Is IPM ready for Microbial Control Agents?

16th Meeting of the IOBC-WPRS Working Group

June 11-15, 2017

Tbilisi, Georgia

Microbial and Nematode Control of Invertebrate Pests

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International Organization for Biological and Integrated Control
Association of Professional Chemists of Georgia
Agricultural University of Georgia

Microbial and Nematode Control of Invertebrate Pests

16th Meeting of theIOBC-WPRS Working Group

PROGRAMME AND ABSTRACT BOOK

June, 11 15,2017
Tbilisi – Georgia

Organizers

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WELCOME MESSAGE

Dear IOBC Members and Colleagues,

We welcome you to the 16th meeting of the IOBC-WPRS Working Group Microbial and Nematode Control of Invertebrate Pests, at the Agricultural University of Georgia, Tbilisi, Georgia.

The general topics of this meeting cover many 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 invertebrate pathogens. The meeting has a special focus on Integrated Pest Management (IPM) and the use of Microbial Control Agents (MCAs). According to EU policies as well as the world-wide endeavor to reduce the risks and application of chemical pesticides, alternatives, such as MCAs, play a more and more important role in agricultural, horticultural and forestry practice. Most if not all MCAs may qualify as low-risk, a new class of plant protection products in the EU legislation, which are favored in the registration process of plant protection product. Thus, MCAs are facing new opportunities and new challenges.

The meeting includes invited and contributed papers, as well as poster and discussion sessions to encourage communication between entomologists, invertebrate pathologists, and experts of other disciplines on the biocontrol of insects. Following IOBC tradition an exciting scientific and social program has been set up.

We are very pleased welcoming you in Tbilisi.

Medea Burjanadze,
Chair Local Organizing Committee

Johannes Jehle,
Convenor of the WG Microbial and Nematode
Control of Invertebrate Pests

CONTENTS

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Prof. Medea Burjanadze - Agricultural University of Georgia

PROGRAMME

JUNE 11, 2017

16:00 - **Registration**

17.30 **- Convenors Meeting**

18:00 **- Mixer**

19:00 - **Transfer to hotels**

JUNE 12, 2017

8.00 **Registration**

9.00 **Opening Ceremony**
(Medea Burjanadze, Johannes Jehle, Giselher Grabenweger)

9:30 **PL-0. Giselher Grabenweger**
Introduction to IOBC

10.00 **Coffee Break**

PLENARY SESSION: BIOCONTROL AND IPM
Convenor: Johannes Jehle

10:30 **PL-1. Tsisia Chkhubianashvili**
Development of Microbial Control Agents to Plant Protection from Pest Insects in Georgia: Past and Future

11:00 **PL-2. Heiri Wandeler**
Including Baculoviruses in Insect Control Strategies – Opportunities and Challenges

11:30 **PL-3. Itamar Glazer**
Efficacy and Persistence of Entomopathogenic Nematodes (EPNs) Against Burrowing and Sedentary Insects

12:00 **PL-4. Stefan Jaronski**
Using Insect Pathogenic Fungi to Manage Insect Pests "Where Are We Going? (Where SHOULD We Be Going?)

12:30 **Lunch**

ORAL SESSION: BACTERIA.

Convenor: Medea Burjanadze

14:00 **OP-1. Ardahan Eski**
Bacterial based biopesticide production for pest management

14:15 **OP-2. Ebru Guney**
Bacterial isolation from Capnodis tenebrionis and determination of the insecticidal effects of these bacteria

14:30 **OP-3. Rami Horowitz**
Effect of UV-stabilized BtK on *Helicoverpa armigera* larvae after exposure to sunlight

14:45 **OP-4. H. M. Kariithi**
Identification of Cultivable Tsetse Gut Microbiota and Assessment of Their Probiotic Potential to Improve Fly Quality and Performance

15:00 **OP-5. Monika Maurhofer**
Root-colonizing fluorescent pseudomonads as biocontrol agents of insects

15:15 **OP-6. Zane Metla**
Analysis of the bacterial community associated to different life stages of the forest pest *Lymantria dispar.*

15:30 **OP-7. Chandrashekhar Patil**
Cloning, Characterization and Expression of a cry1B-type Gene of *Bacillus thuringiensis* to Combat *Capnodis tenebrionis* - a Coleopteran Pest of Stone Fruit Trees

15:45 **OP-8. Luca Ruiu**
Recent findings on *Brevibacillus laterosporus* toxins and virulence factors

