^+/K^+-ATPase inhibitor, and AcMNPV transduction of a wide range of mammalian cells indeed became highly efficient. This study revealed the roadblocks to mammalian cell entry of baculovirus and provides a new strategy for improving baculovirus-based gene delivery and therapy.

CONTRIBUTED PAPERS Wednesday, 18:00-20:00
SLUGS & SNAILS 2 Multispace CD

IPM Toolkit - Barriers, Monitoring and Molluscicides

Chairs: Gordon Port / Nadine Sydow

CONTRIBUTED PAPERS. Wednesday, 18:00 **SS-9**

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 (Molluskit). Discussion of how best to implement the barrier and potential improvements to the design will be presented.

CONTRIBUTED PAPERS. Wednesday, 18:15 **SS-10 STU**

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.

CONTRIBUTED PAPERS. Wednesday, 18:30 **SS-11**

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 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 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.

CONTRIBUTED PAPERS. Wednesday, 18:45 **SS-12**

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, cabling, 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.

CONTRIBUTED PAPERS. Wednesday, 19:00 **SS-13**

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 £43.5million per year. Since it was first launched in 2005 Iron (III) phosphate ($FePO_4$) 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

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.mlapp 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, molluscicidal 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 molluscicidal control methods are not effective for adult slugs before laying eggs. Molluscicidal 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 molluscicidal 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 molluscicide 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, 18:00-20:00
DBI 2 Commission R8

Diseases of managed and wild bees

Chairs: Annette Bruun Jensen / Carrie Hauxwell

CONTRIBUTED PAPERS. Wednesday, 18:00 **DBI-9**

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

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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 sinaiviruses 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 phylosymbiosis 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 symbiont diversity through untargeted metabolomics using gas chromatography-mass spectrometry.

Microbial control **DIVISION BUSINES MEETING**	Wednesday, 20:15-22:00 Auditorium 3
Microsporidia **DIVISION BUSINES MEETING**	Wednesday, 20:15-22:00 Multispace AB
DBI **DIVISION BUSINES MEETING**	Wednesday, 20:15-22:00 Commission R8
SLUGS & SNAILS WORKSHOP	Wednesday, 20:15-21:15 Multispace CD

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

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Nematodes form various associations with terrestrial gastropods including obligatory parasitic, paratenic, phoretic or necromenic mode. There are over 80 gastropod parasitic nematode species from twelve

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.

THURSDAY - 1st August

CONTRIBUTED PAPERS Thursday, 8:30-10:30
MICROBIAL CONTROL 5 Auditorium 3

Microbial control

Chairs: Nguya Maniania / Mary Barbachek

CONTRIBUTED PAPERS. Thursday, 8:30 **MC-32**

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 generalist chewing insects is markedly reduced in the presence of endophytes, likely due to secondary metabolites, produced or induced by these fungi. Furthermore, studies using excised leaves report weaker effects of endophytes than those on intact plants, likely caused by chemical changes being masked by leaf excision. Most surprisingly, endophyte infection of seeds produces the greatest effect on insect herbivores in subsequent mature plants, even though many fungi are transmitted by air-borne spores. In conclusion we demonstrate that these fungi have huge potential in biological control of insect pests and could also alter the structure of natural insect communities.

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 contaminated 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, 9:00 **MC-34**

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.

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%), following by tomato (73.3%), and the lower was detected in cotton plants (40.0%). However, endophytic colonization rate was 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, 9:15 **MC-35**

Purpureocilium lilacinum is also a mycopathogen, although highly specific

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Purpureocilium lilacinum is commonly known as a pathogen of nematodes. However, recently it was shown to behave as an entomopathogen as well. This fungi 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 *Acromyrmex* and one *Atta* species. *P. lilacinum* stopped the growth of the cultivars from *Acromyrmex* LCA but not from *Atta* LCA. Furthermore, we observed cell wall destruction of the cultivar hyphaes from *Acromyrmex* but not from *Atta*. These results are in agreement with a traditional view that cultivar strains differ with LCA genera. However, they disagree with the latest molecular phylogeny of the cultivars of LCA as our cultivars should fall 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 lo local differences. Irrespectively of the reason behind these results, our information is essential from a biological control perspective as 1) *P. lilacinum* seems to control *Acromyrmex* but not *Atta* LCA´s cultivars and 2) *P. lilacinum* behaves as a multifunctional fungi against *Acromyrmex* leaf-cutters positioning itself as an excellent candidate for their biological control.

