



# New Challenges for Biological Control

15th Meeting of the IOBC-WPRS Working Group  
"Microbial and Nematode Control  
of Invertebrate Pests"

**PROGRAMME  
AND  
ABSTRACT BOOK**

**June 7 – 11, 2015, Riga, Latvia**

# WELCOME MESSAGE

Dear Colleagues,

It is our pleasure to welcome you at the 15<sup>th</sup> meeting of the Working Group „**Microbial and Nematode Control of Invertebrate Pests**” (formerly *Insect Pathogens and Insect Parasitic Nematodes*) organised by International Organization for Biological and Integrated Control West Palaearctic Regional Section (IOBC/WPRS) together with Institute of Biology of University of Latvia.

The general topics of this meeting will cover all aspects of biological control of invertebrates, including insects, arachnids, and nematodes, by using biological control agents (BCAs) such as viruses, bacteria, fungi, nematodes and other pathogens.

The meeting will have a special focus on new challenges for microbial and nematode control of invertebrate pests, which originate from new pests and technical developments as well as social, economic and legislative issues.

The meeting encourage communication between entomologists, invertebrate pathologists, and experts of other disciplines on new challenges in the biocontrol of insects.

We are pleased to host you in Riga, the capital of Latvia and a city of great beauty.

Dr. Līga Jankevica

Chair of Local Organizing Committee

Prof. Dr. Johannes Jehle

Convenor of the WG Microbial and Nematode Control of Invertebrate Pests



IOBC  
OILB

WBRS *International Organisation for Biological and Integrated Control  
West Palaearctic Regional Section*  
SROP *Organisation Internationale de Lutte Biologique et Intégrée  
Section Régionale Ouest Paléarctique*

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# PROGRAMME OVERVIEW

	June 7, Sunday	June 8, Monday	June 9, Tuesday	June 10, Wednesday	
8:30		<b>Registration</b>	<b>Viruses 1</b> <i>O-13, O-14, O-15, O-16, O-17</i>	<b>Viruses 2</b> <i>O-34, O-35, O-36, O-37, O-38</i>	
8:45		<b>Opening</b>			
9:00					
9:15					
9:30					
9:45					
10:00		<b>Coffee break</b>	<b>Coffee break</b>	<b>Coffee break</b>	
10:15					
10:30		<b>Plenary section</b> <i>O-1, O-2, O-3, O-4</i> Convenor: Johannes Jehle	<b>Fungi / soil pests</b> <i>O-18, O-19, O-20, O-21, O-22, O-23, O-24, O-25</i>	<b>Nematodes /Soil pests</b> <i>O-39, O-40, O-41, O-42, O-43, O-44</i>	
10:45					
11:00					
11:15					
11:30					
11:45					<b>Closing</b>
12:00					
12:15					
12:30		<b>Lunch</b>	<b>Lunch</b>	<b>Lunch</b>	
13:00		<b>Registration</b>			
13:30					
13:45					
14:00	<b>Fungi</b> <i>O-5, O-6, O-7, O-8, O-9, O-10, O-11, O-12</i>		<b>Soil pests / bacteria</b> <i>O-26, O-27, O-28, O-29, O-29, O-30, O-31, O-32, O-33</i>	<b>Excursion</b>	
14:15					
14:30					
14:45					
15:00					
15:15					
15:30					
15:45					
16:00	<b>Coffee break</b>	<b>Coffee break</b>			
16:15					
16:30	<b>Poster 1</b> <i>Fungi, nematodes</i>	<b>Poster 2</b> <i>Viruses, bacteria, soil pests</i>			
16:45					
17:00					
17:15					
17:30					
17:45					
18:00					
18:30	<b>Guided walking tour</b>				
19:00					
19:30		<b>Get-together</b>		<b>Banquet</b>	
20:00					
20:30					
21:00					
21:30					
24:00					

**PROGRAMME**  
**OF THE 15TH MEETING OF THE IOBC-WPRS**  
**WORKING GROUP**  
**“Microbial and Nematode Control**  
**of Invertebrate Pests”**

Sunday, 7<sup>th</sup> of June

Venue Radisson Blu Daugava Hotel (24 Kugu Street, Riga)

Arrival

13:00 – 19:00     Registration

19:00 – Mixer     Snacks and glass of wine

Monday, 8<sup>th</sup> of June

Venue Radisson Blu Daugava Hotel (24 Kugu Street, Riga)

Time	Code	Presenter	Title
08:00			<b>REGISTRATION</b>
09:00			<b>OPENING</b> Līga Jankeviča, Johannes Jehle, Giselher Grabenweger
10:00			<b>COFFEE BREAK</b>
			<b>PLENARY SESSION</b> Convenor: Johannes Jehle
10:30	O01	Maurhofer M.	The secret life of <i>Pseudomonas protegens</i> CHA0: A switch from antifungal agent to insect killer
11:00	O02	Williams T.	Genotype co-occlusion technology: a novel paradigm for the development of improved alphabaculovirus-based insecticides
11:30	O03	Hill M.	The role of entomopathogenic fungi in the control of citrus pests in South Africa: cause for optimism
12:00	O04	Kahrer A.	5 years of experience in biocontrol of the Western Corn Rootworm, <i>Diabrotica virgifera virgifera</i> by entomoparasitic nematodes
12:30			<b>LUNCH</b>
			<b>WG FUNGI</b> Convenor: Anant Patel
14:00	O05	Garrido-Jurado I.	Colonized plants with entomopathogenic fungi produce mortality on chewing and sucking insects;

Time	Code	Presenter	Title
14:15	O06	Jakobs-Schönwandt D.	Endophytic <i>Metarhizium</i> strains against herbivorous insect pests: development of novel fermentation and formulation strategies
14:30	O07	Gonzalez-Mas N.	Endophytic fungus effect on the probing and feeding behavior of <i>Aphis gossypii</i> Glover and the plant virus transmission by aphids
14:45	O08	Steinwender Bernhardt M.	(In)direct side effects of <i>Metarhizium brunneum</i> on four beneficial predatory arthropods – a resource efficient laboratory approach
15:00	O09	Garrido-Jurado I.	Development of a QuEChERS-based extraction method for the determination of destruxins in potato plants by UHPLC-MS/MS
15:15	O10	Taibon J.	Development of validated analytical methods for the assessment of <i>Metarhizium brunneum</i> metabolites in different matrices
15:30	O11	Quesada-Moraga E.	The entomopathogenic fungi <i>Metarhizium brunneum</i> secretes active compounds toward <i>Rhynchophorus ferrugineus</i> larvae and adults
15:45	O12	Garrido-Jurado I.	Crude extracts secreted by entomopathogenic mitosporic ascomycetes show potential for <i>Ceratitis capitata</i> (Wiedemann) (Diptera; Tephritidae) and <i>Drosophila suzukii</i> (Matsumura) (Diptera; Drosophilidae) control
16:00	<b>COFFEE BREAK</b>		
16:30 17:45	P1- P20	<b>POSTER SESSION 1</b> (Fungi, nematodes)	
18:30 20:00	<b>GUIDED WALKING TOUR</b>		

## Tuesday, 9<sup>th</sup> of June

Venue Radisson Blu Daugava Hotel (24 Kugu Street, Riga)

Time	Code	Presenter	Title
<b>VG VIRUSES I</b>			
Conveor: Miguel Lopez-Ferber			
08:30	O13	Herrero S.	Discovery of novel viral pathogens through transcriptomic studies: Potential application in pest control
09:00	O14	Carbalo A.	Effect of <i>Spodoptera exigua</i> iflavivirus co-inoculation on the insecticidal properties of <i>Spodoptera exigua</i> multiple nucleopolyhedrovirus (SeMNPV)

Time	Code	Presenter	Title
09:15	O15	Murillo R.	Value of mixtures of vertically and horizontally transmitted variant mixtures of <i>Spodoptera exigua</i> multiple nucleopolyhedrovirus (SeMNPV) as the basis for biological insecticides
09:30	O16	Arrizubieta M.	A novel co-occluded binary mixture of <i>Helicoverpa armigera</i> single nucleopolyhedrovirus (HearSNPV) genotypes from Spain is a highly effective insecticide
09:45	O17	Gomez-Valderrama J.	Occurrence and characterization of two betabaculoviruses isolated from <i>Tuta absoluta</i> (Lepidoptera:Gelechiidae) in Colombia
10:00	<b>COFFEE BREAK</b>		
	<b>VG FUNGI</b> Convenor: Quesada-Moraga Enrique		
10:30	O18	Kaiser D.	Biological control of pollen beetles with the entomopathogenic fungus <i>Beauveria bassiana</i> in vegetable oil formulations
10:45	O19	Villamizar L.	Occurrence and characterization of entopathogenic fungi from the complex of sugarcane borer <i>Diatraea</i> spp. in Colombia
11:00	O20	Fernández-Bravo M.	Effect of UV-B radiation on germination, colony growth and virulence of <i>Metarhizium</i> sp. isolates against "Mediterranean fruit fly" <i>Ceratitis capitata</i> (Wiedemann) (Diptera; Tephritidae)
11:15	O21	Quesada-Moraga E.	Can soil application of <i>Metarhizium brunneum</i> (Metsch.) Sorok. (Hypocreales: Clavicipitaceae) toward of immature stages control the olive fruit fly <i>Bactrocera oleae</i> ?
11:30	O22	Pernek M.	Collapse of an isolated outbreak population of <i>Dendrolimus pini</i> caused by a very high infestation of <i>Beauveria bassiana</i>
11:45	O23	Strasser H.	Efficacy assessment of <i>Heterorhabditis bacteriophora</i> (Nematoda: Heterorhabditidae), <i>Metarhizium brunneum</i> (Hypocreales: Clavicipitaceae), and chemical insecticides for <i>Diabrotica virgifera virgifera</i> larval management under real farm conditions
12:00	O24	Hanitzsch M.	Encapsulation of <i>Metarhizium brunneum</i> as basis for an attract and kill strategy within the EU project IN-BIOSOIL
12:15	O25	Rogge S.	Attract and kill kept simple: Fungus colonized barley kernels in cover crops for microbial wireworm control



Time	Code	Presenter	Title
12:30	<b>LUNCH</b>		
<b>WGS SOIL PESTS &amp; BACTERIA</b> Convenor: Tarasco Eustachio			
14:00	O26	Benjamin E.	The economics and environmental benefits and costs of biological control of western corn root-worm <i>Diabrotica virgifera virgifera</i> and wireworm <i>Agriotes</i> spp. in maize and potatoes for selected countries in Europe
14:15	O27	Mävers F.	Evaluation of botanical formulations (Neem) in an "Attract-and-Kill"- strategy under field and laboratory conditions targeting wireworms
14:30	O28	Brandl M. A.	An "Attract & Kill" approach in potato to reduce wire-worm tuber damage
14:45	O29	Tarasco E.	Screening of bacteria isolated from <i>Galleria mellonella</i> (Lepidoptera: Pyralidae) larvae infected with novel entomopathogenic nematodes.
15:00	O30	Metla Z.	Analysis of the bacterial community present in the insect pest <i>Lymantria dispar</i> during the life cycle of insect
15:15	O31	Flury P.	What are the features that plant-beneficial pseudomonads require to become insect pathogens?
15:45	O32	Schneider S.	Influence of multi-year <i>Bacillus thuringiensis</i> subsp. <i>israelensis</i> treatments on the abundance of <i>B. cereus</i> group populations in Swedish riparian wetland soils
15:30	O33	Mossialos D.	Studies on <i>Pseudomonas entomophila</i> : a novel entomopathogenic bacterium
16:00	<b>COFFEE BREAK</b>		
16:30	P21-	<b>POSTER SESSION 2</b>	
17:45	P40	(viruses, bacteria, soil pests, 'low risk' concept)	

## Wednesday, 10<sup>th</sup> of June

Venue Radisson Blu Daugava Hotel

Time	Code	Presenter	Title
<b>VG VIRUSES II</b> Convenor: Trevor Williams			
08:30	O34	Lopez-Ferber M.	Overwinter transmission of CpGV on infected diapausing larvae
09:00	O35	Sauer A.J.	A novel mode of resistance of codling moth against <i>Cydia pomonella</i> granulovirus with a dominant and autosomal inheritance pattern

Time	Code	Presenter	Title
09:15	O36	Gueli Alletti G.	Analysis of the genome sequence of an <i>Agrotis segetum</i> granulovirus
09:30	O37	Moore S.	Determination of reapplication frequency required for the <i>Cryptophlebia leucotreta</i> granulovirus: a factor of rate of virus breakdown and larval behaviour
09:45	O38	Herrero N.	Novel virus discovery in entomopathogenic nematodes
10:00	<b>COFFEE BREAK</b>		
<b>VGS NEMATODES &amp; SOIL PESTS</b> Convenor: Hermann Strasser			
10:30	O39	Jaffuel G.	Low prevalence and strong competition of entomopathogenic nematodes in Swiss agricultural soils imply a need for an augmentation biological control approach for effective crop protection
10:45	O40	Chubinishvili M.	Development of vegetable crops complex protection from <i>Tuta absoluta</i> in greenhouse conditions
11:00	O41	Nježić B.	Control of plum sawflies ( <i>Hoplocampa flava</i> and <i>Hoplocampa minuta</i> ) by three entomopathogenic nematodes
11:15	O42	Tarasco E.	Characterization of heat shock protein 90 gene in native entomopathogenic nematodes from Southern Italy
11:30	O43	Tarasco E.	Nematode fauna of <i>Rhynchophorus ferrugineus</i> (Olivier) in Southern Italy
11:45	O44	White S.	Entomopathogenic nematodes for the control of oak processionary moth in the UK
12:00	Closing		
13:00	Lunch		
14:30	Excursion & Banquet		
24:00	Bus excursion Sigulda, distance from Riga approximately 60 km. Returning to Riga and banquet with music and dances.		

## 5<sup>th</sup> day (Thursday)

Departure or post conference tour.

## POSTER SESSION

Code	Presenter	Title
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Monday, 8<sup>th</sup> of June 16:30 -17:45

P01	Steinwender B.M.	<i>Metarhizium anisopliae</i> or...? – a graphical approach to shed light on a genus
P02	Schuster C.	Group-I intron based strain-specific diagnosis of entomopathogenic <i>Lecanicillium</i> fungi for aphid biocontrol
P03	Groete C. de Azevedo A.	Combined effects of two natural enemies, entomopathogenic fungi and predatory midges, and their effect on a cereal pest
P04	Tabakovic-Tosic M.	<i>Entomophaga maimaiga</i> caused the crash of the gypsy moth outbreak in the forests of central Serbia in the 2014
P05	Tserodze M.	Microbial control of winter moth in Georgia
P06	Przyklenk M.	<i>Metarhizium</i> stationary surface cultivation and encapsulation
P07	Wegensteiner R.	Testing entomopathogenic fungi against <i>Hylobius abietis</i> (Coleoptera, Curculionidae).
P08	Tkaczuk C.	Effect of <i>Beauveria bassiana</i> on the cuticular and internal lipids composition of <i>Hylobius abietis</i> beetles
P09	Wegensteiner R.	Testing effects of natural resin and synthetic terpenes on conidial germination and growth of entomopathogenic fungi
P10	Quesada-Moraga E.	The entomopathogenic fungi <i>Metarhizium brunneum</i> , <i>Beauveria bassiana</i> and <i>Isaria farinosa</i> (Ascomycota, Hypocreales) increase the availability of iron
P11	Enkerli J.	Assessing <i>Metarhizium</i> spp. diversity in three different habitats in Switzerland
P12	Grantina-levina L.	Occurrence of entomopathogenic fungi in agricultural and forest soils and vermicompost samples
P13	Tkaczuk C.	Entomopathogenic fungi in litter samples from <i>Picea abies</i> sites infested with <i>Pachynematus montanus</i> (Hymenoptera, Tenthredinidae)
P14	Pernek M.	When native parasitoids don't work: biological control of an invasive species with introduced parasitoid and entomopathogenic fungi
P15	Kleespies R.G.	A survey of microbial antagonists of <i>Otiorhynchus</i> species from Germany

Code	Presenter	Title
P16	Schuman M.	Entomopathogenic fungal formulations for western corn rootworm control
P17	Laznik Ž.	Can entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) function as an indirect plant defense?
P18	Karimi J.	Pathology of two species of entomopathogenic nematodes, <i>Heterorhabditis bacteriophora</i> and <i>Steinernema carpocapsae</i> on pistachio root beetle larvae, <i>Capnodis cariosa hauseri</i> (Col.: Buprestidae)
P19	Mikaia N.	Evaluation of entomopathogenic nematode <i>Steinernema carpocapsae</i> against fern scale <i>Pinnaspis aspidistrae</i> Sign
P20	Gabroshvili N.	Efficacy evaluation of the <i>Heterorhabditis bacteriophora</i> against Click beetle (Coleoptera: Elateridae)