16:00 **Coffee Break**

16:30 **Poster Session**

19:00 **Transfer hotels**

JUNE 13, 2017

ORAL SESSION: FUNGI

Convenor: Hermann Strasser

08:30 **OP-9. Nona Chkhaidze**
First report of enthomopatogenic fungi on the insect vector *Hyalesthes obsoletus Signoret* in Georgia

08:45 **OP-10. Deborah Kaiser**
Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* – the tricky path to an efficient formulation

09:00 **OP-11. Vivien Krell**
Exoenzymes improvepenetration and colonization of potato plants by endophytic entomopathogenic *M. brunneum*

09:15 **OP-12. Natalia Kryukova**
Possible variants of the interactions on tree-component system: entomopathogenic fungus *Beauveria bassiana* - great wax moth *Galleria mellonella* - ectoparasitoid *Habrobracon hebetor*

09:30 **OP-13. Johanna Mayerhofer**
Non-target effects of *Metarhizium brunneum* on soil microorganisms

09:45 **OP-14. Arne Peters**
Commercial production of conidia emitting granules for the soil application of *Metarhizium brunneum*

10:00 **OP-15. Jaka Razinger**
Evaluation of different strategies employing entomopathogenic and soil fungi against spotted wing drosophila (*Drosophila suzukii*)

10:15 **Coffee Break**

ORAL SESSION: FUNGI
Convenor: Jaka Razinger

10:45 **OP-16. Hermann Strasser**
Biological Control of Adult Diabrotica- Spray experiments with *Metarhizium brunneum* strain BIPESCO 5 under real farm conditions

11:00 **OP-17. Maria Zottele**
Metarhizium brunneum BIPESCO 5: a sustainable and preventive biological control agent to control *Diabrotica virgifera virgifera* larvae

11:15 **OP-18. Eustachio Tarasco**
Potential of entomopathogenic fungi and nematodes against *Drosophila suzukii* in laboratory assays

11:30 **OP-19. Oksana Tomilova**
Entomopathogenic fungi and natural avermectins are interact as a synergists

11:45 **OP-20. Maksim Tyurin**
Entomopathogenic fungi *Metarhizium spp*. from Russia and neighbouring territories, ecological preferences and activity against Colorado potato beetle larvae

12:00 **OP-21. Eustachio Tarasco**
Potential of entomopathogenic fungi and nematodes against the two cryptic species *Parahypopta caestrum* and *Cossus cossus* in laboratory assays

12:15 **OP-22. Francesca De Luca**
Exon-intron structure and sequence variation of the hsp-90 gene in the entomopathogenic nematode *Heterorhabditis bacteriophora*

12:30 **Lunch**

ORAL SESSION: NEMATODE
Convenor: Arne Peters

14:00 **OP-23. Itamar Glazer**
Response of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to physical stimuli by Red Palm Weevil: Behavioral and molecular analysis

14:15 **OP-24. Oleg Gorgadze**
Susceptibility of the cutworm, *Agrotis segetum* (Lepidoptera: Noctuidae) to entomopathogenic nematodes

14:30 **OP-25. Baris Gulcu**
Biological Control of *Naupactus godmani* (Curculionidae), Fuller Rose Beetle, with Entomopathogenic Nematodes

14:45 **OP-26. Javad Karimi**
Challenges toward implementation of microbial pesticide with emphasis on entomopathogenic nematodes as biocontrol agents in Iran

15:00 **OP-27. Ivan Kerchev**
Complex of endoparasitic nematodes of four-eyed fir bark beetle *Polygraphus proximus*, and their impact on its immunity

15:15 **OP-28. Branimir Njezic**
Efficacy of entomopathogenic nematodes against European cherry fruit fly *Rhagoletis cerasi* (L.) in laboratory and field conditions

15:30 **Coffee Break**

ORAL SESSION: NEMATODE/IPM
Convenor: Itamar Glaser

16:00 **OP-29. Martine Rehayem**
New insights in biocontrol strategy against *Cephalcia tannourinensis*, the principal insect defoliator of cedars in Lebanon

16:15 **OP-30. Rostislav Zemek**
Microbial and nematode control of the Colorado potato beetle

16:30 **OP-31. Sigal Braun Miyara**
The use of *Daldinia concentrica*, an endophytic fungus, and its bioactive volatiles against the plant parasitic nematode *Meloidogyne javanica*

16:45 **OP-32. Hilfred Huiting**
IPM in practical wireworm control; struggle or challenge?