CONTRIBUTED PAPERS. Thursday, 9:30 **MC-36**

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 protoxin 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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Metarhizium robertsii isolate CPD6 which was found to be virulent against pepper weevil, *Anthonomus eugenii* and cranberry weevil, *Anthonomus musculus,* is being developed as candidate mycoinsecticide against these two insect pests under the trade name of NoVil. In addition, NoVil was found to endophytically colonize root and stem of pepper plants following seed inoculation, thereby promoting the growth of the plants. Further studies have shown that NoVil is pathogenic against Western Flower thrips, *Frankliniella occidentalis,* whiteflies, stripped cucumber beetle, *Acalymma vittatum,* tarnished plant bug, *Lygus lineoralis,* and two-spotted spider mite, *Tetranychus urticae,* causing mortalities between 80 and 100%. The effect of NoVil was also investigated against beneficial insect such as bumblebee, *Bombus terrestris,* in the laboratory. Spray application of hives, containing approx. 350 bumblebees each, resulted in 9.1 and 25.9% mortality at the concentration of 10^7 and 10^8 conidia mL^{-1}, respectively, after 10 days post-treatment. Mortality of 18.2% was recorded in the control 10 days post-treatment. Preliminary toxicological results indicate that NoVil is safe.

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)**

CONTRIBUTED PAPERS Thursday, 8:30-10:30
VIRUS 7 Multispace AB

Biological control

Chairs: Caroline Knox / Miguel lópez-Ferber

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

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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. Two locally manufactured products, Cryptogran and Helicovir, were registered several years ago (River Bioscience, South Africa) and are now widely used on various crops for the control of *T. leucotreta* (false codling moth) and *H. armigera* (African bollworm) respectively. Despite the successes of baculovirus based products in reducing larval infestations in the field, there are challenges associated with their continuous application in field settings, in particular 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 recovery 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, CrpeNPV and HearNPV-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, 08:45 **V-50**

Step-by-step acquired resistance of *Adoxophyes honmai* passaged 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 F_1 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.

CONTRIBUTED PAPERS. Thursday, 09:00 **V-51**

Tutavir – a new control tool for the tomato leafminer *Tuta absoluta* *Wandeler, Heiri.;* *Dubach, Felix.*

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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 also present in Europe, the Middle East, Africa, parts of Asia and Central America. With its high reproduction capacity and short generation cycle, there is a substantial risk of developing resistance to insecticides. The use of a baculovirus product offers new options for a pest management strategy.

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 in Germany, 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, 09:15 **V-52**

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 (LC_{50}) 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 $1x10^4$ and $1x10^8$ occlusion bodies (OBs)/mL. The mean lethal concentration of the SporNPV and SporGV evaluated independently was greater than that obtained using the natural coinfection 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, 09:30 **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 chrysodeixis 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 codling 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 biological 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 litchi 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, 8:30-10:30
NEMATODES 4 Multispace CD

Novel approaches in the basic and applied research on EPN

Chairs: Duarte Toubarro / Carlos Molina

CONTRIBUTED PAPERS. Thursday, 8:30 **N-25**

Antiprotozoal activity of *Xenorhabdus* and *Photorhabdus* bacteria mutualistically 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 syntethic therapeutics in time, it is extremly 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 oppurtunities 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 protozoans *Acanthomoeba 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**

***Toubarro, D.**[1]; Kenney, E.,[2]; Eleftherianos, I.[2]; Simões, N.[1]*
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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 disclose ESP regulatory functions of the insect immune system.