## Tuesday, 9<sup>th</sup> of June 16:30 -17:45

P21	Eilenberg J.	The 'low risk' concept for plant protection products within the EU: a new opportunity for authorization of biocontrol microorganisms
P22	Rabalski L.	New findings in phylogeny and genome of nun moth ( <i>Lymantria monacha</i> ) baculovirus
P23	Krejmer M.	The genome of recently sequenced white satin moth baculovirus (LesaNPV) shows high level of similarity to douglas-fir tussock moth baculovirus (OpMNPV)
P24	Villamizar L.	Detection and quantitation of alpha and betabaculovirus in <i>Spodoptera frugiperda</i> mixed infections
P25	Villamizar L.	Possible natural betabaculovirus coinfections in Gelchiidae insects from Colombia based on pif gene determination
P26	Demir I.	Identification of four nuclear polyhedrosis viruses from the gypsy moth, <i>Lymantria dispar</i> (Lepidoptera: Lymantridae) in Turkey: variations in their virulence
P27	Gómez-Valderrama J.	Conditions adjustment for <i>in vivo</i> mass production of a Colombian <i>Spodoptera frugiperda</i> nucleopolyhedrovirus
P28	Nalcacioglu R.	Detection of a new insect iridescent virus in the elm leaf beetle, <i>Pyrrhalta luteola</i> (Coleoptera: Chrysomelidae) population
P29	Demirbağ Z.	The esterase/protease gene of <i>Amsacta moorei</i> entomopoxvirus plays significant role on the growth of infectious virus

Code	Presenter	Title
P30	Herrero N.	Prevalence and diversity of viruses in the entomopathogenic and nematophagous fungus <i>Purpureocillium lilacinum</i> in the Czech Republic
P31	Gaganidze D.	<i>Bacillus</i> associated with Colorado potato beetle – <i>Leptinotarsa decemlineata</i> Say and the mottled umber – <i>Erannis defoliaria</i> Clerrck in Georgia
P32	Gomis-Cebolla J.	Proteolytic processing and in vivo binding of the <i>Bacillus thuringiensis</i> Vip3Ca insecticidal protein
P33	Kati H.	Antagonistic effects of <i>Bacillus</i> isolates on phytopathogenic fungi
P34	Muehlbach H.P.	The two faces of <i>Pseudomonas oryzihabitans</i>
P35	Hyrsl P.	Plant alcaloid and probiotics increase resistance of honeybees to nematobacterial infection
P36	Nalcacioglu R.	Cloning and expression of chitinase A, B and C genes from <i>Serratia marcescens</i> originating from <i>Helicoverpa armigera</i> and determining their activities
P37	Kleespies R.G.	Host group adaptation of ‘ <i>Candidatus Rickettsiella isopodorum</i> ’, a lineage of intracellular gamma-proteobacterial pathogens carried by woodlice
P38	Mayerhofer J.	Efficacy of entomopathogenic fungi for wireworm control and their potential effects on microbial communities
P39	Vozik D.	Effectiveness of antimicrobial compounds produced by entomopathogenic nematode symbiotic bacteria to control pests and bacterial plant diseases
P40	Patel A.	Co-encapsulation of <i>Beauveria bassiana</i> or neem extract in CO2 releasing beads attractive towards soil borne pests

**ABSTRACTS  
ORAL  
PRESENTATIONS**

## The secret life of *Pseudomonas protegens* CHA0: A switch from antifungal agent to insect killer

**Monika Maurhofer<sup>1</sup>, Pascale Flury<sup>1</sup>, Peter Kupferschmied<sup>2</sup>, Beat Ruffner<sup>1</sup>,  
Shakira Fataar<sup>1</sup>, Anja Taddei<sup>1</sup>, Maria Péchy-Tarr<sup>2</sup>, Regina G. Kleespies<sup>3</sup>,  
Cornelia Ullrich<sup>3</sup>, Johannes A. Jehle<sup>3</sup>, Christoph Keel<sup>2</sup>**

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*Pseudomonas protegens* CHA0 is a bacterium with manifold skills. It is an excellent root colonizer which promotes plant growth and induces systemic resistance against foliar pathogens. Moreover it efficiently protects plants against the attack of fungal root pathogens by the production of antifungal metabolites. The plant rhizosphere, however, is not the only ecological niche where this bacterium feels at home. If orally taken up by insects CHA0 can efficiently colonize and kill larvae of several insect species, which makes this biocontrol agent even more interesting for use in agriculture. We are currently interested in elucidating how this fascinating bacterium is able to switch its life style from a root colonizer and antifungal agent to an insect pathogen. How can it survive and compete in the ecological hot spot rhizosphere as well as in the insect gut and how does it reach the insect hemolymph where it finally multiplies to high numbers and kills the insect? Interestingly the Fit insect toxin which contributes to insecticidal activity of CHA0 is only expressed in the insect hemolymph but not on roots which indicates the presence of a sensor enabling the bacterium to detect the insect environment and to switch on specific insect virulence factors.

## Genotype co-occlusion technology: a novel paradigm for the development of improved alphabaculovirus-based insecticides

**Primitivo Caballero<sup>1,2</sup>, Inés Beperet<sup>1</sup>, Oihane Simon<sup>1</sup>,  
Arrizubieta Maite<sup>1</sup>, Miguel Lopez Ferber<sup>3</sup>, Trevor Williams<sup>4</sup>**

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Insect pathologists have long recognized that insect pathogenic viruses have evolved to be effective pathogens, and not necessarily effective insecticides. Molecular techniques have begun to reveal the presence of genetic heterogeneity in populations of entomopathogenic viruses, particularly in the case of baculoviruses. We have exploited this diversity to develop a novel technology involving isolate dissection, genotype interaction analyses and assembly of novel genotype mixtures to create unique non-natural combinations of component genotypes with insecticidal characteristics that are better than those of the natural virus populations. The production of mixed genotype occlusion bodies (co-occlusion) and mixed genotype virions (co-envelopment) is an integral part of this technology, that has recently been patented by us and acquired by a multinational agrochemical company. We present the value of this technology for development of non-recombinant virus insecticides, with better-than-wild-type characteristics, based on unique combinations of genotypes. To illustrate this we present examples of SeMNPV, SfMNPV, ChchSNPV, HearSNPV and HearMNPV based insecticides that have been improved in this way. This represents a new paradigm in the development of effective virus-based insecticides which incidentally appears to challenge a number of the established models of baculovirus replication and host range.



## The role of entomopathogenic fungi in the control of citrus pests in South Africa: cause for optimism

Candice Coombes<sup>1</sup>, Veronique Charter-Fitzgerald<sup>2</sup>, Danielle Wiblin<sup>1</sup>,  
Jo Dames<sup>2</sup>, Martin Hill<sup>1</sup> and Sean Moore<sup>1,3</sup>

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Citrus is a highly productive crop in South Africa, but it is damaged by a number of pests that result in yield loss and have the potential to limit market access. Maximum residue limits (MRLs) imposed by importing countries have driven the need for alternative control technologies, including the use of entomopathogenic fungi (EPF). Bioprospecting in citrus orchards and seven years of bioassay trials identified *Beauveria bassiana* G Ar 17 B3 and *Metarhizium anisopliae* G 11 3 L6 and FCM Ar 23 B3 as the most virulent fungal isolates. Preliminary trials with these three fungi against the arboreal pests, California red scale, *Aonidiella aurantii* (Maskell), citrus mealybug, *Planococcus citri* (Risso) and citrus thrips, *Scirtothrips aurantii* Faure, have been promising. False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) is the most important citrus pest in South Africa and while considerable research has been conducted on controlling the insect on the tree, the soil borne stages of this insect have largely been ignored. After laboratory bioassays, the three fungal isolates were taken to field trials. All three isolates persisted for at least six months after application to the soil. A large scale field trial, showed that although all three isolates reduced FCM infestation, isolate *B. bassiana* G Ar 17 B3 performed best, recording a consistent 80% reduction in FCM infestation throughout the trial period. The results of nearly 10 years of research on the potential of EPFs in the control of citrus pests in South Africa is certain cause for optimism.

## 5 years of experience in biocontrol of the western corn rootworm, *Diabrotica virgifera virgifera* by entomoparasitic nematodes

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In many experiments between 2009 and 2013 the efficacy of Dianem™ (containing *Heterorhabditis bacteriophora*) was tested under field conditions. Nematodes were applied to small field plots in five replicates at the time of sowing. Maize plants were artificially infested with *Diabrotica* eggs to provide a homogenous distribution of *Diabrotica* larvae. Treatments were evaluated by (a) recording the emergence of adults under gauze tents, (b) rating the damage of maize roots and (c) recording the yield at the end of the season. The most appropriate method of efficacy evaluation seemed to be the recording of beetles hatch in gauze tents. Root rating was less time consuming but applicable only under conditions of heavy infestation by *Diabrotica* larvae. Artificial infestation of maize plants was successful at rates of approximately 400 eggs per plant. By this method 20–80 emerging beetles per plant could be achieved depending on year and field site. Entomoparasitic nematodes in high concentrations of about 3 Mrd. individuals/ha were able to significantly reduce the emergence of adult corn rootworms. Maximum efficacy obtained in our experiments was 60–70%. It has to be stated that those results were highlights and could not be reached in every year. A very important factor for good efficacy seemed to be the application by so called “Cultanschare”, which are metal tubes that lead the nematode suspension exakt to proximity of the seed corn. Nevertheless metal tubes beneath the soil show a tendency to become plugged with soil particles. High efficacies of Dianem application seemed to be favoured by lower beetle numbers in the untreated control.

## Colonized plants with entomopathogenic fungi produce mortality on chewing and sucking insects

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Laboratory studies were performed to determine the ability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium brunneum* to colonize endophytically plants, and evaluate the effect of their ingestion by *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae and nymphs of *Bemisia tabaci* (Hemiptera: Aleyrodidae). All strains artificially inoculated with conidial suspensions colonized endophytically tomato, alfalfa, and melon plants with results ranging from 4 to 24% of colonization of tissues of the root (24 and 96 h, respectively) and 43 to 98% in the stems and leaves (24 and 72 h, respectively). The pathogenicity and virulence were also assessed by immersion for both insects, ranging between 40 and 95% against *S. littoralis* larvae and 60 and 90% against *B. tabaci* nymphs. Alfalfa, tomato, and melon leaves colonized by the *B. bassiana* EABb 01/33-Su and *M. brunneum* EAMb 09/01-Su strains showed significant mortality of *S. littoralis* larvae after 10 days of chronic exposition. Melon plants were selected for *B. tabaci* host in all the experiments. However, colonized plants with both isolates showed high mortality in *B. tabaci* nymphs only 96h after treatment. On the other hand, adaxial melon leaf surface application with a fungal suspension caused also high mortality of *B. tabaci* nymphs. In both cases, some insects did not show fungal outgrowth but their death could also been caused by the fungi, since destruxin A was detected either in *M. brunneum* colonized plants or insects fed with them.

## Endophytic *Metarhizium* strains against herbivorous insect pests: development of novel fermentation and formulation strategies

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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 organisms 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. The aim of our current investigations is to gain insights into innovative fermentation and formulation approaches of endophytic *Beauveria bassiana* and *Metarhizium brunneum* strains enhancing penetration and colonization of the fungi to systemically protect plants from herbivorous pest insects.

Here we present first data on the submerge mass production of endophytic entomopathogenic *M. brunneum* isolates using agricultural residues. By variation of the cultivation conditions the selective production of fungal spores was achieved. To enhance penetration and colonization of plants, beneficial additives were investigated. Spores were formulated in a novel spray consisting of 0.1% Triton X-114, 1% molasses, 1% titanium dioxide and 10<sup>6</sup> spores/leaf applied on the base of the 4th secondary leaf of tomato plantlets. After seven days, 56 fold more fungal hyphae could be visualized in the leaf tips microscopically. This correlated with PCR results which showed a positive signal for *M. brunneum* in 50% of the samples. Furthermore, a mortality test with whitefly nymphs resulted in a significant decrease in the development rate to adults by 26%.

Based on these promising findings an integrated fermentation and formulation strategy with *M. brunneum* will be developed to broaden the application spectrum of endophytic entomopathogenic fungi.

## Endophytic fungus effect on the probing and feeding behavior of *Aphis gossypii* Glover and the plant virus transmission by aphids

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In the recent years various genera of entomopathogenic fungi have been isolated as endophytes in different plants and have been used as an artificial endophyte, inoculating onto the plants, affecting the survivorship and modifying life cycle, fitness and behavior of the herbivorous pests, while reducing plant damage.

In our study, the cotton aphid (*Aphis gossypii* Glover) probing and feeding behaviour was studied by means of the Electrical Penetration Graph (EPGs) technique to understand aphid response to non colonized and colonized melon plants with a *Beauveria bassiana*.

Preliminary results showed that significant differences were only observed in the duration of the first probe, which was longer in control plants than in treated plants ( $p < 0.02$ ), and in the average duration of standard intracellular punctures (pds), which was significantly longer in control plants than in treated plants ( $p < 0.001$ ). Furthermore, the temporal analysis of aphid feeding behavior indicates that a larger percentage of aphids on treated plant were in non-probe activity (np phase) at the beginning of the recording than those who were on control plants. However, no differences were observed on the phloem-related EPG variables analyzed. Overall, the results indicate that fungus colonization did not have any effect on the feeding behavior of *A. gossypii*.

Moreover, transmission experiments with persistent (*Cucurbit aphid-borne yellow virus*, *Polerovirus*), and non-persistent, (*Cucumber mosaic virus*, *Cucumovirus*) virus were conducted to assess if the fungus treatment on plants can avoid or reduce the virus transmission rate by aphid vectors. The effect of the fungus colonization on the virus transmission will be discussed on the basis of our findings.

## **(In)direct side effects of *Metarhizium brunneum* on four beneficial predatory arthropods – a resource efficient laboratory approach**

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One of the most important steps in considering a new microbial biocontrol agent (either alone or as part of a new formulation) to be ready for field testing is the evaluation of unwanted (direct/indirect) side effects on beneficial organisms. Therefore a resource efficient protocol in the laboratory is needed that helps to decide if the next step – field trials – is worth the efforts or not. In the EU supported project INBIOSOIL we improved such protocols by further incorporating the (field) ecology of the tested organisms. The four model arthropods we chose were: *Aphidoletes aphidimyza* (Insecta, Diptera, Cecidomyiidae), *Atheta coriaria* (Insecta, Coleoptera, Staphylinidae), *Orius majusculus* (Insecta, Hemiptera, Anthocoridae) and *Geolaelaps aculifer* (Acari, Mesostigmata, Laelapidae). These arthropods represent predators with different life cycles, prey and inhabit different strata of the plant and soil in the field.

In this study we used the *Metarhizium brunneum* (Ascomycota: Hypocreales) isolate BIPE-SCO5 (aka F52) to proof the feasibility of our protocol. We evaluated the direct (mortality) and indirect (fecundity) side effects on our model predatory arthropods and developed a decision tree that we recommend as a guideline for future side effect evaluations.

## Development of a QuEChERS-based extraction method for the determination of destruxins in potato plants by UHPLC-MS/MS

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Ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) with electrospray ionization has been proposed for the determination of fifteen natural destruxins (A, B, C, D, E, Ed, Ed1, A2, B2, D2, E2, Cl, DesmA, DesmB, and DH-A), secondary metabolites with insecticidal and phytotoxic activities produced by *Metarhizium species* fungus, which are being studied as biological agents in pest control. Therefore, procedures to control them in the food chain are required, starting with crops. As a consequence, in this study, a simple QuEChERS-based destruxin (dtx) extraction procedure has been developed and validated in four different parts of potato plant (tuber, root, stem and leaves) for the first time. For dtx A, the limits of detection obtained, ranged between 0.5 and 1.3 µg/kg, and for quantification, ranged between 1.7 and 4.2 µg/kg. Precision values were below 8.5%; and in all cases, recoveries were higher than 91%. Considering these complex matrices, good recoveries and precision values were obtained. Finally, the method has been successfully applied in potato samples inoculated by EAMa 01/58-Su strain, where dtxs A and B were detected and quantified. In all cases, dtx B concentration was higher than dtx A. To sum up, the results demonstrate the applicability of the proposed method to monitor these secondary metabolites in potato samples at trace level. Moreover, considering the advantages of the QuEChERS procedure, it could be a good option to use in routine analysis in future risk assessments of dtxs.

## Development of validated analytical methods for the assessment of *Metarhizium brunneum* metabolites in different matrices

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The entomopathogenic fungus *Metarhizium brunneum* (formerly *Metarhizium anisopliae* var. *anisopliae*), especially the *Metarhizium* production strain BIPESCO 5/F52 listed in the regulation (EU) No. 540/2011, plays an important role as a biological control agent (BCA). It is well known, that fungal BCAs are able to secrete a wide variety of metabolites, mostly products of the secondary metabolism. The main secondary metabolites produced by *Metarhizium* are destruxins (dtxs), cyclic hexadepsipeptides, which exhibit a wide variety of biological activities. There are concerns that the use of *Metarhizium* sp. as a BCA or its secondary metabolites entails risks to humans and the environment, one of the most important questions is whether dtxs enter the food chain. However, there is a lack of information concerning BCA risk assessment, the development of validated analytical assays for the monitoring of dtx traces in crops, is of great necessity.

A novel QuEChER-based sample preparation protocol for the extraction of destruxins from different crop matrices as strawberry and maize was developed and combined with a fast and selective UHPLC-QTOF-MS method for the quantitative determination of the main metabolites dtx A, B and E. The whole procedure allows destruxin trace analysis down to ppb range.