17:00 **OP-33. Milan Pernek**
Comparison of pathogen abundance between high and low level populations of *Ips typographus* in Croatia after an ice storm disaster

17:30 **Transfer to Old Tbilisi. City Tour**

20:00 **Transfer to hotel**

JUNE 14, 2017

ORAL SESSION: VIRUS
Convenor: Johannes Jehle

08:30 **OP-34. Zihni Demirbag**
Functional Analysis of Putative glycosyl transferase gene (AMV248) of *Amsacta moorei entomopoxvirus*

08:45 **OP-35. Cihan Inan**
Protein-Protein Interactions Between Attachment (AMV248) and Entry Fusion Complex Proteins of *Amsacta moorei entomopoxvirus*

09:00 **OP-36. Michael D. Jukes**
Baculovirus synergism: investigating mixed alphabaculovirus and betabaculovirus infections in the false codling moth, *Thaumatotibia leucotreta*, for improved pest control

09:15 **OP-37. Miguel Lopez-Ferber**
Measuring the fitness cost of type I resistance breaking by CpGV

09:30 **OP-38. Sean Moore**
The isolation of a novel alphabaculovirus and its potential for microbial control of key tortricid moth pests

09:45 **OP-39. Marcel Tanner**
Integratet Pest and Resistance Management of *Helicoverpa zea* (Lepidoptera: Noctuidae) with a *Helicoverpa armigera nucleopolyhedrovirus*

10:00 **Coffee Break**

PLENARY SESSION: NOVEL TECHNIQUES FOR BIOLOGICAL CONTROL

Convenor: Giselher Grabenweger

10:30 **PL-5. Johannes Jehle**
Deciphering different mechanisms of resistance to CpGV products in codling moth field populations

11:00 **PL-6. Stefan Vidal**
From lab to registration: an Attract & Kill control strategy for wireworms in field crops: achievements and problems

11:30 **Business Meeting and Closing**

13:30 **Lunch**

14:30 **Excursion to Jvari and Mtskheta**

19:00 **Banquet**

ABSTRACTS:
PLENARY REPORTS

PL-0. An Introduction to IOBC

Giselher Grabenweger

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

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

IOBC-WPRS (West Palearctic Regional Section) currently comprises 20 working groups (WGs) focusing on specific crops (e.g. citrus, olives, viticulture, fruit crops, oilseed crops, vegetables), pest organisms (e.g. mites, plant pathogens), and methods (e.g. plant resistance breeding, application of pheromones and semi-chemicals, landscape management). WGs usually consist of about 40 to more than 100 members, including scientists, students, and representatives of governmental institutions, advisory services and the biocontrol business. Meetings take place every second or third year to help exchange recent scientific findings, draw attention to newly emerging plant protection issues, or share experience from laboratory and field tests. Lively discussions and excellent networking opportunities contribute significantly to the popularity of IOBC-WPRS WG meetings.

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

The conference in Georgia's Capital Tbilisi is already the 16th meeting of this truly international and very active WG. It is currently embedded in a very favorable environment, with a worldwide demand for reducing risks and application of chemical pesticides, and at the same time a growing interest in biological control. The main challenge of the WG remains nonetheless unchanged: to prove the potential of microbial and nematode control agents, to show the opportunities they provide in IPM, and to certify their safety to the user, the consumer and the environment.

PL-1. Development of Microbial Control Agents to Plant Protection from Pest Insects in Georgia: Past and Future

Tsisia Chkhubianishvili

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Georgia, a relatively small mountainous country in the Caucasus, has a variety of landscape zones and is rich by flora and fauna. It has a well-developed forestry, horticulture, viticulture, vegetable gardening, citrus, tea growing industry, subtropical crops and ornamental plants. The great damage to economy of Georgia cause the pest organisms (insects, rodents, weeds, fungi, bacteria, viruses etc.) distributed on the different plants. The needs for environment protection on the global level initiated the development of natural, biological means for pest control. Biological agents number regulation of pest insect populations and their use for plant protection have studied at Georgian Scientific Research Institute of Plant Protection from the day of its foundation (1930) and continue to the present. As a result of many years of research, we have a rich collection of entomopathogenic viruses, bank of different strains *Beauveria sp.* (storage place EBCL, France) and local entomopathogenic nematodes (EPNs). The initial efforts in this area were directed with searching the local "natural resources" - entomopathogens (fungi, viruses, nematodes) in pest populations, testing the local, introduced strains and commercial formulations. Currently the EPNs are considered as the most promising means among the biological control agents for pest organisms number regulation and therefore predominate data prevail regard to them. The investigations were conducted in different agrocenosis – vegetable cultures (closed and open grounds), vineyards, potato sowings, ornamental plants, sunflower crops. There is the preliminary data on biotechnology of nematode formulation on the base of local EPN, *Steinernema feltiae* "Georgian strain" (Identified by Prof. P. Stock, USA), tentatively called *"Geo-nema*". *S. feltiae* was tested to main pest of vine the grape berry moth, *Lobesia botrana, to* quarantine pest insect, the fall webworm, *Hyphantria cunea,* to the new, invasive quarantine insects, the South American tomato moth, *Tuta absoluta and* the serpentine leaf miner, *Liriomyza* sp. and etc. The possibility of join action - *S. feltiae* and parasitoid *Encarsia, - Encarsia formosa* to the greenhouse whitefly, *Trialeurodes vaporariorum* in greenhouse conditions has established. As the results, the *"Geo-nema" is* considered as the perspective bioformulation to control of different pest insects. The tested environmentally safe means will take important place in integrated pest management (IPM) system in closed and upon ground. Biological means are not produced in Georgia and the import from foreign countries is very expensive. Therefore, it is necessary to carry out self-production of biological means, as the potential possibilities have been already created in Georgia.