The infection of *Drosophila* 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 *totA* and *totM*. Thus causing the negative regulation of the proliferation of hemocytes and the differentiation of lamellocytes. Moreover, in flies challenged with inactivated *X. nematophila*, nematode ESPs strongly supress key genes in immune response, namely the *pgrp-lc* receptor and the polyphenol oxidase genes, *ppo1* and *ppo2*, 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 — Commission R8

Microsporidia-host interactions: from organism to molecular levels

Chairs: Ronny Larsson / Yuliya Sokolova

CONTRIBUTED PAPERS. Thursday, 8:30 **MS-1**

Co-infection of paramyxid and microsporidian parasites in feminised amphipod crustaceans

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Within populations of the amphipod crustacean *Orchestia aestuarensis*, co-infection with the paramyxid *Paramarteilia orchestiae* and the microsporidian *Dictyocoela cavimanum* occurs more frequently than expected by chance. Both parasites occur more frequently in female *O. aestuarensis* hosts than in males, suggesting that one or both of these parasites causes feminisation of *O. aestuarensis*. *O. aestuarensis* 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. cavimanum* does not. This indicates that the paramyxid, rather than

the microsporidian, is the cause of feminization in *O. aestuarensis*.

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 germination process for two microsporidian species, *Anncaliia algerae* and *Encephalitozoon hellem*. The timescale of the firing event is less than two seconds with the increase of the speed over time. We found the reduction of velocity and acceleration when the tube is fully extended. To follow how the nucleus and other organelles are transferred through the polar tube, we are optimizing the use of fluorescent dyes for live-cell imaging. For over a century, it has been known that the nucleus of the parasite must be translocated through the tube. However, this presents a paradox, as the tube diameter is more than 10-fold smaller than that of the nucleus, raising the question of how the nucleus may fit through the tube. We found that the nucleus is translocated through the tube slightly after the tube is fired, and undergoes a remarkable degree of reorganization to fit through the tube, which retains approximately constant diameter throughout the process. These studies begin to provide insights into the initial stages of microsporidia polar tube firing and infection.

CONTRIBUTED PAPERS. Thursday, 9:00 **MS-3**

Cancelled

CONTRIBUTED PAPERS. Thursday, 9:15 **MS-4**

PtdIns(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 PtdIns(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 *gvp36*Δ strain, a BAR protein knocks out strain of *Saccharomyces cerevisiae*, could recover the phenotype about vacuole biogenesis defection. These findings suggest that NbSWP12 may serve as an import regulator of membrane in microsporidia proliferation. Further research will uncover how NbSWP12 binded with PtdIns(3)d 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 sporont was wrapped 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.

Coffee Break	Thursday, 10:30-11:00 Foyer
SIP BUSINES MEETING	Thursday, 11:00-13:00 Auditorium 3
Lunch	Thursday, 13:00-14:30 Multispace 2
IOBC BUSINES MEETING	Thursday, 13:00-14:30 Auditorium 3
JURY STUDENT COMPETITION	Thursday, 13:00-14:30 Multispace AB
CONTRIBUTED PAPERS BACTERIA 4	Thursday, 14:30-16:30 Auditorium 3

Bacterial symbionts of invertebrates

Chairs: Luca Ruiu / Patricia Hernández

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. This 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 depauperate 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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The gut of the European honeybee *Apis mellifera* is the site of exposure to multiple stressors such as pathogens and ingested chemicals. Therefore, the gut microbiota, which contributes to the host homeostasis, may be altered by these stressors. The abundance of the major bacterial taxa of the gut was evaluated in response to infection with the intestinal parasite *Nosema ceranae* or to a chronic exposure to low doses of neurotoxic regularly found in the hives (coumaphos, fipronil, thiamethoxam and imidacloprid). Experiments were performed in laboratory conditions on adult workers collected from hives in winter and in summer and revealed season-dependent changes in the bacterial community composition. *N. ceranae* and a lethal fipronil treatment increased the relative abundance of both *Gilliamella apicola* and *Snodgrassella alvi* in surviving winter honeybees. Furthermore, the parasite and sublethal exposure to all insecticides led to a decreased abundance of *Bifidobacterium* spp. and *Lactobacillus* spp. whatever the season. The similar effects induced by insecticides belonging to distinct molecular families suggested a shared and indirect mode of action on the gut microbiota, possibly through aspecific alterations of the gut homeostasis. These data showed an impact of the pathogen and chronic exposures of insecticides, even at low concentrations, that may have effects on the whole honeybee holobiont. The gut microbiota should therefore be considered as an important parameter in bee health studies.