## The entomopathogenic fungi *Metarhizium brunneum* secretes active compounds toward *Rhynchophorus ferrugineus* larvae and adults

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is the most important pest for many species of palm trees in the Mediterranean basin. The restrictive legislation about the use of chemical products and the society preoccupation in environmental protection make necessary the search of alternatives for the red palm weevil control. One of the most promising alternatives is the use of entomopathogenic fungi and their extracts. The crude extract of EAMb 09/01-Su *Metarhizium brunneum* isolate was tested by ingestion on L2 and L5 instar larvae on two different types of artificial diet containing or not coconut fiber (CF). The mortality of L2 and L5 larvae fed with diet containing or not CF was clearly higher than the controls after 15 days of treatment, and their food intake and development were affected. On the other hand adults of *R. ferrugineus* were also exposed to *M. brunneum* extracts. Their mortality was 100% after 9 days of treatment but interestingly feeding reduction and changes in their behavior were observed, such as repellent effects and alterations in their movement capacity.

## **Crude extracts secreted by entomopathogenic mitosporic ascomycetes show potential for *Ceratitis capitata* (Widemann) (Diptera; Tephritidae) and *Drosophila suzukii* (Matsumura) (Diptera; Drosophilidae) control**

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The insecticidal activity of the crude extract of 12 entomopathogenic fungal strains that have been isolated from different ecosystems was studied against *C. capitata*, a key Mediterranean pest, and *D. suzukii*, a new invasive pest. Furthermore, the insecticidal activity of the different fractions (dialyzed, adialyzed, and protein fraction) of the more virulent crude extracts were studied. The crude extracts from the isolates EAMa 10/07-Su of *Metarhizium guizhouense* and EAPI 10/01-Fil of *Purpureocillium lilacinus* produced high *per os* mortalities (100 and 76.7%, 95.9 and 75%) against *C. capitata* and *D. suzukii* respectively. The dialyzed fraction of the EAMa 10/07-Su crude extract caused 100 and 59% mortality of *C. capitata* and *D. suzukii* adults respectively. Nevertheless, the adialyzed fraction of the crude extract of EAPI 10/01-Fil strain was the responsible of the insecticidal activity with a 43.3 and 98.3 % mortality of *C. capitata* and *D. suzukii* respectively. Additionally, the protein fraction of the EAPI 10/01-Fil crude extract presented high *per os* activity against *C. capitata* and *D. suzukii* with mortalities values of 83.3 and 70.7 % respectively. On the other hand, the protein fraction of the EAPI 10/01-Fil crude extract was demonstrated to be quite thermostable (at 60 °C for 2 h and 120 °C for 20 min) and photoresistant (2, 4, and 6 h to 1200 mW/m<sup>2</sup> of UV-B radiation). Our results indicate high potential of both the secondary metabolites produced by the EAMa 10/07-Su strain and the insecticidal proteins secreted by the strain EAPI 10/01-Fil for the control of *C. capitata* and *D. suzukii*. However more investigations are necessary to further purify of both extracts in order detect the molecules responsible for the activity.

## Discovery of novel viral pathogens through transcriptomic studies: Potential application in pest control

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Traditionally, insect virus discovery relies on the screening and identification of individuals showing clear symptoms of infection, limiting the discovery to highly pathogenic viruses. These methods are not appropriated for the discovery of viruses causing latent or covered infections as well as for those with not clear detrimental effect on the insect fitness. During the last decade, high throughput sequencing technologies have allowed the fast and cost-effective generation of genetic information of non-model organisms, becoming a very useful tool for the discovery of novel microbes and viruses from animals and plants. Here, we report the generation of the larval transcriptome from two different species of Lepidoptera and its use for the discovery of novel viral pathogens with potential applications in pest control. In a first study, we have used 454-FLX sequencing platform to generate the larval transcriptome of the *Spodoptera exigua* (beet armyworm), an important pest of vegetable and ornamental crops worldwide. Transcriptome mining revealed the presence of three novel viruses from the order *Picornavirales* and one novel Cypovirus that were further sequenced, isolated, and characterized. In a second study, the transcriptome of one of the most important pine pest, *Thaumetopoea pityocampa* (pine processionary moth) was generated using Illumina sequencing platform. Sequence mining rendered the complete sequence of two novel viruses, one Iflavirus and one Cypovirus. Although all these new viruses do not produce drastic effect, they can play an important role on the insect ecology and population dynamics opening new set of possibilities for pest control that will be discussed.

## Effect of *Spodoptera exigua* iflavirus co-inoculation on the insecticidal properties of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV)

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Baculovirus are natural pathogens that can produce epizootics in agricultural ecosystems. The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) can efficiently control this pest in sweet pepper crop systems of southern Spain. Studies on SeMNPV persistence in covertly infected adults revealed the presence of RNA viruses in insect colonies. These were identified as two iflaviruses (SeIV1 and SeIV2). Subsequent experiments indicated that SeMNPV and SeIV could be detected in both field and laboratory insect populations using PCR-based techniques. In this study, we evaluated the effect of SeIV1 co-infection on the insecticidal characteristics of SeMNPV. For this, feeding-drop bioassays were performed on second instar larvae by inoculating at the same time one of five SeMNPV inoculum concentrations and one of the following iflavirus treatments: i) SeIV1; ii) SeIV2; iii) SeIV1+SeIV2; and mock-infected control. Overall, iflavirus co-inoculation consistently reduced median lethal concentrations (LC50) for SeMNPV compared to larvae infected with SeMNPV alone. However, the speed of kill of SeMNPV was similar in the presence or absence of the iflaviruses. Adults survivors of a sublethal SeMNPV treatment were examined for covert infection and SeMNPV DNA were found to be present at a high prevalence in all treatments. In conclusion, simultaneous SeMNPV-SeIV co-infection resulted in improved pathogenicity of SeMNPV and did not alter the capacity of SeMNPV for vertical transmission.

## Value of mixtures of vertically and horizontally transmitted variant mixtures of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as the basis for biological insecticides

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The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) is a highly pathogenic host-specific virus that can be used to control its natural host, the beet armyworm *S. exigua* (Lepidoptera: Noctuidae). Recent studies have demonstrated that distinct genotypes of SeMNPV differ in their insecticidal properties and could be associated with different routes of transmission. In the present study we evaluated the value of using mixed genotypes of SeMNPV that had favorable insecticidal properties (Se-G25), or the capability for vertical transmission (Se-A11). Interestingly, mixed genotypes containing 25 and 75% of Se-G25 improved the pathogenicity of mixed genotype occlusion bodies (OBs) compared to Se-A11 genotype OBs, although no differences were observed in speed of kill or OB production (OBs/larva). The capacity to produce covert infections was evaluated in adult survivors of a sublethal dose. The Se-A11 genotype was the most efficient in producing sublethal infections (90% infection) and the mixture of 50% A11 + 50% G25 (87%), which were significantly more efficient than the Se-G25 (51%) genotype alone. The mixture of 25% A11 + 75% G25 and 75% A11 + 25% G25 resulted in 72% and 80% of adults positive for covert infection by qPCR, respectively. A greenhouse experiment revealed that sublethal infections in adult survivors increased with the proportion of Se-A11 genotype present in inoculum OBs. We conclude that the presence of vertically-transmitted variants is of limited interest for the development of biological insecticides based on SeMNPV.

## A novel co-occluded binary mixture of *Helicoverpa armigera* single nucleopolyhedrovirus (HearSNPV) genotypes from Spain is a highly effective insecticide

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The cotton bollworm, *Helicoverpa armigera*, is a major polyphagous pest. The *H. armigera* single nucleopolyhedrovirus (HearSNPV) can be an effective agent for control of this insect. In the present study, the genotypic diversity present in two Spanish HearSNPV isolates was evaluated with the aim of identifying mixtures of genotypes with improved insecticidal characteristics. Biological characterization of the different genotypes and co-occluded mixtures revealed that a co-occluded mixture of two genotypes in equal proportions named HearSP1B:LB6 showed the highest insecticidal activity, and therefore was selected as the basis for a bioinsecticide. In order to optimize the conditions for scaled virus production, the effects of several factors on the production of HearSP1B:LB6 occlusion bodies (OB) were evaluated. Results indicated that inoculation of newly molted L5 larvae with an LC80 concentration of OBs followed by rearing at 30 °C resulted in the highest production of OBs. Finally, the insecticidal efficacy and persistence of HearSP1B:LB6 OBs in greenhouse and open-field tomato crops in the Iberian Peninsula were compared to those of two commercial biorational insecticides. Application of HearSP1B:LB6 resulted in similar levels of crop protection to those provided by commercial insecticides against *H. armigera* in both types of crops. OBs showed good persistence in both crop settings, although persistence was markedly longer in greenhouse crops. We conclude that HearLB6:SP1B deserves to be registered as a biological insecticide for control of *H. armigera* on tomato crops in this region. The unique HearSP1B:LB6 binary genotypic mixture has been the subject of a patent application (P201430956).

## Occurrence and characterization of two Betabaculoviruses isolated from *Tuta absoluta* (Lepidoptera:Gelechiidae) in Colombia

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*Tuta absoluta* is a devastating invasive pest of tomato plants, causing losses of up to 100%. The high use of chemical pesticides for its control, the risk of contamination and the generation of resistance make it necessary to find control alternatives. An interesting tool is the use of entomopathogenic viruses of Baculoviridae family, which are insect-specific and do not generate environmental impacts. Despite its high potential, its use on *T. absoluta* larvae has been poorly studied. In this work, two Betabaculoviruses (granuloviruses) were isolated from 1186 *T. absoluta* larvae sampled in tomato crops in Colombia (virus occurrence: 0.3%), which were coded as VG012 and VG013 and morphological, biological and molecularly characterized. The granules of these isolates showed ovoid shape with an approximate size of 514 nm × 250 nm, with a single virion enclosing one nucleocapsid. Restriction endonuclease analysis showed the same pattern compared to *Phthorimaea operculella* granulovirus but different compared to the granulovirus isolated from *Tecia solanivora*, although they were classified in the same monophyletic group. The estimated genome size was 130 kbp. The mean lethal concentration (LC50) of viral isolates was  $1.6 \times 10^4$  and  $1.9 \times 10^4$  OBs/mL for VG012 and VG013 respectively and LC90 of  $7.0 \times 10^6$  and  $8.5 \times 10^6$  OBs/mL, with an average productivity of  $3.0 \times 10^{10}$  OBs per gram of tissue. Considering the high potential of using GV to control *T. absoluta*, the characterized isolates VG012 and VG013 are a promising tool for developing a new biopesticide.

## Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* in vegetable oil formulations

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Pollen beetles are a major pest in oilseed rape (OSR) throughout Europe. Substantial yield loss is caused by adult beetles feeding on pollen in spring during bud stage of inflorescences. There is currently no possibility to control pollen beetles in organic OSR cultivation and increasing resistance of pollen beetles to common insecticides further emphasizes the need for alternative control possibilities.

A laboratory screening of Swiss isolates of entomopathogenic fungi (EPF) revealed high potential of *Beauveria bassiana* isolates for biological pollen beetle control (Kuske et al. 2011). Field treatments showed comparable results regarding pollen beetle reduction, but did not result in significantly increased yield so far. To improve the efficacy of EPF applications, we explored synergistic interactions between EPFs and other natural compounds which have been shown to reduce pollen beetle abundance in OSR, such as stone dusts or vegetable oils (Dorn et al. 2013).

The combined application of *Beauveria bassiana* submerge spores and an oil formulation in a spray chamber showed an increase in Abbott corrected pollen beetle mortality of up to 40%, relative to single treatments. This was 12% more than expected by mere addition of the effects of single-treatments, suggesting a synergistic interaction between the EPF and the oil formulation. The latter seems to foster the fungus' reproductive phase since twice as much mycosed pollen beetles were recorded in the combined treatment.

First results of a field trial point in the same direction with increased yield and reduced pollen beetle abundance and damage of OSR plants after application of *Beauveria bassiana* submerge spores formulated in oil.



## Occurrence and characterization of entopathogenic fungi from the complex of sugarcane borer *Diatraea* spp. in Colombia

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Panela is a solid piece of unrefined sucrose obtained from evaporation of sugarcane juice, a very important industry and source of employment in Colombia. Panela yield depends of sucrose content in sugarcane, characteristic seriously affected by the presence of the stem borers complex, difficultly controlled by chemical insecticides. Biological control by using microorganisms is an interesting tool for controlling this pest which has been poor developed. The aim of this work was to isolate and characterize entomopathogenic fungi naturally infecting *Diatraea* spp. larvae in sugarcane crops for panela production in Colombia. Larvae were collected from three different production areas and maintained in quarantine until dead. A total of 445 larvae were collected and 23 presented fungal growth after dead. Nineteen fungal sporulated isolates from larvae corresponded to *Fusarium* sp., three to *Aspergillus* sp. and one to *Beauveria* sp. (Bc3j-22). Bc3j-22 isolate caused 83.3% and 73.3% of mortality of *Diatraea indiginella* and *Diatraea saccharalis* larvae, respectively. Sequence analysis of three genomic regions (rDNA 18S, ITS and EF1- $\alpha$ ) allowed to classify Bc3j-22 isolate as *Beauveria bassiana*. This isolate showed high susceptibility to ultraviolet radiation, with 33, 49 and 81% of inactivation after 30, 40 and 50 minutes of irradiation, respectively. Optimal conditions for fungal growth were 25°C and pH 7.0. Isolate Bc3j-22 showed high potential for controlling different species of the sugarcane borer and constitute the base for the development of a new biopesticide.

## Effect of UV-B radiation on germination, colony growth and virulence of *Metarhizium* sp. isolates against “Mediterranean fruit fly” *Ceratitis capitata* (Wiedemann) (Diptera; Tephritidae)

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UV-B is one of the main factors limiting the success of field applications of entomopathogenic fungi as aerial treatments targeting medfly adults. This work investigates the effect of UV-B radiation on the *Metarhizium* sp. conidia viability, germination, culturability, colony growth and virulence. Eighteen isolates of *Metarhizium* sp. were exposed to 1200 mW m<sup>-2</sup> for 2, 4 and 6 hours. After that, all isolates showed a significant decrease in germination, colony growth and culturability, which resulted in a conidia inactivation after 6 hours of exposure, indicating a quite much higher susceptibility of this fungal genus than the one observed in *Beauveria bassiana* in previous experiments. Beside the effect of UV-B radiation on virulence of the *Metarhizium brunneum* EAMa 01/58-Su isolate was evaluated. For that, conidia were irradiated with 1200 mW m<sup>-2</sup> during 6 hours before and after medfly adults' exposition, with final dosage of  $1.7 \times 10^6$  conidia per insect. In the former treatment, *C. capitata* adult mortality was significantly reduced (from 100% for non-irradiated conidia to 91.43% for irradiated conidia), and average survival time was increased (from 4.57 days for non-irradiated conidia to 5.91 days for irradiated conidia). However, such an effect was not observed in the later treatment (UV-B exposition after treatment). Even if a high decrease in viability was observed (10 and 70% of viability for irradiated and non-irradiated conidia, respectively). A further bioassay was developed to inspect whether a dose dependent UV-B effect on virulence could exist. For that, adult flies were exposed to a range of  $10^4$  conidia/ml (1.6 conidia/insect) to  $10^8$  conidia/ml ( $1.6 \times 10^4$  conidia/insect) and then irradiated at 1200 mW m<sup>-2</sup> and 6 hour, which allow detecting a LC50 reduction from  $5.7 \times 10^7$  to  $4.2 \times 10^7$  conidia/ml for irradiated and non-irradiated conidia respectively.

## Can soil application of *Metarhizium brunneum* (Metsch.) Sorok. (Hypocreales: Clavicipitaceae) toward of immature stages control the olive fruit fly *Bactrocera oleae*?

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Entomopathogenic fungi (EF) have an important role in IPM programs as an alternative to conventional chemical control for biological control of tephritid flies. The strategic options in using entomopathogenic fungi in medfly control are aerial applications and autodissemination targeting adults. However the current work explores the use of EF in soil applications targeting pupariating larvae and puparia. Before the field application of the fungus, the virulence of the strain EAMa 01/58-Su was examined against *B. oleae* adults and pupae under laboratory conditions. When *B. oleae* adults were sprayed with a conidial suspension, the strain EAMa 01/58-Su caused 95.2% of total mortality with 91.8% of fungal outgrowth, mean lethal time (LT<sub>50</sub>) of 6.2 days, and mean lethal concentration (LC<sub>50</sub>) of  $7 \times 10^6$  conidia per milliliter. In soil treatments, *B. oleae* pupae mortality reached 68.3 % of mortality with 38.3% showing fungal outgrowth and LC<sub>50</sub> of  $1 \times 10^7$  conidia per milliliter. Then, the strain was applied in the field under trees canopy during 3 years. The fungus persisted in the soil in all years of application during the development of the experiment. The fungal concentrations in the soil after 4 months of treatment were  $6.1 \times 10^2$ ,  $0.8 \times 10^2$ , and  $1.1 \times 10^3$  conidia per g of soil in 2011, 2013, and 2014 respectively. In all years of treatment, the *B. oleae* population density that emerged from the treated plots was almost 70% lower than that from the control ones. These results indicate that *M. brunneum* EAMa 01/58-Su strain is a promising tool to be used in an integrated pest management olive fruit fly management strategy.