PL-2. Including Baculoviruses in Insect Control Strategies – Opportunities and Challenges

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Baculoviruses (BV) are successfully used for many years for an efficient control of insect pests in agriculture. BV insecticides are highly specific and thus, beneficials are not harmed. Therefore, they are mainly used for the control of key pests. However, there is an increasing demand for BV insecticides also in integrated pest management due to the residual situation and the increasing occurrence of resistance problems.

In close collaboration with researchers and distributors, Andermatt Biocontrol is developing strategies, which allow using BV products for the control of pests in complexes. Field trials are performed in the USA with the two products Spexit® (SeNPV) and Loopex® (AcNPV), applied alone or in combination.

In fresh market tomato, high rates of Spexit as well as low rates of Spexit in tankmix with Xentari (Bta) showed the best results with around 70% efficacy. Furthermore, Spexit was an excellent rotation partner for Spinosad. In a second trial with Loopex in broccoli, efficient cabbage looper (*Trichoplusia.ni)* control was found. The development of late larval instars, which are mainly responsible for feeding damage, was successfully avoided. Likewise, good efficacy of Loopex in combination with Btk was shown, also controlling secondary pest. Both products Spexit and Loopex were applied in a third trial in cabbage in combination with organic and conventional products.

Results show that NPV's are valuable tools for IPM production systems with similar efficacy to growers standard.

Key words: baculovirus, control strategies, pest complex, Spexit, Loopex, SeNPV, AcNPV.

PL-3. Efficacy and Persistence of Entomopathogenic Nematodes (EPNs) Against Burrowing and Sedentary Insects

Itamar Glazer

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Burrowing and sedentary insects provide a challenge for efficient use of entomopathogenic nematode (EPN) since the later need to allocate and reach them from a distance. In our lab we gained substantial experience in use of EPN against such insect. The white grub *Maladera matrida,* the Red Palm Weevil *Rhynchophorus ferrugineus* and the Peach Flatheaded Rootborer *Capnodis tenebrionis*.

The presentation will describe the serious of laboratory and field trials for control of these pests. For control of *Maladera matrida* the EPN were applied using different application methods: Spray, irrigation and soil injection. The presence of nematodes in the soil was evaluated using '*Galleria* traps'. Application of *Heterorhabditis bacteriophora,* resulted in 80% reduction in damage to the peanuts with no affect on the yield.

We studied the attraction behavior of the EPNs to the RPW under simulated natural conditions in columns to evaluate their infective potential. In all experiments a major proportion (38 to 48%) was attracted to the host. Both *H. bacteriophora* and *S. carpocapsae* were efficient crawlers, climbing up and descending when locating their insect host. They were efficiently attracted to the various larval sizes and stages of the RPW life cycle. Host localization by ascending movement was more prominent in *S. carpocapsae* than in *H. bacteriophora*.

The efficacy of the EPN to control of *C. tenebrionis* larvae inside the tree root system. The experiments were conducted in a commercial plantation. Nematodes (*S. carpocapsae, S. feltiae* and *H. bacteriophora*) were applied at rates of $1\text{-}3*10^6$ infective juveniles per tree. The results indicate substantial reduction of insect infection by 70-80%. In all trails, nematodes appeared to be active during the entire growing season. Towards the end of the season, nematode activity was also detected in the un-treated control plots.

PL-4. Using Insect Pathogenic Fungi to Manage Insect Pests: Where Are We Going? (Where Should We Be Going?)