CONTRIBUTED PAPERS. Thursday, 15:30 **B-29**

Gut microbiota of *Aedes aegypti* shift in response to host blood meal source

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Bacterial communities that colonize the guts of disease vectors such as mosquitoes are considered potential tools for mosquito-borne disease control. These microbes vary markedly within and between mosquito species but the factors responsible for these variations are poorly understood. We used MiSeq sequencing of the 16S rDNA gene to investigate the impact of host blood meal source on the gut microbiota of the yellow fever mosquito *Aedes aegypti*. Adult mosquitoes were fed on human, rabbit or chicken blood and their gut microbiota compared to those of sugar-fed and newly emerged adults. Microbial diversity was significantly reduced in blood-fed and sugar-fed mosquitoes but was restored to the levels of newly emerged adults' 7-days post blood meal. Microbial composition was strongly influenced by host blood meal source. *Leucobacter* spp., *Chryseobacterium* spp., *Elizabethkingia* spp. and *Serratia* spp. were characteristic of newly emerged adults and adults fed on chicken, rabbit, and human blood respectively. Sugar-fed mosquitoes had higher abundance of *Pseudomonas* spp. and unclassified Acetobacteraceae. Shifts in gut microbial communities in response to host blood meal source may fundamentally impact pathogen transmission and vector susceptibility to a variety of mosquito-borne pathogens and may be a key determinant of individual and population variation in vector competence.

CONTRIBUTED PAPERS. Thursday, 15:45 **B-30**

Gut bacteria activate hypoxia-inducible transcription factors that impact growth and metabolism of mosquito larvae

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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

Valicente, F.H.[1]*; Carvalho, K.S. de*[2]*; Nunes, Gabriel H.F.*[2]*; Machado, D.H.B.*[2]*; Lana, U.G., de P.*[1]*; Aguiar, F.M.*[1]*; Modesto, F.; Geraldo, L.*[2]*; Pinho, J.M.R.*[1]
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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 a 100 baculovirus isolates that have been tested against: *Spodoptera frugiperda*, *S. cosmioides*, *S. eridania*, *Helicoverpa zea*, *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 sugarcane pest. These strains harbor some *cry1*, *2*, *8* genes and also *vip1*, *2* and *vip3Ah, vip3Ba1, vip3Aa2, vip3Af1* genes.

CONTRIBUTED PAPERS Thursday, 14:30-16:30
FUNGI 5 Multispace CD

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. Plantlets 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 robertsii* C25 in baits against *Drosophila suzukii*

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Entomopathogenic fungi (EPF) are a potential alternative to chemical products against *Drosophila suzukii*. As full-field application is costly, developing a bait station may be an affordable alternative. In baits, either a fast-killing EPF, or an EPF that quickly reduces oviposition (sub-lethal) is needed to prevent oviposition and thus damage. Furthermore, a substantial proportion of the population of flies has to be infected, which means that baits need to be attractive.

Experiments with *Metarhizium robertsii* (C25) indicated that mortality sets in slowly and sub-lethal effects do not occur. Blueberries treated with *M. robertsii* were less preferred oviposition-sites than berries treated with kaolinite, *Beauveria bassiana* GHA or untreated berries. Ranking of preferences was consistent for choice and no-choice tests. However, the fungus did not repel the flies. Fewer eggs were deposited and fewer flies developed in berries treated with *M. robertsii*.

In northern Europe, there is a prolonged period of time between the onset of activity of the flies and ripening of fruits. During this period, baits do not compete with commercial fruits and can attract, infect and kill the flies. This opens possibilities for *D. suzukii* control. Field trials are needed to investigate whether a sufficiently large proportion will become infected.