## **Collapse of an isolated outbreak population of *Dendrolimus pini* caused by a very high infestation of *Beauveria bassiana***

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Pine-tree lappet moth, *Dendrolimus pini* is a pest known in some regions in Europe (e.g. Germany, Poland) where it can completely defoliate pine trees. In Croatia this pest is sometimes present in the continental region but not in high abundance, so no damage has been reported up to now. In the Mediterranean region the pest has never been recorded until autumn 2014, as an unexpected total defoliation occurred in several localities around Skradin on Aleppo pine, *Pinus halepensis*. After overwintering as larvae in soil they climb in the spring to tree crowns where they feed on needles but also on the bark of young shoots causing serious damages on pine trees. In winter, larvae have been counted per square meter of soil surface. On average, more than 10 larvae per square meter were found which is considered as critical number, but it was surprising that they were all killed by *Beauveria* cf. *bassiana*. Unusual weather conditions with high precipitation is the probable reason for unusual *D. pini* occurrence on *P. halepensis*, but the same conditions were also very favorable for unusually high infestation of *B. bassiana* which caused the pest population decline. Such rapid population break-down is in Croatia known only in gypsy moth populations (*Lymantria dispar*) caused by nuclear polyhedrosis virus (NPV) or recently recorded *Entomophaga maimaiga*.

## **Efficacy assessment of *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae), *Metarhizium brunneum* (Hypocreales: Clavicipitaceae), and chemical insecticides for *Diabrotica virgifera virgifera* larval management under real farm conditions**

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A two-year field study using a blend of entomopathogens in conjunction with chemical insecticides was carried out to determine to which extent they affect indigenous western corn rootworm (WCR; *Diabrotica virgifera virgifera* LeConte) survival, maize root damages, and grain yield. The products applied consisted of formerly in Austria registered clothianidin dressed maize seeds (Poncho<sup>®</sup>), the currently approved products Belem<sup>®</sup> (active ingredient cypermethrin) and dianem<sup>®</sup> (nematodes of the species *Heterorhabditis bacteriophora*), and of two innovative products based on the insect pathogenic fungus *Metarhizium brunneum*. The experiments were conducted in two (2013) and four (2014) naturally heavily WCR infested maize fields in the southeast of Styria, Austria. The lowest number of WCR adults was found, when the two biological control agents were combined with clothianidin dressed maize seeds.

Apart from this promising result, further IPM management strategies and operational necessities aiming to bring WCR population below its stipulated economic threshold are discussed.

## Encapsulation of *Metarhizium brunneum* as basis for an attract and kill strategy within the EU project INBIOSOIL

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Several soil-borne herbivorous insect pests such as wireworms and western corn rootworm larvae cause tremendous losses in different crops like potato and maize. The INBIOSOIL project aims at developing innovative beads containing entomopathogenic fungi (EPFs) as well as novel synergistic co-formulations of EPFs with efficacy enhancing agents to control soil-borne insect pests.

We developed “attract and kill” formulations that are based on biopolymer beads or granules combining CO<sub>2</sub>-releasing baker’s yeast, an effective attractant compound for various insect pests, with *M. brunneum* asexual spores BIPESCO5 or ART 2825 as a kill components. Both components were encapsulated separately as well as co-encapsulated to implement an “attract and kill” – strategy to control wireworms and other soil-borne insect pests in agricultural fields.

Here we report on the encapsulation and drying of novel formulations from lab to technical scale. Furthermore data will be presented on capsule characteristics such as re-swelling of dried capsules, on the influence of nutrients on sporulation and of fillers on aw value, survival of dried encapsulated asexual spores and shelf life and virulence against *Tenebrio molitor* larvae. Field tests of the collaborating Vidal group demonstrated that selected novel formulations are capable to significantly reduce the wireworm populations in the field.

## Attract and kill kept simple: Fungus colonized barley kernels in cover crops for microbial wireworm control

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The multiannual belowground development of wireworms is characterized by a sequence of active and inactive phases. During the former, wireworms are found feeding and foraging in upper soil levels. It follows that a control measure against wireworms may hit its target only when applied during active phases of the pest insect.

Wireworms typically show two peaks of activity per year, one in springtime and a second one in late summer. Considering this seasonal activity, we developed a simple “attract and kill” approach, which was tested in a semi-field pot experiment. *Metarhizium*-colonized, sterile barley kernels were applied into the upper substrate layers of the pots in late summer, immediately before sowing of summer oat as a cover crop. The CO<sub>2</sub>-emitting roots of the oat seedlings should attract artificially released late instar larvae of *Agriotes obscurus* into fungus infested upper substrate layers during their late summer activity peak. After hibernation in deeper substrate layers, wireworms were assumed to migrate upwards again during springtime, facing the risk of getting infected a second time, right before planting of a main crop.

Establishment of the fungus was estimated by counting colony forming units in substrate samples. The efficacy of the control measure was evaluated by assessing the mortality of recaptured wireworms from the pots. Preliminary results proved establishment of the fungus in the substrate after a few weeks and showed up to 70% of wireworm mortality, depending on fungus inoculum dose applied. The simple yet promising biocontrol strategy against wireworms is currently tested in a follow-up experiment under field conditions.

## The economics and environmental benefits and costs of biological control of western corn rootworm *Diabrotica virgifera virgifera* and wireworm *Agriotes spp.* in maize and potatoes for selected countries in Europe

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The application of biological control in Europe for reducing yield losses caused by the western corn rootworm (WCR; *Diabrotica virgifera virgifera*) and wireworms (*Agriotes spp.*) in maize and potatoes has economic and environmental implications. This study quantifies important economic and environmental impacts of biological control agents: entomopathogenic fungi, entomopathogenic nematodes, and semiochemicals (based on on-going research) on their efficacy to control WCR and wireworms in Austria, Germany, and Switzerland. Parameters of the supply and demand function of the aforementioned staple crops are analyzed using data from EUROSTAT and field trials. Environmental impacts of these biological control agents are estimated using the environmental impact quotient (EIQ) as well as irreversible standards while adoption by farmers are derived using available secondary literature.

Preliminary results suggest that the use of these biological control agents results in a reduction in the rate of pesticide application, which reduces environmental externalities in European agricultural production. However, the effectiveness of aforementioned biological agents to control WCR and wireworm depends on factors ranging from climatic conditions to pest density. Adoption of an efficacious biological control may provide an environmental-friendly crop protection alternative with benefits to society over a 50-year period of ca. €600 million and €120 million for potatoes and maize, respectively. Finally, while the adoption of such biological control measures in a number of selected European countries could result in welfare gains, this may be moderate given the cost and level of adoption associated with biological control strategies.



## Evaluation of botanical formulations (Neem) in an “Attract-and-Kill”- strategy under field and laboratory conditions targeting wireworms

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Wireworms, the larvae of click beetles, are an important taxon of soil dwelling pests. They attack the subterranean parts of a wide range of crop plants and have recently evolved into a serious problem in many cultures, mainly due to the lack of specific control options. The joint project ATTRACT focusses on the development of an innovate technology, taking advantage of the fact that wireworms orientate towards their host plants via detecting root CO<sub>2</sub>-exudates. Providing an alternative CO<sub>2</sub>-source (“Attract”-component) combined with a “Kill”-component may be the key for an efficient wireworm control, especially since insecticides in susceptible crops like potatoes and maize are on the brink of being phased out. Baker’s yeast, acting as CO<sub>2</sub>-source was combined with nutrient additives and encapsulated in Ca-Alginate (“Attract”-formulation) as well as NeemAzal® technical (“Kill”-agent). Experiments performed on a laboratory scale have shown that “Attract-and-Kill”-formulations affect wireworm vitality and have the potential to kill them. These preliminary results have been confirmed in field experiments conducted at four sites in Lower Saxony. The damage level of wireworm infestations in organically managed potato fields were significantly (although not always) reduced by the application of “Attract-and-Kill”-formulations. Our current aim is to improve present formulations and enhance the attractiveness of the capsules by adding phagostimulants, aiming at arresting the wireworm towards the capsules and provoking a bite reflex. The development of a refined “Attract-and-Kill”-strategy to control wireworm infestations will contribute to reduced pesticide inputs in sustainable agricultural-horticultural systems and offers potential savings for growers.

## An “Attract & Kill” approach in potato to reduce wireworm tuber damage

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Wireworms (Coleoptera: Elateridae) are polyphagous root herbivores causing significant damage to multiple crops. Several control options may be available for farmers, including agronomical, chemical or biological measurements; yet the efficacy of these measures is rather inconsistent. The implementation of the EC regulation 1107/2009 and Directive 2009/128/EC supporting integrated pest management (IPM) approaches promoting sustainable agricultural production systems opens new opportunities for biological control strategies. However, new and efficient control strategies are required to meet these requirements.

Entomopathogenic fungi (EPF), such as *Beauveria* sp. or *Metharizium* sp., are potential agents, whereas the latter is more efficient in terms of rhizosphere competence, essential for controlling wireworms as they represent a cryptic living and prolonged larval stage with 3–5 years.

In the last two years we aimed at implementing an “Attract & Kill” strategy under field conditions in potato (*Solanum tuberosum*) cultures. The studies were performed under on-farm conditions in Germany, Lower Saxony. The concept focusses on targeted trapping (“Attract”-component) by modification of random movements during host location and a coincidentally contact with a killing agent (“Kill”-component) at the same spot. For the “Attract”-component an artificial CO<sub>2</sub>-emitting source is used, whereas for the “Kill”-component the *M. brunneum* isolate (ART2825) is used. Both components were applied on a formulated basis for stabilizing-, improved shelf-life-, and application reasons. Tuber damage was significantly reduced, however, several constraints need to be taken into account. This study is funded by the EU Project INBIOSOIL (Innovative biological products for soil pest control; <http://inbiosoil.uni-goettingen.de/>).

## Screening of bacteria isolated from *Galleria mellonella* (Lepidoptera: Pyralidae) larvae infected with novel entomopathogenic nematodes

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Major bacterial species developing on *Galleria mellonella* larvae infected with different entomopathogenic nematodes (EPN) were isolated by plating serial dilutions of insect haemolymph. On average 1, and at most 2 different bacterial isolates, were collected from each insect-EPN association. Nematodes involved in the research were collected from soil in different areas of Italy and Algeria, and were previously identified as *Steinernema* and *Heterorhabditis* species.

Bacterial cultures were screened against insects in different orders, including Lepidoptera (*Malacosoma neustria* and *Lymantria dispar*) and Diptera (*Musca domestica* and *Ceratitis capitata*). In total 80 bacterial isolates were assayed by incorporating either the cell fractions or the culture supernatants in comparative experiments involving reference strains of *Bacillus thuringiensis*, *Photorhabdus luminescens* and *Xenorhabdus nematophila*. The majority of bacterial isolates were toxic to lepidopteran larvae, while around 20 of them showed significant toxicity against fly larvae and/or adults. Bacteria showing the highest mortality levels (>50 %) on flies were submitted to identification through 16S rRNA gene sequencing, which highlighted the effectiveness of species belonging to different genera including *Serratia*, *Pseudomonas*, *Alcaligenes*, and *Stenotrophomonas*.

## Analysis of the bacterial community present in the insect pest *Lymantria dispar* during the life cycle of insect

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Microbiome of larval midgut community is dynamic in time and space. Presence of microorganisms in host can be influenced by ecological relationships among the gut microorganisms. The objective of this work was to characterize the bacterial midgut community of the important forest insect pest *L. dispar* and to monitor changes in diversity during the life cycle and within individual larvae. They were first screened based on a) culture dependent approach; b) culture independent method with PacBio (Single molecule real time sequencing (SMRT)) technology by sequencing full length 16S genes of bacterial gut communities. Results showed relatively simple composition of the gypsy moth midgut community when conducted with culture dependent approach. Diversity of community significantly increased when culture independent method was applied. PacBio sequencing approach lead to better classification at species level and allowed to identify OTU's that are consistently detected or persistent in all life stages of larvae – core community species. We observed differences between individual larvae from the same time point and structural changes of diversity in bacterial communities over the season of larval development. This project is conducted within the frame of a SCIEX project with ETH Zürich and the University of Daugavpils as partners.

## What are the features that plant-beneficial pseudomonads require to become insect pathogens?

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The *Pseudomonas fluorescens* group harbors many root-associated plant-beneficial bacteria that suppress soil-borne fungal diseases and promote plant growth. These bacteria cluster in three phylogenetic subgroups, the *P. fluorescens* subgroup, the *P. chlororaphis* subgroup and the *P. corrugata* subgroup. Remarkably, strains of the *P. chlororaphis* subgroup were shown to have oral insecticidal activity, which is associated with the Fit insect toxin and unknown GacA-regulated traits. To date insecticidal activity has only been studied in a few strains. To obtain an overview on occurrence and frequency of this trait throughout all three subgroups we selected 25 strains and tested them for injectable and oral insecticidal activity. Whereas none of the strains of the *P. corrugata* subgroup caused lethal infections, we discovered that in addition to the *P. chlororaphis* subgroup also the strains of the *P. fluorescens* subgroup are able to kill insect larvae. However, these strains, which do not have the *Fit* genes, were less virulent than the strains of the *P. chlororaphis* subgroup. Still, the *P. fluorescens* and *P. chlororaphis* subgroups might share factors that contribute to their insecticidal activity. By next generation sequencing and subsequent comparative genomics we searched for genes that are common and unique to insecticidal strains. In this way we identified several candidate genes, e.g. a specific chitinase, which we further studied for their impact during the infection of insect larvae. Thus, we provide not only an extensive survey on insecticidal activity in the *P. fluorescens* group, but also try to untangle the mechanisms underlying this trait.

## Influence of multi-year *Bacillus thuringiensis* subsp. *israelensis* treatments on the abundance of *B. cereus* group populations in Swedish riparian wetland soils

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*Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is a soil-born bacterium affiliated to the *B. cereus* group (*Bcg*, a group including the pathogens *B. cereus*, *B. thuringiensis*, and *B. anthracis*) and used in biocontrol products against nematoceran larvae. However, knowledge is limited on how long-term *Bti* application affects the structure of indigenous *Bcg* communities as well as the overall abundance of *Bti*. Based on new primers, group-specific quantitative PCR assays for *Bcg* and *Bti* in environmental samples were developed. On six occasions during the vegetation season, soil samples were collected in forest swamps and wet meadows which had been treated with *Bti* during the preceding 11 years as well as in untreated forest swamps, wet meadows and well-drained forests. Abundances of *Bcg* and *Bti* varied among the different sampling occasions. The highest abundance of *Bcg* was found in forest swamps and differed significantly from wet meadows while no such variation was found for the *Bti* abundance. The *Bti* treatments had no effect on the overall *Bcg* abundance whereas for *Bti*, the abundances were significantly higher in the treated than in the untreated sites. However, abundances of *Bti* and *Bcg* didn't correlate with the number of *Bti* applications, indicating that *Bti* use influenced abundances of *Bti* on the short term while on the long term the number of treatments had only a limited effect. The findings illustrate the value of such investigations for understanding the ecology of *Bti* applications, which can facilitate environmental risk assessment as well as approval of biological control agents.

## Studies on *Pseudomonas entomophila*: a novel entomopathogenic bacterium

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*Pseudomonas entomophila* was recently identified to be the only pseudomonad that naturally infects and induces mortality of *Drosophila melanogaster* upon ingestion (Vodovar *et al.*, PNAS 2005; 102:11414–19). Complete sequencing of the 5.9-Mb *P. entomophila* genome revealed putative virulence factors involved in entomopathogenicity but experimental evidence for most of them is still lacking (Vodovar *et al.*, Nature Biotech. 2006; 24: 673–679). Recently we demonstrated that *P. entomophila* produces HCN *in vitro* placing it among the few cyanogenic bacteria (Ryall *et al.* Lett. Appl. Microbiol. 2009; 49: 131–135). In order to investigate a possible implication of HCN in entomopathogenicity exerted by *P. entomophila*, a mutant impaired in HCN production was constructed and compared to wild type strain in bioassays using *Drosophila melanogaster* but no significant difference in mortality was observed. Therefore we conclude that HCN production does not promote entomopathogenicity in *P. entomophila*. Regarding the ecology and insect host range of *P. entomophila* few data are available so far. In order to facilitate studies aiming to identify insect hosts of *P. entomophila* as well as its distribution in diverse habitats we developed a PCR-based method to detect *P. entomophila* in environmental samples. (Papagiannoulis *et al.* Lett. Appl. Microbiol. 2010; 50(3): 241–245). Using our PCR method we screened specimens of *Bactrocera oleae* (olive fruit fly) captivated in natural habitats from all over Mediterranean region but *P. entomophila* was not detected. Moreover putative entomopathogenicity exerted by *P. entomophila* against major agricultural pests in Mediterranean region such as *Ceratitis capitata* (adults).

## Overwinter transmission of CpGV on infected diapausing larvae

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Different Granuloviruses are used as biological control against insect pests. Among them, CpGV is widely sprayed in apple orchards to limit codling moth proliferation. Resistance to CpGV-M has developed in some European orchards. A better understanding of the biology of the virus should allow a better rational use leading to increased durability of this control method.

Transmission of CpGV from one year to the next in natural conditions relies in the presence of reservoirs in orchards, allowing young larvae of the first generation in the spring to enter in contact with the virus. A possible way is the survival over winter of infected larvae that could die upon metamorphosis or yield infected adults able to vertically transmit the virus. We have explored using qPCR the level of infection in overwintering five instar codling moth larvae that survive to the late summer treatments in commercial orchards. About 8% of such larvae were found to be positive for virus.