CONTRIBUTED PAPERS. Thursday, 14:45 **F-35**

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

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Experimental preparations of *Metarhizium brunneum* (Petch) strain EAMa 01/58-Su consisting of granule formulations made using in vitro produced microsclerotia (MS), which are intended to produce infective conidial spores after soil application to control olive fruit fly, were evaluated under different abiotic factors such soil type, moisture, temperature and UV-B. In a first series of experiments, the ability of this strain to form microsclerotia in a liquid culture and then conidia, once formulated, was confirmed, with 3×10^4 MS/ml. A first experimental product of the strain produced 8×10^8 conidia/g. High storage temperature (25˚C), during four months, slightly reduced MS capacity to produce conidia if compared with low temperatures with 0.1×10^8, 1×10^8, 1×10^8, and 5×10^8 conidia/g of experimental preparation for 25, 4, -18 and -80˚C respectively. The soil type affected time course of conidia production by the MS, with infective conidial spores produced by MS in all soils from the first week and onwards. However, MS produced more conidia in soils with high sand content. The optimal combination of temperature and soil moisture was 22.7˚C and 7.28 (%) respectively, with a 1.4×10^8 conidia/g of experimental preparation. Finally, the experimental preparation was shown to be quite photo resistant.

CONTRIBUTED PAPERS. Thursday, 15:15 **F-36**

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.

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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 (*Metarhizium*, *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 strains in a broad spatial matrix, combined with crop heterogeneity, represents a practical solution for long term proactive biopesticide resistance management.

CROOSS-DIVISIONAL SYMPOSIUM Thursday, 14:30-16:30
DBI-MICROSPORIDIA Commission R8

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. Thélohan (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 ceratomyxae*, *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, *Ovipleistaphora diplostomuri,* 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 characteristics 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-inferred 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.

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Metchnikovellideans (genera *Metchnikovella*, *Caulleryetta*, *Amphiamblys* 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 *Amphiamblys* is an exception, producing rod-shaped spores in the sac-bound sporogony. The free sporogony is by plasmotomy or budding. The sac-bound sporogony proceeds as vacuolation, in the cytoplasm of the sporont, and the cell wall of the sporont remains as a spore-sac. In the genus *Amphiamblys* the presence of synaptonemal complexes indicates meiotic division. The wall of the sporont 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 enveloped 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.

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

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

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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 mitochondriate relatives belonging to Rozellosporidia (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 *Amphiamblys* spp. and *Amphiacantha* spp. the sequenced species of *Metchnikovella* 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

Corsaro D.[1]*; Walochnik J.*[2]*; Wylezich C.*[3]*; Hauröder B.*[4]*;* Müller K.-D.[5]*;* ***Sokolova Y.***[6]*; Michel R.*[4]

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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 chytrid-like endoparasites of water molds and algae. Amoeba parasites, 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 mitochondrial 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*.

SLUGS & SNAILS SYMPOSIUM — Thursday, 14:30-16:30, Multispace AB

Future of Integrated Pest Management for Mollusc Control

Organisers / Chairs: Jenna Ross / Jirka Nermut

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

Riding the Slime Wave: Global Perspective of Slug Control

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Terrestrial slugs are major agricultural and horticultural pests around the world. Current methods for controlling these animals involve the use of chemical molluscicide pellets, such as metaldehyde and Iron (Ferric) phosphate, as well as biological and cultural control methods. However, with tighter restrictions being place on the chemical tool box, the future of slug control is uncertain. The aim of this project was to collate global information on slugs, and their various control options, in order to enhance farming methods. The project was commissioned as part of the Nuffield Farming Scholarship scheme, and the author spent 24 weeks travelling to Australia, Belgium, Brazil, Canada, France, Japan, Kenya, New Zealand, Norway, Ukraine, United Kingdom, United States and South Africa, to review control options, monitoring systems, direct and indirect economic impact, biosecurity threats, and to evaluate the future of malacology. A mixture of researchers, farmers, government officials, entrepreneurs and businesses were interview, generating both qualitative and quantitative data. This presentation highlights the key findings from the report, and opens up opportunities for future collaborative projects in order to address key knowledge gaps.