## A novel mode of resistance of codling moth against *Cydia pomonella* granulovirus with a dominant and autosomal inheritance pattern

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The codling moth (CM, *Cydia pomonella*) is the major insect pest in most pome fruit production areas. *Cydia pomonella* granulovirus (CpGV, *Baculoviridae*) are worldwide used as biocontrol agents of CM. CpGV products have the advantage over the application of chemical insecticides because of their narrow host range and environmental safety. CpGV is registered as biological control agents in both, organic and integrated fruit production. In 2005, the first case of resistance of CM populations to CpGV-M, the so-called Mexican isolate, was observed in Germany and France. Since then, resistant CM populations have been identified in 38 orchards in seven European countries. For many of these tested CM populations, the resistance could be traced back to a single, dominant allele that is linked to the sex chromosome Z. Current resistance management strategies are based on the application of improved products, containing CpGV isolates that are breaking the resistance. However, one CM field population showed even resistance to most of these resistance breaking isolates, suggesting a second mode of resistance. To obtain a genetically homogenous line, this field population was reared by mass crossing under virus pressure for five generations. The obtained lines CpR5M and CpR5S were used for single pair crossing with a sensitive laboratory strain CpS to elucidate the mode of this new inheritance. The results of the reciprocal crossing experiments indicate a new dominant and autosomal inheritance pattern.

In addition bioassays with different instars (L1-L5) of the CpR5M strain were done to investigate, if the new virus resistance is related to specific larval stages.

## Analysis of the genome sequence of an *Agrotis segetum* granulovirus

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Larvae of *Agrotis segetum*, the common cutworm (Denis & Schiffermüller) and *A. ipsilon*, the black or greasy cutworm (Hufnagel) are polyphagous pests, which cause severe damage on roots and stems of numerous cultivated plants. They are of significant economic importance in Africa, Europe and North America. Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *Agrotis segetum* nucleopolyhedrovirus B (AgseNPV-B), *Agrotis ipsilon* nucleopolyhedrovirus (AgipNPV), and *Agrotis segetum* granulovirus (AgseGV), belonging to the genera *Alpha-* and *Betabaculovirus*, respectively, are known to infect larvae of the lepidopteran pests *A. segetum* and *A. ipsilon* and may be used as efficient and highly specific biocontrol agents. Infections with these baculoviruses were characterized in dose-response bioassays and with molecular biological methods. Mixed infection of single *A. segetum* larvae with AgseNPV and AgseGV have been reported. As a further step to improve our understanding of the potential molecular interaction of AgseNPV and AgseGV in mixed infections, the genome of AgseGV was completely sequenced. The genome sequence of AgseGV will be presented and compared to sequences of other isolates from Europe and China.

## Determination of reapplication frequency required for the *Cryptophlebia leucotreta* granulovirus: a factor of rate of virus breakdown and larval behaviour

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The *Cryptophlebia leucotreta* granulovirus (CrLeGV) has now been used commercially for the control of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), on citrus in southern Africa for more than 10 years. Farmers need improved clarity on frequency of application required. Consequently, the rate of breakdown in the field, determined to be mainly due to ultraviolet radiation, was determined. This was done at regular intervals from zero to 28 days after application, by washing occlusion bodies off Navel orange fruit from both the northern (sunny) and southern aspects of trees and conducting dose-response bioassays against neonate *T. leucotreta* larvae in the laboratory. At 21 days after application, LD50 of CrLeGV recovered from the northern sides of trees was 15 times higher than from the southern sides of trees. By 28 days after application, virulence of CrLeGV on the northern sides of trees was indeterminable, whereas on the southern sides of trees, there was still a clear dose response. In a separate study, neonate *T. leucotreta* larvae were placed onto molasses-treated and water only-treated Navel oranges and behaviour filmed, recorded and analysed. Although distance traversed by the larvae on the fruit surface was significantly greater without molasses, larvae on molasses-treated fruit fed more actively, thus increasing the probability of viral ingestion before penetration. Combining the rate of breakdown of virus and larval feeding behaviour with and without molasses, a model is proposed for estimating necessary time intervals between CrLeGV sprays.

## Novel virus discovery in entomopathogenic nematodes

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Viruses have been largely viewed as pathogens; nonetheless, even if most of the studied viruses are detrimental to their hosts, some of them have been reported to be also beneficial or symptomless. They are ubiquitous, and have been described infecting almost all types of organisms, from other viruses and bacteria, to animals, plants, fungi, or even protozoa. Surprisingly, little is known about viruses which naturally infect nematodes, even if they are the most abundant animals on earth. Nevertheless, RNA viruses infecting *Caenorhabditis* species and the soybean cyst nematode have been recently detected thanks to next generation sequencing technologies.

Many viruses associated with persistent and symptomless infections are known to have double-stranded RNA (dsRNA) genomes. The presence of dsRNA molecules of sizes ranging from 1 to 14 kbp have been used as indicator of virus infection in plants and fungi. In this work a collection of entomopathogenic nematodes was screened for the presence of dsRNA virus-like molecules. At the present time a total of 58 strains belonging to 22 different species were analyzed. Two dsRNA virus-like molecules of approximately 2.4 and 2.3 kbp were detected infecting one of the analyzed species, *Steinernema huense*. These molecules correspond to two different viruses apparently asymptomatic, a new member of *Partitiviridae* family, *Steinernema huense* Virus 1 (ShV1), and a new member of *Narnaviridae* family, *Steinernema huense* Narnavirus (ShN). These findings open a new field of research that could help to better understand the biology and ecology of entomopathogenic nematodes, and for instance, to improve their use as biocontrol agents.

## Low prevalence and strong competition of entomopathogenic nematodes in Swiss agricultural soils imply a need for an augmentation biological control approach for effective crop protection

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In the context of a research consortium that studies beneficial microorganisms and their use in agriculture, we investigated factors that determine the efficacy of entomopathogenic nematodes (EPN) as biocontrol organisms against soil dwelling insects in maize and wheat. We investigated how crop type (wheat, maize and grass) and management practices (conventional, organic, biodynamic and control) shape the natural occurrence of EPN and key soil food web members in a long-term Swiss field trial (DOK). The randomized plots were sampled twice (April and October 2013, 96 plots per event). Traditional (insect bait) and molecular (real time qPCR) techniques were combined to evaluate EPN abundance and selected members of the soil food web (free living nematodes, nematophagous fungi and ectoparasitic bacteria). Studies on the microbial biomass (Cmic and Nmic) and soil properties were linked with the information on the EPN food webs, including spacial association analyses (SADIE). Molecular analysis revealed six species of EPN, three free-living nematodes (FLN) and four nematophagous fungi (NF). Management practices did not affect the natural occurrence of soil food web members ( $P > 0.05$ ). However, crop type significantly shaped the nematodes (EPN and FLN) occurrence as NF, with significantly higher quantities in wheat plots ( $P < 0.05$ ). EPN represented 12.5% of the nematodes recovered from baiting insect cadavers, whereas *Oscheius* spp (FLN) represented 87.54% showing strong competition between nematode guilds. Overall, very few EPN were found in the soils, and they were strongly outcompeted implying that an augmentative approach is highly recommended.

## Development of vegetable crops complex protection from *Tuta absoluta* in greenhouse conditions

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A new invasive insect pest, the South American tomato moth, *Tuta absoluta* (Povolny) (Lepidoptera: Gelechiidae) is causing the great damage (80–90%) of vegetable crops in Georgian greenhouse farmings. The complex protection of tomato plants from the pest insect was elaborated by using of biological and biotechnical means. The experiments and observations at the stationary plot (200–250 sq.m greenhouse, village Misaktsieli, Mtskheta region) planted by Dutch tomato cultivar “Rose pinkinum” and cucumber cultivar “Georgian Mukhranuli”, were carried out. The biotechnical mean – insect yellow traps for pests monitoring were placed in greenhouse. Towards *T. absoluta* the new generation of highly efficient biological formulations – Proclaim (spending rate of 0.4 kg/ha), Vertamectin Forte WG (spending rate of 0.16 kg/ha) and entomopathogenic nematodes – “Geo-nema”, *Steinernema feltiae* Georgian strains (spending rate 25 million IJs/ha) were tested. The treatment of vegetable plants with mentioned solutions was carry out two times. The biological effectiveness (B.E.) of formulations – Proclaim and Vertamectin Forte WG – 71.8–82.7% and 91.2–72.5%, respectively was established. The B.E. of nematode suspension was 53.2–54.1%, while the B.E. of chemical product – Aktar (etalon) 91.1–97.8% has been achieved. The preliminary data in laboratory and natural conditions, on the based of tested environmentally safe, biological and biotechnical means, will be developed for *T. absoluta* complex control in greenhouse farms. The elaborated approach for complex protection will take the determined and important place in the integrated pest management system (IPM) of vegetable crops from pest organisms.

## Control of plum sawflies (*Hoplocampa flava* and *Hoplocampa minuta*) by three entomopathogenic nematodes

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Plum sawflies are among the most damaging pests of plum. Current control strategy implies insecticide application. No other measures are reported for commercial application in plum sawflies management. Considering high efficacy of entomopathogenic nematodes (EPN) against soil dwelling stages of some pests, these strategies were evaluated against plum sawflies. Three species of EPN *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were tested in laboratory and field conditions to assess effectiveness against larval and adult stages of plum sawflies. Laboratory tests were performed in petry dishes filled with sterile sandy soils. 500, 1000 and 2000 infective juveniles (IJ) were applied against 10 sawflies larvae per petry dish. Age of larvae was considering by testing larvae 1 day after they exit last infested fruit, and 10, 20 and 40 days older larvae. In treatments with larvae 1 day after they exit last infested fruit mortality was 92–100 %, whereas no mortality was observed for older larvae. To set up field test against adult sawflies plum trees were covered by insect proof net. Same nematode species were applied to soil surface at the dosage of 0.5 million IJ per square meter before anticipated day of adult emergence. In 2013 reduction in fruits infestation was 90–98%, whereas in 2014 30–90%. Lower efficacy from 2014 is explained by late application of EPNs. Forecasting of adult emergence is essential for high control. EPN are highly effective against sawflies larvae before they make the cocoon and against adult sawflies.

## Characterization of heat shock protein 90 gene in native entomopathogenic nematodes from Southern Italy

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Heat shock protein 90 (*hsp90*) family is an evolutionarily conserved, abundant cytosolic protein that has been shown to be involved in chaperone function, cell cycle control. It also plays an essential role in response to stress conditions such as heat or cold shock and presence of heavy metals. The partial *hsp90* genes of *Steinernema apuliae*, *S. ichnusae* and *Heterorhabditis bacteriophora* were amplified by using degenerate primers and sequenced. Expression analysis of *hsp90* gene will be determined at different temperatures by qRT-PCR.



## Nematode fauna of *Rhynchophorus ferrugineus* (Oliver) in Southern Italy

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This study reports a survey conducted to evaluate the nematode fauna associated to the Red Palm Weevil *Rhynchophorus ferrugineus* (RPW) in Southern Italy, in order to select potential biological control agents. Several infested *Phoenix canariensis* exemplars were sampled in different locations, particularly in the Apulia region, collecting each stage of RPW. Insects were dissected and nematodes were isolated using the water bait method. Nematodes individuals were identified by morphological and molecular traits. Results reveal that the RPW shows a own nematode fauna, including some new species.

## Entomopathogenic nematodes for the control of oak processionary moth in the UK

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The oak processionary moth (OPM), *Thaumetopoea processionea* is native to central and southern Europe but has spread north since the mid-1900s and was first confirmed in west London in 2006. OPM can defoliate oak trees and the older larvae have hairs that can cause serious skin and eye irritation and respiratory problems in humans and certain animals. OPM has established in several London boroughs, where it is now considered that eradication with currently approved control measures is not feasible. Current management policy is focussed on containment and minimising spread, by controlling numbers in known infested areas, and on eradication in any new outbreaks and on infested imported trees. Two insecticides and one biopesticide are currently approved in the UK for OPM control on amenity trees. The biopesticide *Bacillus thuringiensis* (Bt) is the preferred treatment as it kills only caterpillars and thus has less side-effects on non-target insects. However, Bt is only effective against young caterpillars and is washed off by heavy rain so application timing is critical. Spraying Bt or any insecticide to the entire tree canopy is a further challenge. Laboratory tests on alternative, integrated control methods have highlighted the potential of biological control with entomopathogenic nematodes. Results will be presented of experiments investigating the efficacy of different strains and rates of *Steinernema feltiae* against OPM larvae.



**ABSTRACTS  
POSTER  
PRESENTATIONS**

## ***Metarhizium anisopliae* or...? – a graphical approach to shed light on a genus**

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The first mention of *Metarhizium anisopliae* was in 1883, when Sorokin transferred *Entomophthora anisopliae* Metchnikoff (1879) to the new genus *Metarhizium* using the species name that Metchnikoff originally used for the insect disease “green muscardine”. 132 years later, the name *M. anisopliae* is still in use, is one of the most widely used names for entomopathogenic fungi and is frequently found in research literature. What many of us tend to forget is that today there are 35 additional species within the genus *Metarhizium*! This should serve as a reminder that the taxonomy of *Metarhizium* has been in a continual state of flux since its original description. In the first 100 years the genus was expanded to include 10 species; however, a critical review in 1976 reduced the genus to only two morphologically distinct species. With the advent of molecular tools for identification again the genus has grown as subspecific taxa have been raised to species status and cryptic species have been resolved. Finally with the implementation of the “one fungus one name” rule (2013) many teleomorphic species have been transferred from the genera *Metacordyceps*, *Nomuraea* and *Chamaeleomyces* to *Metarhizium* – resulting in a total of 36 recognized *Metarhizium* species.

Conflicting species concepts among taxonomists have made it especially challenging for researchers to keep track of what name pertains to which species at what time. Over the years species and subspecific names have come and gone, and come again. With this graphical overview we provide a detailed review of the history of the genus *Metarhizium*, its changes and the most current taxonomy.

## Group-I intron based strain-specific diagnosis of entomopathogenic *Lecanicillium* fungi for aphid biocontrol

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Mitosporic fungal entomopathogens of the genus *Lecanicillium* (Ascomycota; Hypocreales) are of particular interest as biological control agents for phloem-sucking plant pests including aphids. Bioprospection for these fungi in Argentina has given rise to a set of isolates from a wide range of hosts. Molecular taxonomic analysis using a Multilocus Sequence Typing approach has confirmed previous morphology based assignments to the genus *Lecanicillium*. For further research in *Lecanicillium* fungi and development of a bio-aphicide, a PCR-based diagnostic tool allowing the reliable and fast differentiation of strain CEP419 from the further Argentine isolates was highly solicited. Screening of this full set of fungi and additional reference strains from other geographic origins for the presence or absence of self-splicing group-I introns disrupting the 18S and 28S rRNA encoding genes at previously identified intron insertion hot-spots revealed a unique intron constellation for the strain of particular interest. In contrast to all other *Lecanicillium* isolates investigated, the rRNA genes of CEP419 were found to comprise at least two group-I introns each. These findings were exploited in the development a double identification assay for CEP419. Primer pairs hybridizing against the 18S and 28S rRNA intron sequences, respectively, were designed and used to amplify partial rRNA gene sequences in a strain specific manner. Each of both diagnostic PCR reactions alone unambiguously identified strain CEP419 across the full set of *Lecanicillium* isolates investigated. In conclusion, the feasibility of strain-specific identification based on group-I intron sequences has been demonstrated for a potential biocontrol strain of *Lecanicillium*.

## Combined effects of two natural enemies, entomopathogenic fungi and predatory midges, and their effect on a cereal pest

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Both the predatory midge, *Aphidoletes aphidimyza* and the entomopathogenic fungi, *Metarhizium brunneum* have been successfully used for regulating populations of pest in many crops including aphids, such as *Rhopalosiphum padi*. However the interaction between two natural enemies and how that affects the biological control awaits further investigation. As part of the EU supported project INBIO SOIL we designed a pot-trials to assess these interactions. The study was conducted in a greenhouse at an experimental farm of Copenhagen University, at Taastrup, Sjælland (Denmark). Sweet corn (*Zea mays* var. *saccharata*) seeds were grown in pots (40 cm in diameter, 20 L) filled with the natural soil. The experiment included four treatments: *R. padi* with *A. aphidimyza* pupae and *M. brunneum*, *R. padi* with *A. aphidimyza* pupae, *R. padi* with *M. brunneum* and just *R. padi* (control). Each treatment was replicated in ten pots containing one maize plant at the three to five leaf stage. For keeping the replicates isolated and prevent insects from escaping, cages (40 cm diameter and 1 m height) made of plastic Mylar film were placed on each pot. The experiment was checked daily, noting date of adult midge emergence and recording the number of living adult midges. The final assessment was destructive allowing a full count of the number of alive aphids and their developmental stage, dead aphids with signs of being preyed upon by *A. aphidimyza*, number of *A. aphidimyza* eggs and larvae. The presence of the conidia in the soil and on the leaves was assessed during the experiment. Results will be presented and discussed.

## ***Entomophaga maimaiga* caused the crash of the gypsy moth outbreak in the forests of central Serbia in the 2014**

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*Entomophaga maimaiga* Hamber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) is not native entomopathogenic fungus in Europe. In 1999, it was introduced for the first time in Bulgaria. Recent data suggest that *E. maimaiga* is getting spread in Europe. Since 2011 the fungus has been found in several other European countries. First time this fungus was reported from the European part of Turkey in 2011 and in the same year it was also found in Serbia.