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-

rope and the recent discovery of *Phasmarhabditis hermaphrodita* in the western US. Infectivity trials with this US strain confirm that it is lethal to key gastropod pests in North America including *Lissachatina fulica*. The recent development of novel attractants for key slug and snail pests is also a compelling option as these lures could be used in both trapping, and attract and kill strategies to manage established pests but also to detect new invasive populations. The development of these and other novel approaches will be key to the successful management of invasive gastropods in North America in the future as the threat of molluscicide bans loom large.

SYMPOSIUM. Thursday, 15:30 **SSS-3**

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*.

SYMPOSIUM. Thursday, 16:00 **SSS-4**

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.

Coffee Break Thursday, 16:30-17:00

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*

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The insecticidal bacterium, *Bacillus thuringiensis*, was registered in the U.S. and Europe over fifty years ago, and since then isolates of several subspecies have been used widely in commercial products for controlling larvae of lepidopteran pests and biting flies in biological control and IPM programs. The most successful subspecies for lepidopterans are *B. thuringiensis* subsp. *kurstaki* (Btk) and *B. thuringiensis* subsp. *aizawai* (Bta), whereas *B. thuringiensis* subsp. *israelensis* (Bti) is used commonly for controlling mosquito and black fly larvae. The isolates used in successful products share several important traits; they all have a broad activity spectrum against target insects yet are safe for most non-target invertebrates and vertebrates, they are easy to mass produce using relatively inexpensive fermentation technology, they can be packaged and applied using techniques similar to those used for synthetic chemical insecticides, and have a shelf life of at least two years. An important feature of their broad activity spectrum against targeted insect groups is that mechanisms evolved in each to aggregate insecticidal proteins with slightly different target spectra and efficacies into a single parasporal body (PB). The PB of Btk contains Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa, whereas Bta contains Cry1Aa, Cry1Ab, Cry1Ca, and Cry1Da. In both, the Cry1 c-terminal halves are highly conserved enabling cocrystallization forming a stable crystal. Bti also aggregates four proteins, Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1A, but within a fibrous matrix. Thus, the evolution of natural Bt isolates, so much more environmentally safe than chemical insecticides, provides examples for improving other insecticidal bacteria.

SYMPOSIUM. Thursday, 17:20 **BS-2**

***Brevibacillus* as an insecticidal bacterium and source of pesticidal proteins**

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Brevibacillus laterosporus is a widespread bacterial entomopathogen adapted to a variety of habitats and morphologically characterized by a canoe-shaped lamellar body attached to one side of its spore, although not all strains show this morphology. According to the results of numerous studies involving diverse strains, this bacterium shows toxicity and pathogenicity against a variety of major insect pests in different orders, including Coleoptera, Lepidoptera and Diptera, as well as activity against a range of other organisms. Recent genome sequencing and annotation work revealed its potential to produce polyketides, nonribosomal peptides, and toxins. Gene expression and proteomic studies led to the identification and characterization of specific bacterial proteins involved in the pathogenic process, among which, diverse enzymes (i.e., chitinases, proteases), toxins, and other putative virulence factors. The implication of

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, 18:20 **BS-4**

Recent advances in *B. thuringiensis* physiology and infection features

Chen,X.[1], Jin ,Lv.[1], Peng,Q [1], Zhang, J[1]., **Song,F**[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. *CwlB* and *cwlC* hydrolases gene transcription are controled by SigmaK and positively regulated by the transcriptional factor GerE. The disruption of *cwlB* delayed MCL while the mutation of *cwlC* completely blocked MCL. Suggesting that CwlC is essential for MCL in this strain. The *sigK* mutation also blocked the MCL in HD73. In addtion the SpoIIID positively regulates *sigK* transcription. However, MCL in the spoIIID mutant was medium-dependent. CwlC is conserved in all sequenced *B. cereus* group strains but the regulation can be strain dependent.