During the culmination phases (2014) of the latest outbreak (2009–2014) of the gypsy moth in the central Serbia, the greatest area (339,988.90 hectares) was subject to the very high infection rates (tens of thousands gypsy moth egg masses was laid on the unit of area). The weather plays an important role in the anticipation of the effectiveness of *E. maimaiga*. Like most fungi, its spores need moisture and high humidity to germinate. April and May 2014 were the favourable months to the germination of the resting spores and to the infection of the gypsy moth larvae. Especially, the frequency of the rainy days and the average daily air temperature around 21°C in the first half of the month, caused the massive epizootics and mortality of the young gypsy moth larvae (L2 stage). By the microscopic analysis of the dead caterpillars, the presence of the conidiospores and zigospores of the entomopathogenic fungus *E. maimaiga* was confirmed.



## Microbial control of winter moth in Georgia

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Winter moth is a common pest in the east part of Georgia. Winter moth is serious pest of many trees (Apple, Cherry, Hazel, Chestnut, Oak and so on). The larvae feed upon leaves and may cause complete defoliation of trees.

Possibilities to control of winter moth with *Metarhizium anisopliae* were tested. Entomopathogenic fungi *Metarhizium anisopliae* are applied for biological control of *Operophtera brumata*. Different concentrations of *Metarhizium anisopliae* was tested against 1st, 2<sup>nd</sup> and 3<sup>rd</sup> larval instars of winter moth. First and second larval instars were more susceptible than the third instar. At the highest concentration ( $1 \times 10^{10}$  spores/ml) of *Metarhizium anisopliae* mortality of first larval instars after 10 th days were 100%. Also was tasted affectivity of fungus with different methods of infection. The best infection method of larvae is the method of applying dry preparation of the fungus on the integument. After 10–15 days of infection begins the mass death of pest larvae.

## ***Metarhizium* stationary surface cultivation and encapsulation**

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Entomopathogenic fungi (EPFs) such as *Metarhizium brunneum* invade their host by direct penetration of the host exoskeleton or cuticle. That is why in the scope of the EU funded project INBIO SOIL we will aim at developing novel mechanically stable beads containing EPFs as active biocontrol agent. Fytovita, a producer of aeroconidia of filamentous fungi as powder product, uses a technology based on the method of stationary surface cultivation on a thin layer of a liquid nutrient medium (Kybal, J., Sikyta, B., 1986). Suitability of this technology has been validated for *Metarhizium brunneum* strains ART 2825 and Bipesco 5. The production of conidia with this method yields a product with a concentration of more than  $5 \times 10^9$  spores per gram and at the end of this process the produced aeroconidia showed very low humidity.

Here we report on the influence of amorphous silica (Leland, J.E. *et al.*, 2005), as a drying agent in different concentrations on *Metarhizium* the viability of unformulated *Metarhizium* spores during storage. First results showed that different amounts of amorphous silica don't have a significant impact on the spore viability. Further drying agents will be tested. Additionally, first results will be presented on the influence of different concentrations of encapsulated spores per bead on growth, amount of newly formed aeroconidia and their performance against *Tenebrio molitor* larvae.

## Testing entomopathogenic fungi against *Hylobius abietis* (Coleoptera, Curculionidae)

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Control of *Hylobius abietis* is still an unsolved problem even this beetle species causes a lot of damage in afforestation and reforestation areas. Till now, several chemical insecticides, anti-feedants and mechanical control measures (physical barriers) were tested; in addition, some attempts were made to use entomopathogenic nematodes. Some few entomopathogenic fungi were also subject of preliminary investigations. This was the reason for testing the effects of some entomopathogenic fungi on this beetle in the laboratory. *H. abietis* was collected with bark traps on clear-cut areas in Carinthia (Austria) in 2012–2014. Isolates of entomopathogenic fungi *Beauveria bassiana*, *Isaria fumosorosea* and *Metarhizium anisopliae* originated from soil samples in Austria, *Metarhizium flavoviride* from soil samples in Poland, one *B. bassiana* bark beetle isolate and one *Beauveria brongniartii* isolate from infected *H. abietis* larvae both from Austria. A mix of three fungal species (*B. bassiana*, *I. fumosorosea* and *M. anisopliae*) was tested once. Beetles were inoculated with different concentrations of aqueous fungal spore suspensions. 20 to 30 beetles per group were incubated in Petri dishes (13.5 cm Ø) together with fresh spruce bark chips in an incubator at 22 °C, at long day conditions. Beetles mortality was checked every day, till the death of all beetles (in 2012 and 2014) or after 10 weeks in 2013. Dead beetles were incubated in moist chambers to stimulate fungal growth at room temperature. In a first preliminary test (in 2012) beetles inoculated with *B. bassiana* (60.1%) and with *M. anisopliae* (56.3%) had relatively high infection rates; *I. fumosorosea* inoculated beetles showed very low infection (22.6%), no infection was found in the control group. In a second test series (in 2013, n = 25 in all tested variants) high infection rates were found with *B. bassiana* (76.0%), and low with *M. anisopliae* and *I. fumosorosea* (each 32.0%), with the mix of three fungal species 84% died of a fungal infection (56.0% by *B. bassiana*, 24.0% by *M. anisopliae* and 4.0% by *I. fumosorosea*) and no infection was found in the control group. In a third series of infection experiments (in 2014, n = 30 in all tested variants) 100% infection was found with the *B. bassiana* soil isolate and with the *B. bassiana* beetle isolate, 73.3% infection with *M. flavoviride*, 50% infection with *B. brongniartii* and no infection in the control group.

## Effect of *Beauveria bassiana* on the cuticular and internal lipids composition of *Hylobius abietis* beetles

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*Hylobius abietis*, the large pine weevil, is known as an economic pest in various parts of Europe, because it feeds on phloem of young conifers in reforestation and afforestation areas, leading in some cases to the complete destruction of new plantations. Control of *H. abietis* is still an unsolved problem and at present, chemical insecticides provide the only method of protecting transplants from this pest feeding damage. New EU legislation is promoting the use of integrated pest management programmes with preference to be given to non-chemical, (especially biological) methods of control. Entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* are already commercially available and show considerable potential for the control of various soil and foliar insects. Their development as biocontrol agents requires appropriately applied research on the interactions between the pest and biological agent species. Therefore, it was decided to check the fungus *B. bassiana* impact on the lipid composition of *Hylobius abietis* beetles. Insects were collected with the pine trunk discs on clear-cut areas in Sokółów Podlaski forest district (Poland). The study was performed on male and female *H. abietis*, both infected and uninfected by *B. bassiana*. The extraction lipid composition, cuticular and internal, were performed by the method of Folch. The GC-MS analysis allowed for the initial identification of compounds founded before and after the beetles infection. *H. abietis*, infected and uninfected by fungus, were cultured under identical conditions but infection took place after the soak in a mixture of spores. The preliminary research has showed the large changes in the lipid composition between infected and uninfected insects. Compounds belonging to free fatty acids, alcohols, fatty acid methyl esters and sterols were identified and quantified. For example, the extracts of *H. abietis* female after *B. bassiana* exposure contained free fatty acids from C8:0 to C24:0. Major fatty acids were C16:0, C18:1 and C18:0. In the future, these results may affect the quality of the bio-insecticides based on *B. bassiana*.

## Testing effects of natural resin and synthetic terpenes on conidial germination and growth of entomopathogenic fungi

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The effects of liquid resin, solid resin and colophony of *Pinus nigra* and the chemically synthetic terpenes (-)- $\alpha$ -Pinen, (-)- $\beta$ -Pinen, Myrcen and R-Limonen on conidial germination and growth of three entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* were tested on Agar plates by disk diffusion tests.

Solid resin, colophony and all selected terpenes significantly inhibited the conidial germination of the fungi. However, the inhibition of substances varied depending on the distance between substance (applied on a filter disc) and fungus and with regard to the time period post application.

Germination of spores and fungal growth was significantly inhibited with liquid resin in *B. bassiana* and *M. anisopliae*. In contrast, no germination and fungal growth inhibition was found in *I. fumosorosea*. Furthermore, only *B. bassiana* was influenced in growth by solid resin and colophony.

Conidial germination of all three fungal species was inhibited by (-)- $\beta$ -Pinen and R-Limonen. Fungal growth was inhibited in *B. bassiana* by R-Limonen and Myrcen, whereas *M. anisopliae* fungal growth was reduced only by Myrcen. No germination and fungal growth inhibition was found in *I. fumosorosea* with any of the substances.

## The entomopathogenic fungi *Metarhizium brunneum*, *Beauveria bassiana* and *Isaria farinosa* (Ascomycota, Hypocreales) increase the availability of iron

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Although entomopathogenic fungi such as *Beauveria*, *Metarhizium* and *Isaria* (Ascomycota, Hypocreales) are mainly used as commercial biopesticides, additional unexpected roles are being identified about their wide ecology affecting the availability of soil nutrients in the rhizosphere and therefore to plant nutrition. Among the soil nutrients, Fe plays a crucial role in iron chlorosis either its content or particle size of Fe oxides, and in the biological control of some soil-borne plant diseases. The main objective of this study was to evaluate the Fe mobilization produced by the fungi in Petri plates using 10 Fe oxides that differ in composition, particle size and crystallinity. For that, the Fe oxides were added to Czapek-Dox (CD) medium and three different strains of *Beauveria bassiana*, *Isaria farinosa* and *Metarhizium brunneum* were grown under in vitro conditions. On the other hand, the three strains were grown on CD medium supplied with three sources of iron (Ferrihydrite, Hemathites and Goethite), selected according to their specific surface area. Besides, calcium carbonate was supplied to CD medium to approach to calcareous soils, and Fe mobilization was also evaluated under the new conditions. The results showed that all three fungi increase the Fe available (soluble and labile) in respect to the control (without fungus). For all sources of iron, *Isaria* and *Beauveria* increased the pH of the culture medium while *Metarhizium* decreased in some cases. The presence or absence of calcium carbonate affects the metabolism of the fungus and its ability to increase the availability of iron. The fungi mobilized more Fe when Ferrihydrite was used as source of Fe independently of the calcium carbonate content, due to their high specific surface. To sum up, entomopathogenic fungi are able to mobilize the iron and can be used to prevent iron chlorosis.

## Assessing *Metarhizium* spp. diversity in three different habitats in Switzerland

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Insect pathogenic fungi of the genus *Metarhizium* play an important role as natural regulators of insect populations. They have the ability to infect and kill insects including economically important soil pests, which provides them with a great potential for use as biocontrol agents. Recent taxonomic revisions resulted in the recognition of various new species within *Metarhizium* (Bischoff et al. 2009). For instance, the former *Metarhizium anisopliae* species complex is now divided into nine species. Knowledge on how abundance and diversity of the different *Metarhizium* spp., particularly in the light of the recent taxonomic changes, are affected by different environmental factors or agricultural practices is limited.

The goal of this study was to establish isolate collections from three habitats in Switzerland, i.e., permanent grassland, wheat, and forest and to assess *Metarhizium* population structure by investigating species and genotype diversity.

*Metarhizium* isolates were collected according to a strict scheme, i.e., twenty soil cores were drawn along four parallel transects of 100 m each in each habitat. A selective medium (Strasser et al. 1996) was used for isolation of *Metarhizium* spp. A total of 70 isolates from permanent grassland, 74 from wheat, and 77 from forest were collected. Colonies were assigned to three different morphotypes. The three morphotypes were equally represented among isolates from grassland and wheat. Among the forest isolates 50% displayed one morphotype and 25% each the second and the third. EF1 $\alpha$  sequence and SSR marker analysis is used to determine species and genotype diversity and correlations with the detected morphotypes.

## Occurrence of entomopathogenic fungi in agricultural and forest soils and vermicompost samples

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During several investigations members of entomopathogenic fungi have been isolated from agricultural and forest soils in Latvia as well as from vermicompost samples. From the soil of European aspen (*Populus tremula* L.) and hybrid aspen (*P. tremuloides* Michx. × *P. tremula* L.) stands several fungal species were isolated that are entomopathogenic according to the literature: *Beauveria geodes*, *Tolypocladium geodes*, *Metarhizium anisopliae*, *Paecilomyces carneus*, *P. marquandii*, *Isaria fumosorosea*, *Lecanicillium kalimantanense*; or pathogens of soil nematodes: *Plectosphaerella cucumerina*. From the soil of Norway spruce (*Picea abies*) stands *Beauveria bassiana*, *Tolypocladium cylindrosporum* (synonym *Beauveria cylindrospora*), *B. caledonica*, *B. geodes*, *M. anisopliae*, *P. carneus* have been isolated from particular sampling plots. From the samples of agricultural soil such species as *P. carneus*, *P. marquandii*, *M. anisopliae*, *Beauveria* spp. have been isolated. *Metarhizium flavoviride* was isolated from the vermicompost produced from starchless potato pulp and composted grass. *Paecilomyces* spp. were isolated from the vermicompost produced from cow manure and tree leaves with organic waste. Quantitative data about numbers of colony forming units of mentioned species in various soils were also obtained. Representatives from all mentioned fungal strains were deposited in the Microbial Strain Collection of Latvia (Riga, Latvia).



## Entomopathogenic fungi in litter samples from *Picea abies* sites infested with *Pachynematus montanus* (Hymenoptera, Tenthredinidae)

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*Pachynematus montanus* passes an outbreak in the foreland of the Alps of Austria and Bavaria. Little is known about natural enemies occurring in that sawfly species. This lack of knowledge was the reason to search for the presence of entomopathogenic fungi in litter samples from *Picea abies* sites infested with *P. montanus*.

Litter samples were collected from six sites having different outbreak situation, five in the Austrian foreland of the Alps (Jeging, Gennersberg, Mondseeberg, Hasenkopf, Grafenholz) and one in Bavaria (HeiningerLohe, Germany). Investigating the presence of entomopathogenic fungi two methods were used, the *Galleria* bait method, with *Galleria mellonella* as a bait insect and on the other hand water suspended litter samples were spread onto the surface of selective medium to assess the species and the density of entomopathogenic fungi (colony forming units) in each litter sample.

Results look to be more accurate using the selective medium compared to *Galleria* bait method. In total five entomopathogenic fungal species were isolated by means of selective medium from investigated litter samples: *Beauveria bassiana*, *Isaria farinosa*, *Isaria fumosorosea*, *Metarhizium* sp. and *Tolypocladium* sp.

*B. bassiana* was found in litter from all sampling sites, *Metarhizium* sp. in litter from three sites, *I. farinosa* in two and *I. fumosorosea* and *Tolypocladium* sp. in litter from one sampling site. Highest density of colony forming units was recorded with *B. bassiana* and with *M. anisopliae* both in one site (Grafenholz). Fungal diversity was highest in three sites (Jeging, Mondseeberg and Grafenholz) with three different fungus species. In the litter samples from the study sites, natural infection of *P. montanus* larvae in cocoons caused by *B. bassiana*, *Isaria farinosa* and *Lecanicillium* sp. were found.

## When native parasitoids don't work: biological control of an invasive species with introduced parasitoid and entomopathogenic fungi

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*Dryocosmus kuriphilus* is a globally invasive insect pest, spreading very quickly in new habitats and making seriously damage to sweet chestnut forests in Croatia. Indigenous parasitoid species trophically associated with oak gallwasps have adapted to this new host but cannot effectively regulate its population density. Classical biological control using introduced parasitoid *Torymus sinensis* has been proven to be the only effective method of controlling the populations of *D. kuriphilus*. *T. sinensis* can successfully control the population density of *D. kuriphilus*, slowing down the spread and mitigating negative impact of this invasive chestnut pest and keeping the damage of *D. kuriphilus* at acceptable level. High specificity of *T. sinensis* suggests that it has limited potential of exploiting native hosts. In Croatia, during 4 years 15 species of native parasitoids have adapted to new invasive host but have no impact on lowering the population density. *T. sinensis* has been released in sweet chestnut forests in Croatia as promising biological control agent. *Fusarium proliferatum* induces larval mortality on *D. kuriphilus* and could be used in combination with native and introduced parasitoids for lowering high population densities of invasive *D. kuriphilus*.

## A survey of microbial antagonists of *Otiorhynchus* species from Germany

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Several species of the genus *Otiorhynchus* Germar (Coleoptera: Curculionidae) are serious polyphagous pests in horticulture. Not much is known about their natural microbial antagonists. During 2011 and 2013 a survey of natural occurring *Otiorhynchus* species in Germany and their antagonists was conducted. Altogether, 3,821 beetles of 9 selected species were collected. 178 dead specimens were diagnosed by light microscopy for insect pathogens: 31 beetles contained unspecified bacterial infections, and 86 specimens displayed fungal growth with 33 infected by the entomopathogenic taxa *Beauveria bassiana* (15), *Isaria* sp. (1) and *Lecanicillium* sp. (17). On one beetle, a nematode infecting fungus, *Arthrobotrys* sp., was found, and another one carried an infection by an Entomophthoralean species. Microsporidia, a protist of the genus *Mattesia*, and nematodes were observed in two dead individuals each, and 57 individuals showed no pathogen infections. The role of these entomopathogens as control factors in the field is still unknown.