SYMPOSIUM. Thursday, 18:40 **BS-5**

Bacillus thuringiensis: 50 years of safety to vertebrates

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The *Bacillus cereus* group contains vertebrate pathogens 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.

ICTV STUDY GROUP	Thursday, 17:00-19:00 Multispace AB
Organizer: Robert Harrison	

CONTRIBUTED PAPERS MICROBIAL CONTROL 6	Thursday, 17:00-19:00 Multispace CD
Entomopathogenic fungi	
Chairs: Todd Kabaluk / Edith Ladunder	

CONTRIBUTED PAPERS. Thursday, 17:00 **MC-39**

Efficacy of *Beauveria bassiana* strain ATCC 74040 (Naturalis®) against the leafhopper *Scaphoideus titanus* Ball under open-field conditions

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The leafhopper, *Scaphoideus titanus* Ball (Cicadellidae), native to North America, was introduced accidentally to Europe, where it has become a key pest by acting as the principal vector of the grapevine flavescence dorée phytoplasma (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 infective, i.e. before they reach the 4th-5th instar. However, due to the

prolonged egg-hatching period of the target pest, repeated sprays may be necessary to effectively control the pest. The most effective insecticides against this *S. titanus* allowed in organic viticulture, are products based on pyrethrins. Due to their low persistence and their toxicity to non-target organisms, research on alternative control tools and measures, to be included not only in organic, but in sustainable plant protection strategies in general, is ongoing. The aim of these studies was to investigate the efficacy of the microbial pest control agent *Beauveria bassiana* strain ATCC 74040 (formulated product: Naturalis®), applied twice by itself, in comparison to organic and chemical reference insecticides under open-field conditions. The microbial control agent did not provide complete pest control, but reached final efficacy values in reducing the number of live *S. titanus* ranging from 63 up to 84%. Perspectives and options for the inclusion of *B. bassiana* strain ATCC 74040 in *S. titanus* control strategies are discussed.

CONTRIBUTED PAPERS. Thursday, 17:15 **MC-40**

Efficacy test of entomopathogenic fungi for controlling chili thrips (*Scirtothrips dorsalis*)

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Chili thrips (*Scirtothrips dorsalis*) is an important pest of chili crops and is a major vector of viral plant pathogens. Due to the widespread outbreak of the thrips, chemical insecticides have been heavily used in the last few decades. In order to reduce the utilization of chemical pesticides, alternative biocontrol agents such as entomopathogenic fungi have been screened against the thrips. Laboratory screening revealed that 2 insect fungi isolates, *Beauveria bassiana* BCC48145 and *Purpureocillium lilacinum* TBRC 10638 were the most effective isolates against chili thrips. Efficacy validation in the greenhouse showed that *P. lilacinum* TBRC 10638 was more effective than *B. bassiana* BCC48145 and could control the thrips up to 80% when using the fungus at 10^8 spores/ml. The LC_{50} values of *P. lilacinum* TBRC 10638 against chili thrips based on total thrips count from two experiments were $1.17x10^8$ and $1.12x10^7$ spores/ml when the fungal spores were sprayed once a week. The optimal concentration of *P. lilacinum* TBRC 10638 spore for effective control of chili thrips was determined at 1.41 $x10^9$ spores/ml. Average efficacy of *P. lilacinum* TBRC 10638 for thrips control from 3 field trials were 30.08%, 14.39% and 29.92%. This result was not significantly different from the chemical insecticide treatment group that showed efficacy at 19.27%, 14.92% and 19.97%. However, there was no difference in productivities among the different treatment groups. Our results demonstrated that *P. lilacinum* TBRC 10638 is a promising biocontrol agent that could be used as an alternative to chemical insecticide for controlling chili thrips.