## Entomopathogenic fungal formulations for western corn rootworm control

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The western corn rootworm (WCR) is an invasive maize pest in Europe. The below ground feeding of the larvae on maize roots causes disruption of water uptake and eventually plant lodging. Entomopathogenic fungi (EPF) are promising microbial antagonists for WCR larvae, but do not cause a high efficacy for effective larval control. This study looked into the possibility to enhance the efficacy of *Metarhizium brunneum* (Strain: BIPESCO5) for a successful implementation as a biological control agent against WCR larvae.

Different *M. brunneum* capsules were screened as a wet formulation to enhance EPF viability and sporulation. Furthermore *M. brunneum* capsules were combined with semiochemicals to manipulate WCR larval behaviour and increase contact with EPF spores. Semiochemicals may therefore act as „efficacy enhancing agents“ (EEA) for an EPF infection. The study demonstrates the suitability to use innovative EPF formulations with EEA for an improved WCR control with *M. brunneum*.

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## Can entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) function as an indirect plant defense?

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In a laboratory investigation, we have tested the chemotactic responses of 4 EPN species (*Steinernema* and *Heterorhabditis*) to 6 compounds released by insect (wireworms and grubs) damaged and undamaged carrot roots. Our results indicate that all of the tested EPN species exhibited attraction (or repulsion) to volatiles, irrespective of their foraging strategy. Our current results suggest that responses to distinct volatile cues are a species-specific characteristic. Terpinolene (VOC released from undamaged roots) was a repellent for *Steinernema* and *Heterorhabditis* species in our investigation. Our results suggest that healthy plant roots release specific VOCs into the soil, which signal to natural insect enemies (EPNs) to keep away.

## Pathology of two species of entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on pistachio root beetle larvae, *Capnodis cariosa hauseri* (Col.: Buprestidae)

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The pistachio root beetle, *Capnodis cariosa hauseri* causes general weakness in the pistachio trees and may even kill the infected trees. This insect was distributed in many pistachio growing regions of Iran and causes heavy damage to pistachio trees, particularly when the infection is accompanied by gummosis. Cryptic behavior of the larvae reduce efficacy of common control methods. Due to hidden habitat of pest and penetration ability of entomopathogenic nematodes (EPNs) to hidden habitat of some insects, we investigated efficacy of EPN species, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* against last instars larvae of the pest in laboratory. Pathogenicity test in petri dish were performed with five concentrations: 5, 10, 25, 50 and 100 infective juveniles (IJs) per larva. The LC50 values indicated that *S. carpocapsae* (12.6 IJs larva<sup>-1</sup>) was comparatively more virulent than *H. bacteriophora* (17.1 IJs larva<sup>-1</sup>) against *C. cariosa* larvae after 72 h. Larval mortality was significantly influenced by species and concentration. The highest mortality were recorded at the concentration of 50 IJs larva<sup>-1</sup> for *S. carpocapsae* (97.1%). Both EPNs successfully penetrated and reproduced in the *C. cariosa* larvae at the concentrations of 5 and 25 IJs larva<sup>-1</sup>. The highest reproduction was recorded for *H. bacteriophora* at 5 IJs larva<sup>-1</sup> in *C. cariosa* (562881±7773 IJs) and the highest penetration in *C. cariosa* was observed for *S. carpocapsae* at 25 IJs larva<sup>-1</sup> (19 IJs). The current study demonstrated susceptibility of *C. cariosa* to both nematode species as well as their reproduction capability and penetration on the pest larva.

## Evaluation of entomopathogenic nematode *Steinernema carpocapsae* against fern scale *Pinnaspis aspidistrae* Sign.

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The aim of this study was to determine the biological control effect of entomopathogenic nematode *Steinernema carpocapsae* on fern scale *Pinnaspis aspidistrae* in the laboratory condition. The experiments were conducted in 10 cm Petri dishes lined with a moistened filter paper. One infested Laurel plant leaf containing approximately 100–120 *P. aspidistrae* was placed in each Petri dish and the nematodes were applied 0, 500, 1000 and 1500 infective juveniles/ml. Insect mortality was checked 3, 5 and 7 days after the treatment. The data showed that *S. carpocapsae* was highly virulent against *P. aspidistrae* and the mortality was related with time and nematode concentrations. At 7 days after treatment, 500, 1000 and 1500 IJs/ml applications exhibited 22, 62 and 82 % mortality, respectively. As conclusion, it was determined that *P. aspidistrae* can be controlled by *S. carpocapsae* but further studies should be conducted in greenhouse and field conditions.

## Efficacy evaluation of the *Heterorhabditis bacteriophora* against Click beetle (Coleoptera: Elateridae)

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This paper will discuss the advances in understanding in application of entomopathogenic nematode *Heterorhabditis bacteriophora* to control Click beetles (Coleoptera: Elateridae). Wireworms, the larvae of click beetles are destructive to a wide range of plants. Adult wireworms, or click beetles, are hard-shelled and cause no crop damage at this stage of their life cycle but single larva can successively destroy several plants.

*H. bacteriophora* was tested against different stages of a click beetle - wireworms, pupae and young beetles. Insects were treated with EPN suspension containing 1000 IJs/ml water (i.e. dose 100 IJs per insect). The experiments were carried out under laboratory conditions at the temperature  $22 \pm 2$  °C and 80% RH. The mortality rate was checked after 5 and 7 days. The efficiency of *H. bacteriophora* on the adults reached 60%, on the pupae – 88 % and the highest mortality of wireworms achieved 100%.

We also evaluated the nematodes activity into wireworm's organism after treatment (2, 4, 8, 24, 48 hours) and histological changes in the fatty tissues as well. Experiments have shown that degradation of larvae tissues has already begun after 4 hours. The first were observed increase number formed elements of haemolymph, then changing cells of fat-body, intestines and other organs of larva.



## The 'low risk' concept for plant protection products within the EU: a new opportunity for authorization of biocontrol microorganisms

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In the EU, microbial plant protection products have to be authorized according to Regulation (EC) 1107/2009 and provisions in Directive 2009/128/EC on the sustainable use of pesticides. Microorganisms are subjected to the same approval system as chemical pesticides, and the approval for a microorganism is mostly long lasting. The EU aims to reduce the use of chemical pesticides and encourage the use of non-chemical plant protection methods. A novel tool within Regulation (EC) 1107/2009 to achieve this goal is the category of "low risk products", which will pose only a low risk to human and animal health and the environment. One main questions remaining is how microorganisms can be included into the new concept of 'low risk' and whether their approval after classification as low risk substances can be facilitated in practice.

A working group consisting of European and national regulatory authorities, stakeholder organizations and scientists was established in 2013 to develop guidelines for this concept. We have participated in this work, which is still ongoing, and will present some main issues considered: 1) Which safety criteria are most relevant, and also feasible to apply, for determining when an organism (or more specific, an isolate) qualifies for inclusion as a 'low risk' compound?, 2) Should the same set of criteria apply equally to virus, fungi, and bacteria?, 3) How to get the traditional ecotoxicological perception of risk to meet with especially inoculation biological control?, 4) Is it possible from the standpoint of the biology of plant-beneficial microorganisms to unequivocally distinguish between "Biostimulants" and "Biological control agent"?

## New findings in phylogeny and genome of Nun moth (*Lymantria monacha*) baculovirus

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Nun moth (*Lymantria monacha*) belonging to Lepidoptera, Lymantriidae is currently one of the most important pest of conifers in Poland. This folivore usually feeds on older and healthy pines, spruces and larches, but in the absence of these species and during characteristic gradation period (that occurs every 10 years) can attack deciduous trees. Nun moth is widely present throughout Europe. The main natural enemy of this pest is *Lymantria monacha* Nucleopolyhedrovirus (LymoNPV) from *Baculoviridae* family that is able to control its host population. To date only small regions of three genes (*polh*, *lef-8*, *pif-2*) from two different LymoNPV isolates are available in the NCBI database. On the basis of those small genome fragments Jehle *et al.* in their work from 2006 classified LymoNPV as a member of Alphabaculovirus group II. During our studies we isolated, sequenced on next generation platform and prepared the draft of full genome of Polish LymoNPV strain. Our analyzes have confirmed previously established phylogeny position where LymoNPV cluster together with Gypsy moth (*Lymantria dispar*) and Casuarina moth (*Lymantria xyliana*) baculoviruses.

## The genome of recently sequenced white satin moth baculovirus (LesaNPV) shows high level of similarity to douglas-fir tussock moth baculovirus (OpMNPV)

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The white satin moth *Leucoma salicis* L. (Lepidoptera, Lymantriidae) is a dangerous defoliator occurring mainly in Europe and Asia, but it was also introduced to North America in 1920s. The caterpillars feed principally on the leaves of poplar, cottonwood, aspen and willow trees (family Salicaceae) and less commonly on oak. They attack healthy trees, what make them the primary pest and can lead to massive defoliations and weakened trees. One of the main natural enemies of this insect is baculovirus, LesaNPV (*Leucoma salicis* Nucleopolyhedrovirus). For population control of white satin moth the biopesticides based on LesaNPV baculovirus can be used. This type of control agents are claimed to be very safe for human and animals, because they do not infect organisms other than arthropods and cannot replicate outside their natural host. It has been reported in the literature that LesaNPV is infective against the close relative of its host, *Orgyia pseudotsugata* L., but OpMNPV does not infect *Leucoma salicis* L. According to short nucleotide sequences of three genes (polh, lef-8, pif-2) available in the NCBI database LesaNPV was annotated as Alphabaculovirus group I (Jakubowska et al., 2008). In our studies we have prepared the draft of full genome of LesaNPV which we found highly similar to genome of OpMNPV. We identified 153 ORFs (open reading frames) in LesaNPV genome, few were not present in OpMNPV (like *he65*) or were fused from two OpMNPV ORFs (Op82-Op83). The phylogenetic relationship of LesaNPV and other baculoviruses will be presented.

## Detection and quantitation of alpha and betabaculovirus in *Spodoptera frugiperda* mixed infections

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The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is an important pest in America. This insect has a very wide host range but the most frequently affected crops are maize, cotton and shorgum, causing important economic impact. *Alphabaculovirus* and *Betabaculovirus* (nucleopolyherovirus-NPV and granulovirus-GV: *Baculoviridae*) have been isolated causing co-infection in fall armyworm populations in Colombia. This condition have a considerable potential as biological control strategy, due to in some cases GVs are able to enhance the infectivity and virulence of NPVs. In this work a sensitive tool for detection and quantitation of NPVs and GVs in mixed infections in *S. frugiperda* was developed. A multiplex PCR real time method was developed based on highly specific oligonucleotides and Taq-man probes that recognize fragments of polyhedrin (polh) and granulin (gran) genes. The specificity of individual probes showed non-cross amplification between NPVs and GVs. Besides, each probe was able to detect NPVs or GVs from different geographical origins. Samples of mixed SfMNPV/SfGV genomes at different ratios were analyzed and interferences were not observed. The minimum detection limit of the technique was  $6.4 \times 10^{-4}$  ng / $\mu$ l of DNA, equivalent to  $1.25 \times 10^3$  gene copies (gran and polh) and minimal variation inter-assays showed that the technique was reproducible. This technique is essential in the process of biopesticide developing based on alpha and betabaculoviruses, including the detection of infected larvae in field until the quality control of final formulated product.

## Possible natural betabaculovirus coinfections in Gelechiidae insects from Colombia based on pif gene determination

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Baculoviruses are pathogens worldwide distributed and classified in four genera (*Alpha*-, *Beta*-, *Gamma*- and *Deltabaculovirus*) which infect insects many of them considered as pests in agriculture. These entities are excellent candidates to formulate bioinsecticides. *Tecia solanivora*, *Phthorimaea operculella* and *Tuta absoluta* are invertebrates belonging to the potato tuber moth complex (Lepidoptera: Gelechiidae) and are considered the main pests in potato and tomato crops. Thus, the characterizations of baculoviruses that can multiply in these insects comprise a relevant focus required to assist in the development of biological control strategies. Considering the above, three betabaculoviruses recovered in Colombia from *T. solanivora*, *P. operculella* and *T. absoluta* respectively were studied to add information with respect to the host range specificity associated with the identity of the *per os* infectivity complex, constituted by seven proteins encoded in all baculovirus genomes (P74, PIF1, PIF2, PIF3, ODV-e28, ODV-e56 and PIF6) which are essential to support the primary infection into larvae. For this, fragments of the aforementioned seven *pif* genes were amplified by PCR and sequenced to assist in virus classification and to study sequence factors related to virus host range. Results showed that three isolates were closely related to the previously described *Phthorimaea operculella* granulovirus (PhopGV) based on sequence data of six *pifs*, but surprisingly some amplicons of *pif1* also revealed a sequence relationship with *Helicoverpa armigera* granulovirus (HearGV). This observation could suggest the presence of natural coinfections and/or horizontal gene transfer processes associated to expand the host range.

## Identification of four nuclear polyhedrosis viruses from the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantridae) in Turkey: variations in their virulence

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*Lymantria dispar*, commonly known as the gypsy moth, is one of the most destructive pests of forest, shade, fruit and ornamental trees. Gypsy moth caterpillars cause extensive defoliation, leading to reduced growth or even mortality of the host tree. Also, urticacious hairs on larvae and egg masses cause allergies in some people. The gypsy moth has a variety of natural enemies (predators, parasites, and diseases) that naturally control populations. In addition, entomopathogenic viruses offer another important option for biological control of this pest. The aim of this study is to characterize new entomopathogenic viruses from this pest. For this purpose, *Lymantria dispar* larvae, collected from different localities (Samsun, Bingöl, Yozgat and Trabzon) in Turkey, were examined for the presence of inclusion bodies under light microscope. The larval samples contained inclusion bodies were subjected to polymerase chain reaction using the conserved primers for polyhedrin (*polh*) and late expression factor 8 (*lef-8*) genes. Sequence analysis, confirming the light microscopy results, showed that larval samples from four different localities contain nucleopolyhedroviruses (NPV). These isolates were designated as S-LdNPV, B-LdNPV, Y-LdNPV and T-LdNPV. Phylogenetic analysis of viral isolates according to target genes were also performed. Insecticidal activities of these isolates, performed with  $10^6$  OBs/ml, yielded insecticidal effects between 100 – 60 % on the third instar *L. dispar* larvae under laboratory conditions.

## Conditions adjustment for *in vivo* mass production of a Colombian *Spodoptera frugiperda* nucleopolyhedrovirus

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The Colombian *Spodoptera frugiperda* nucleopolyhedrovirus isolate SfNPV003, was used for developing a biopesticide with great potential for the control of this pest in the field, becoming an alternative to replace chemical pesticides. However, its commercialization has been limited due to high costs for *in vivo* virus production, which have a significant impact on the product price. Traditional *in vivo* production involved feeding the virus to insect larva, temperature should be maintained carefully to keep the larva alive and to produce the highest yield and quality of virus, larvae must be harvested at the proper time. In this sense, the aim of the present work was to adjust the conditions of SfNPV003 multiplication system, in order to increase yield and reduce costs. Initially, the effect of larval age and viral inoculum concentration on viral productivity was determined. Subsequently, the effect of diet for larvae rearing and incubation temperature was established. Finally, the effect of harvest time on productivity and contaminant content was evaluated. Sixteen days-old larvae were selected for inoculation with a viral suspension adjusted to a concentration of  $1 \times 10^7$  OBs/mL and wheat germ-based diet was selected for larvae rearing. Implementation of a temperature control system for incubation did not improve viral production. Seven days post- inoculation was selected as the optimum time for infected larvae harvest. Setting these conditions, an increase in viral production capacity was achieved, which allowed a 20% of reduction in the price of the product, improving its economic feasibility.

## Detection of a new insect iridescent virus in the elm leaf beetle, *Pyrrhalta luteola* (Coleoptera: Chrysomelidae) population

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The elm leaf beetle, *Pyrrhalta luteola* (Coleoptera: Chrysomelidae) is a leaf-chewing pest of elm trees. It infests all species of elms, however, American elm *Ulmus Americana*, Siberian elm *U. pumila*, and European elms *U. minor*, *U. glabra*, *U. laevis*, *U. procera* are severely damaged by this beetle. Adults chew entirely through the leaf, often in a shot-hole pattern. Larvae skeletonize the leaf surface, causing damaged foliage to turn brown to whitish. Elm leaf beetles, when abundant, can entirely defoliate large elm trees, which eliminate summer shade and reduce the aesthetic value of trees. Repeated, extensive defoliation weakens elms, causing trees to decline. A new invertebrate iridescent virus (designated as PIIV) was detected in *P. luteola* larvae collected from the area around Igdir, Turkey. Field samples were brought to the laboratory and screened for viral infection. Polymerase chain reactions were carried out using major capsid protein (*mcp*) and DNA polymerase (*dnapol*) specific primers of iridoviruses. Sequence analysis of the amplified products (435 bp for *mcp* and 900 bp for *dnapol*) showed that the virus discovered is an iridovirus. These sequences were submitted to the GenBank with accession numbers KP219401 (*mcp*) and KP219400 (*dnapol*). Phylogenetic analysis was performed using partial sequences of *mcp* and *dnapol* genes. The resulted trees located the PIIV near iridoviruses from Coleoptera and Isopoda. Scanning electron microscope studies also confirmed the general iridovirus structure. This is the first report of an iridescent virus isolated from elm leaf beetle, *Pyrrhalta luteola*, collected from the field.



## The esterase/protease gene of *Amsacta moorei* entomopoxvirus plays significant role on the growth of infectious virus

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*Amsacta moorei* entomopoxvirus (AMEV) is the type species of genus betaentomopoxvirus encompassing viruses infect insects belong to Lepidoptera (moths) and Orthoptera (grasshoppers). AMEV has an important potential to be a microbial control agent against some harmful insects in agriculture and a gene expression and therapy/delivery vector. This virus can be readily studied and manipulated in cell culture. It has been shown that AMEV replicates in nearly the entire body of insect, especially in the adipose tissue. Lipolytic genes have been investigated in several viral genomes and play a role in various functions including the production of DNA replication metabolites, rescue from endosomes, and membrane fusion. AMEV has an open reading frame, *amv133*, which encodes an active esterase enzyme with protease activity. Therefore, *amv133* may plays an essential role in virus growth. In this study, we investigate the effect of *amv133* on virus growth first time. For this purpose, we constructed *amv133* knockout virus by homolog recombination, purified the recombinant virus through serial plaque assays and determined the virus titer on 0, 24, 48, 72 and 96 hours post infection via end point dilution assay. Our results showed that deletion of *amv133* gene from virus genome dropped the infectious virus production significantly (68%) compare to wild type.

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## Prevalence and diversity of viruses in the entomopathogenic and nematophagous fungus *Purpureocillium lilacinum* in the Czech Republic

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*Purpureocillium lilacinum*, widely known as *Paecilomyces lilacinus*, is a ubiquitous saprophytic fungus commonly isolated from soils. This fungus has a cosmopolitan distribution and is used as biological control agent against invertebrates in agriculture. Additionally, it can infect humans and other vertebrates, and asymptotically colonize plants as an endophyte.

The presence of double-stranded RNA (dsRNA) elements indicative of viral infections has been discovered in numerous fungal species, but unlike most plant or animal viruses, they are rarely related with deleterious effects on their hosts. The knowledge about viruses infecting entomopathogenic fungi is very limited and only few species harboured by *Beauveria bassiana* and *Tolypocladium cylindrosporium* have been described. Mycoviruses add complexity to the knowledge of entomopathogenic and nematophagous fungal ecology, which is crucial for understanding their role in managed and natural ecosystems, and for their successful development as biocontrol agents.

In the present study, 88 *P. lilacinum* isolates obtained at different locations and from different habitats around the Czech Republic were analysed for the presence of dsRNA elements. Results revealed that around 30% of the isolates harboured dsRNA elements with viral characteristics. The different electropherotypes indicated a wide virus diversity among infected isolates. There was no relation between particular virus species or dsRNA profiles and specific locations or habitats. One of the most common dsRNA elements was sequenced. It represents a new virus taxon of mycoviruses with a partitivirus-like lineage, and forms a distinct clade from previously known viruses. The new virus was termed *Purpureocillium lilacinum* nonsegmented virus 1 (PINV-1).

## ***Bacillus* associated with Colorado potato beetle – *Leptinotarsa decemlineata* Say and the mottled umber – *Erannis defoliaria* Clerrck in Georgia**

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For the identification of endemic species of *Bacillus* in agro- and forest ecosystems of Georgia infected and healthy individuals of Colorado Potato Beetle – *Leptinotarsa decemlineata* Say and caterpillars of the Mottled UMBER – *Erannis defoliaria* Clerrck were collected at different age of larvae. Ten of the emitted 20 isolates (BZ1, BZ2, BZ3, KM1, KM2, KM3, KM4, KM5, KM5 (1), M5 (2)) were Gram positive. For the evaluation of spore formation ability isolates were cultivated in liquid media (Nutrient Broth and Selective Media) and the spore formation was analyzed under the microscope

For the purpose of establishment of formation of crystal proteins of *Bacillus*, the proteinaceous range of gram positive isolates was studied by SDS-PAGE polyacrylamide gel electrophoresis. Isolates – BZ1, BZ2, KM2, KM3, KM4, KM5, are distinguished with high protein content which size ranged between 130 kD and 66 kD, and each strain has different proteinaceous range. The microscopic analysis revealed the existence of spores in KM2, KM3, KM5 isolates.

With the purpose of identification of crystal inclusions the noted isolates were transferred on Selective media. The 24-hours microscopic analysis of culture didn't show the existence of crystal inclusions.

## **Proteolytic processing and *in vivo* binding of the *Bacillus thuringiensis* Vip3Ca insecticidal protein**

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A screening of collections of *Bacillus thuringiensis* strains led to the discovery of novel *vip3* genes encoding the new family of Vip proteins Vip3Ca (Palma et al., 2012, Appl. Environ. Microbiol. 78: 7163). The present study examines the insecticidal spectrum of the Vip3Ca protein and reports the first results on the mode of action of this protein. The proteolytic processing of Vip3Ca was studied by incubating the protoxin with midgut juice from a susceptible insect (*Mamestra brassicae*), a moderately susceptible insect (*Agrotis ipsilon*) and nonsusceptible insect (*Ostrinia nubilalis*). In all cases, the ca. 90 kDa protoxin was converted into a ca. 60 kDa toxin, suggesting that the activation is not critical in determining the susceptibility of an insect species. Binding of Vip3Ca to the epithelial membrane of *M. brassicae* midgut larvae was shown after ingestion of the protoxin and further detection with an anti-Vip3 protoxin polyclonal antibody. The binding experiment was carried out in parallel with Vip3Aa, a more potent toxin against this insect species, and with a nontoxic Vip3 protein. Histopathological inspection showed swelling of the epithelial cells with further disruption, which suggests that the mode of action of Vip3Ca is similar to that described for Vip3Aa.

## Antagonistic effects of *Bacillus* isolates on phytopathogenic fungi

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*Chitin* is an important cell wall component of pathogenic *fungi*. Also, it is the main structural component of exoskeleton in insects. Enzymes known as chitinases degrade chitin. Chitinase enzymes can be used as biological fungicide against phytopathogenic fungi, as well as an insecticide against insect pests.

In this study, fifteen *Bacillus* isolates obtained from soil were identified as chitinase producers on colloidal chitin plates. These isolates were identified based on biochemical and molecular tests. Antagonistic activity assays of these isolates were performed against *Fusarium culmorum*, *F. graminearum*, *F. subglutinans*, *Trichoderma* sp. and *Aspergillus niger* which are known as phytopathogenic fungi. Antagonistic activities were assayed using dual-culture method.

According to the results of molecular and biochemical tests, these bacteria were identified as members of *Bacillus cereus* group. Between these, T5, T8 and T29 isolates showed antagonistic activity. While T5 and T8 isolates showed antagonistic activity against *F. culmorum*, *F. graminearum*, *F. subglutinans*, and *Trichoderma* sp., T29 isolate only showed against *F. culmorum* and *F. graminearum*. T8 isolate exhibited higher activity on *Fusarium culmorum*, *F. graminearum* and *F. subglutinans* compared to other fungi. Also the effects of the T8 and T29 isolates on these fungi is greater than the effects of T5 isolate.

Further research will be done to identify the component caused antagonistic effect is chitinase or another antifungal component.

## The two faces of *Pseudomonas oryzihabitans*

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Studies on the bacterial community associated with the Indian rosewood (*Dalbergia sissoo* Roxb.) in Bangladesh revealed isolates related to the *Pseudomonas* group in 75.5% of trees affected by the disastrous dieback disease, but only in 21% of non-symptomatic trees. The close association with dieback prompted us to characterize these isolates in more detail. Taxonomic grouping of 33 selected *Pseudomonas* isolates was addressed by sequencing the nearly complete 16S rDNA (1506 of 1537 bases) and by 'Amplified Ribosomal DNA Restriction Analysis' (ARDRA). Blast search revealed 20 isolates as *P. oryzihabitans*, while 10 were related to *P. putida* and three to other pseudomonads. Almost the same clustering was obtained with grouping by ARDRA. Interestingly, previous studies from our laboratory had shown that the isolates related to *P. oryzihabitans* exhibited pathogenic activity on indicator plants as well as on *D. sissoo* seedlings (Valdez *et al.*, Bangladesh J. Bot. 42: 1–16, 2013). *In situ* localisation studies using broad spectrum antisera against *Pseudomonas* revealed a scattered distribution in root parenchyma. *Pseudomonas oryzihabitans* was therefore discussed as pathogenic agent in the dieback disease of *D. sissoo*.

In contrast, *P. oryzihabitans* was shown as potential biological control agent against *Fusarium oxysporum* and root knot nematodes (Vagelas & Gowen, Pak. J. Phytopathology 24: 32–38, 2012). These obvious contradictory properties provoked the idea that different strains of *P. oryzihabitans* are responsible for such divergent activities, by harbouring genes for nematicidal compounds on one hand and for a plant pathogenic potential on the other hand.

## Plant alkaloid and probiotics increase resistance of honeybees to nematobacterial infection

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Entomopathogenic nematodes (EPNs) are obligate insect parasites that are symbiotically associated with entomopathogenic bacteria. These nematobacterial complexes are highly pathogenic and therefore used in biological control. As was shown previously, EPNs can be used also as a natural infection model and a powerful tool to study insect immunity. In this study we show that also honeybee larvae are suitable hosts for nematobacterial complex that offer an excellent environment for successful development of both nematodes and their symbiotic bacteria. Here we used EPNs for evaluation of the overall immune resistance of honeybee larvae treated with potentially immuno-modulating substances (plant alkaloid sanguinarin and probiotics). Honeybee larvae were infected with *Heterorhabditis bacteriophora* or *Steinernema feltiae*, both carrying their symbiotic bacteria, and then scored for mortality. In comparison to untreated honeybee larvae we observed the decrease in mortality of approx. 35% in case of *H. bacteriophora* and approx. 10% upon *S. feltiae* infection in larvae treated by selected substances. Both sanguinarin and probiotic treatment showed similar protective effect that was less significant in highly virulent *S. feltiae*. This is the first record that the nematobacterial infection was used for evaluation of immune status of beneficial insect. We suggest and propose this method as a valuable tool for immunity testing in honeybees as well as in other insects. Our research is supported by grant from the Ministry of Agriculture of Czech Republic (project No. QJ1210047) and by the program CZ.1.07/2.3.00/30.0009 co-financed from European Social Fund and the state budget of the Czech Republic.

## Cloning and expression of chitinase A, B and C genes from *Serratia marcescens* originating from *Helicoverpa armigera* and determining their activities

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Three genes encoding chitinase A (*chiA*), B (*chiB*) and C (*chiC*) were amplified by PCR using degenerate primers from *S. marcescens* that isolated from a naturally dead *Helicoverpa armigera* larvae and identified as *Serratia marcescens* based on 16S rDNA sequence analysis (with KF823633 accession number). The open reading frames (ORF's) were identified as 1692, 1500 and 1443 base pairs for *chiA*, *chiB* and *chiC* genes, respectively. These sequences were submitted to the GenBank with accession numbers KF823630 (*chiA*), KF823631 (*chiB*) and KF823632 (*chiC*). Comparison of the deduced aminoacid sequences with those of other bacterial chitinases revealed that three chitinases contain the catalytic domain. Furthermore, all three chitinases showed 99% similarity to the *Serratia marcescens* WW4 strain at aminoacid level. Chitinases were overexpressed in *Escherichia coli*. Expressed proteins were purified and their activities were tested using colloidal chitin as a substrat. Reasonable pH and temperature ranges were also determined as 7–11 and 33–37°C, respectively. Insecticidal activities of these proteins were tested on *Malacosoma neustria* and *Helicoverpa armigera* larvae. Test results showed that, 1000 U ml<sup>-1</sup> chitinase A, B and C have 47, 50 and 66 % insecticidal activities on *Malacosoma neustria*, and 80, 45 and 50% insecticidal activities on *Helicoverpa armigera* larvae within 10 days, respectively.



## Host group adaptation of *Candidatus Rickettsiella isopodorum*, a lineage of intracellular gamma-proteobacterial pathogens carried by woodlice

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The taxonomic genus *Rickettsiella* (*Gammaproteobacteria*; *Legionellales*) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans that are currently under intensive evaluation as possible sources of new biocontrol agents. A multilocus sequence analysis approach has been employed to independently evaluate the previously established 16S rRNA based phylogeny for two *Rickettsiella* strains associated with isopods from California and Germany. Phylogenetic reconstruction from three protein-encoding marker genes, namely *ftsY*, *gidA*, and *sucB*, confirms the very tight phylogenetic relationship of *Rickettsiella* bacteria from closely related hosts, but distant geographic origins. These findings are generally indicative of stable host adaptation and, therefore, challenge the currently adopted view of rather unspecific host – pathogen relationships within the *Rickettsiella*.

Reference: Kleespies, R.G., Federici, B.A. & Leclerque A. 2014: Ultrastructural characterization and multilocus sequence analysis (MLSA) of *Candidatus Rickettsiella isopodorum*, a new lineage of intracellular bacteria infecting woodlice (Crustacea: Isopoda). *Systematic and Applied Microbiology* 37(5): 351–359.

## Efficacy of entomopathogenic fungi for wireworm control and their potential effects on microbial communities

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The entomopathogenic fungus *Metarhizium brunneum* is a natural antagonist of *Agriotes* spp. larvae (wireworms) and has potential as biological control agent (BCA). The efficacy of *M. brunneum* to control *Agriotes obscurus* larvae was investigated in pots planted with potato. Various treatments were tested including two different formulations of *M. brunneum* (fungal colonized barley kernels [FCBK] and newly developed fungal capsules), fungal spore powder, garlic capsules (repellent) and combinations of *M. brunneum* and garlic capsules. The latter treatments were included to assess potential synergistic interactions. A granular formulation of the insecticide clothianidin was used as positive control. *M. brunneum* densities in fungal treatments ranged from  $4 \times 10^3$  to  $1 \times 10^4$  colony forming units (CFU) per g soil dry weight compared to on average less than  $5 \times 10^2$  CFU per g soil dry weight in pots that were not treated with *M. brunneum*. Fungal application resulted in a 60 to 95% reduction of *A. obscurus* larvae and significantly reduced potato damages. Application of FCBK was the most efficient control method. No synergistic effects in the combined *M. brunneum* / garlic capsule treatments were observed.

Assessment of potential effects on non-target organisms is an important aspect in the development of biological and chemical control agents. The established pot experiment provides a well-characterized setup to investigate potential effects of the treatments on soil microbial community structures. Microbial community structures in the different treatments will be determined and compared 7 and 15 weeks after application using a metagenomics approach.

## Effectiveness of antimicrobial compounds produced by entomopathogenic nematode symbiotic bacteria to control pests and bacterial plant diseases

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Entomopathogenic nematode-bacterium symbiotic complexes (EPN-EPB) provide several options for environmentally friendly biological pest control. In the last decades EPN formulations become commercially available for agricultural applications. Relatively less attention was dedicated to the antimicrobial potential of the symbiotic bacteria. The role of the EPB partner is essential for the successful colonization of the insect host cadaver via their production of antibiotics and hydrolytic exoenzymes, and process the insect's cadaver into utilisable nutrient for the nematodes. The broad-spectrum antibiotics of peptide nature produced by EPB non-ribosomally keep monoxenic conditions in insect cadavers in soil. These compounds show significant antagonistic activity against numerous plant pathogenic bacteria and fungi *in vitro*. Potato wilt caused by *Ralstonia solanacearum* is one of the most challenging plant diseases in several countries, including Hungary. In this project we established some parameters needed prior to experimental field application of antibacterial substances from EPB strains *Xenorhabdus budapestensis* (EMA) and *X. szentirmaii* (EMC) in *Ralstonia* control. We quantitatively determined the (i) optimum inoculum size needed for successful *Ralstonia* infection; (ii) the minimum phytotoxic concentration and (iii) the minimal inhibitory concentration of EMA cell free conditioned medium (CFCM). The bactericidal effect of EMA CFCM against *Ralstonia solanacearum* test organism was determined *in vitro* circumstances. At the light of the results we consider the antibacterial component(s) of EMA CFCM potential tool(s) of *Ralstonia* control.

## Co-encapsulation of *Beauveria bassiana* or neem extract in CO<sub>2</sub> releasing beads attractive towards soil borne pests

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Soil-dwelling larvae of pest insects like western corn rootworm or wireworms cause considerable economic damage in agricultural production systems. As CO<sub>2</sub> is known as an attractant for such pests there is a high interest in CO<sub>2</sub> emitting formulations for an “attract-and-kill” strategy. Ca-alginate encapsulation of baker’s yeast has previously been shown to result in a slow release of CO<sub>2</sub> in soils. The objective was to improve the formulation aiming at a prolonged CO<sub>2</sub> release and the co-encapsulation of a killing agent.

Addition of starch extended and increased CO<sub>2</sub> release resulting in the formation of significant CO<sub>2</sub> gradients in a defined soil during several weeks. The missing starch degrading activity of baker’s yeast was compensated by random soil microorganisms, by the co-encapsulation of a technical amylase, or by co-encapsulated entomopathogenic fungus *Beauveria bassiana* producing exoenzymes with amylase activity in order to tackle two issues at the same time.

Experiments performed on a laboratory scale have shown that “attract-and-kill”-formulations are principally attractive towards western corn rootworm larvae or wireworms. However, the co-formulation with *B. bassiana* was not able to control western corn rootworm larvae. We speculate that incubation time of six days was too short for fungal sporulation, which is needed for a successful infection. The laboratory experiments have also demonstrated that the co-formulation with neem extract affected wireworm viability. These preliminary results have been confirmed in field experiments and will be further investigated.

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