CONTRIBUTED PAPERS. Thursday, 17:30 **MC-41**

Multitrophic interactions among endophytic *Beauveria bassiana,* aphid prey and its natural enemies on melon

González-Mas, N.; *Cuenca-Medina, M.; Gutiérrez-Sánchez, F.; Quesada-Moraga, E.*
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Entomopathogenic fungi are now known to act as endophytes and induce several changes in the composition of plant nutrients and/or defensive compounds. These changes could influence plant interactions with the insect community. In this study, the predation/parasitism efficacy of larvae of the lacewing, *Chrysoperla carnea*, and the braconid parasitoid, *Aphidius colemani*, when offered aphids that had been challenged by the entomopathogenic fungus *Beauveria bassiana* were investigated. Aphids were either inoculated directly with a fungal suspension (lacewing bioassay only) or had been feeding on melon plants endophytically-colonized by *B. bassiana*. Our results indicate that *B. bassiana* application did not significantly influence the number of aphid prey consumed by lacewings and the time took them to consume each aphid. In a choice bioassay, *C. carnea* larvae preferred to feed on aphids reared on *B. bassiana*-colonized plants compared with control plants. In another choice assay, the number of aphids parasitized by *A. colemani* and their sex ratio were not influenced by whether the aphids had been feeding on *B. bassiana*-colonized plants or not. Our findings support the use of endophytic entomopathogenic fungi in combination with other natural enemies, such as predators and parasitoids, in Integrated Pest Management programs.

CONTRIBUTED PAPERS. Thursday, 17:45 **MC-42**

Effects of entomopathogenic fungi against the crapemyrtle bark scale and its natural enemies

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Crapemyrtles, *Lagerstroemiae* sp., are one of the most common ornamental plants in Southeastern U.S. In 2004 the crapemyrtle bark scale (CMBS), *Acanthococcus lagerstroemiae* was detected, leading to an increase in management costs. CMBS directly impacts crapemyrtle'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, 18:00 **MC-43**

Apples and oranges: Standard non-target tests are unsuitable for entomopathogenic fungi - a proposal for a new guideline

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The value of a plant protection product, chemical or biological, depends on its effectiveness against a target species, as well as its safety for the environment. Risk assessment schemes have therefore been developed to facilitate classification and regulation. These guidelines however are directed towards chemical substances and are in many cases not suitable for the specific requirements of biocontrol organisms. In this study we developed a protocol for non-target testing of soil applied entomopathogenic fungi with the predatory mite *Gaeolaelaps (Hypoaspis) aculeifer. G.aculeifer* is frequently found in arable and grassland worldwide and is, due to its habitat in soil, at a high risk of exposure. The protocol assesses lethal and sublethal effects of the recommended field concentration and tenfold field concentration. It adapts to fungal biology in terms of duration, end points and quality control. As a representative of entomopathogenic fungi we chose *Metarhizium brunneum* ART2825, a

promising new biocontrol organism against wireworms.

CONTRIBUTED PAPERS. 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.*
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The false carmine cochineal *Dactylopius 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, *Cryptolaemus montrouzieri* 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*

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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.

CONTRIBUTED PAPERS. Thursday, 18:45 **MC-46**

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 140×, 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 videoed 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.

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'

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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 specialisms, 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 protists to humans. In particular, the *Enterocytozoon* group Microsporidia (EGM) 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

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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

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Global food security is highly dependent on pollinators. Pollinators such as bees and hoverflies (Syrphidae) 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 augementative biocontrols in selected low to lower-middle income countries

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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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Mantas	Adomaitis	SS-15 STU	Lithuania
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Wafa	Al Arimi	DBI-8 STU	United Kingdom
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Joel	Gonzalez-Cabrera	V-6 STU, PMI-9 STU	Spain
Natalia	González-Mas	MC-20 STU, MC-34, MC-41	Spain
Giselher	Grabenweger	MC-7, MFCS-4, MC-18 STU, PB-34 STU, PMC-14 STU, PMC-16, MC-43	Switzerland
Christine	Griffin	N-1, N-19 STU	Ireland
Ekaterina	Grizanova	F-11, B-26	Russia
David	Grzywacz		United Kingdom
Dawn	Gundersen-Rindal	PMC-13	France
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Beomku	Han	PV-22	South Korea
Robert	Harrison	V-25, V-53, B-30	Usa
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