Stefan T. Jaronski

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Abstract. Since the initial efforts to take advantage of entomopathogenic Ascomycetes in the 19th Century, with the work of Metchnikoff with *Metarhizium* in Russia and the Kansas Department of Agriculture in the U.S. with *Beauveria*, practical exploitation of these fungi has steadily increased to the present day, slowly at first, then with increasing rapidity during the past three decades. Today, these fungi are a significant component of microbial biopesticides. A 2007 survey recorded over 110 commercial products using Ascomycete; today there are about 160. How are we using them? All too often, we use them as inundative, catastrophic mortality factors, i.e. like chemicals, frequently leading to user disappointment. In response, new and often ingenious methods have been devised to improve efficacy, through formulations, application methods, even genetic modification. In recent years new, potentially exciting uses have also appeared, changing our perception of these microorganisms — as plant endophytes that can affect herbivorous insects either directly or via induced systemic resistance. Regardless, how should we be using them? The answer lies in the context of integrated pest management, in which biopesticides are just one component of a holistic, sustainable agriculture and not in a chemical paradigm.

Key words: integrated pest management, *biopesticides*, *Beauveria*, *Metarhizium*

PL-5. Deciphering different mechanisms of resistance to CpGV products in codling moth field populations

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

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Cydia pomonella granulovirus (CpGV) products are of eminent importance for the environmentally safe control of codling moth (*Cydia pomonella*, L., CM) in organic and integrated pome fruit production. During the last decade, more than 40 orchards with CM populations resistant to CpGV products have been identified in different European countries. Thorough genetic analyses and resistance testing revealed that not all CM populations follow the same mode of resistance. Indeed, three types of resistance (type I-III) can be distinguished and differ strongly in their susceptibility to CpGV isolates of different genome groups A-E and their inheritance pattern. Type I resistance is Z chromosomal inherited and is targeted only against CpGV belonging to genome group A. Type II resistance is autosomal inherited and also targeted against A, D and E. Finally, type III resistance appears in certain aspects like a mixture of both type I and type II resistance. This presentation will provide an update on these novel findings on CpGV resistance in CM field.

Key words: Codling moth, granulovirus, resistance, inheritance, resistance testing

PL-6. From lab to registration: an Attract & Kill control strategy for wireworms in field crops: achievements and problems

Stefan Vidal[1]*, Anant Patel[2], Wilhelm Beitzen-Heineke[3]

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Wireworms are a major pest problem in many arable and vegetable cropping systems in most European countries, including potato, corn, carrots, or lettuce, just to mention a few, both in organic and conventional farming systems. Control options targeting wireworms are limited, due to the banning of effective insecticides (i.e. organophosphates) or phasing out decisions (i.e. fipronil) in Europe. Even more dramatic is the current situation for organic growers, because only ineffective or expensive options are available. Within an EU funded project, we aimed at developing a new control strategy, combining behavioral traits of wireworms with an effective biocontrol agent.

Wireworms use carbon dioxide gradients, established by growing roots, as long-distance cues to locate their host plants. We made use of this orientation cue by developing a dry capsule system, which, following application into the soil, re-hydrates and starts to produce CO_2 (i.e. providing the attract component). Simultaneously an entomopathogenic fungal isolate was formulated into the capsules (i.e. providing the kill component) allowing the hyphae to grow out of the capsules and to sporulate on the surface following re-hydration. When coming into contact with this A&K capsules, wireworms will be infected and will be killed after a certain time. To make this strategy work under field conditions, the capsules need to build up CO_2 gradients significantly higher than the background CO_2 concentrations in the soil for at least several weeks. Moreover, the entomopathogenic fungal isolate needs to target a set of different wireworm species, typically found together in potato or crop fields to guarantee high mortality rates.

In the meantime, this capsule type, developed by the EU Project, has been registered (ATTRACAP®) based on clause 53 of EU regulation 1107/2009 and has been proven effective in farmer fields in 2016 with an average efficacy above 60%. However, the use of the product is constraint by some abiotic and biotic parameters, which need to be addressed in future research during further field tests. Moreover, although the final registration of ATTRACAP® is on the way, several time-consuming restrictions, such as data acquisitions, and additional field evaluations, will delay final registration.

Based on the results compiled so far from field tests the A&K strategy offers potential for the control of wireworms; however, several adaptations of this strategy to other crops than potato need to be assessed and will be discussed in detail.

Acknowledgements. The development of this product was funded by the EU project INBIOSOIL No. 282767

ABSTRACTS:
SECTION BACTERIA

OP-1. Bacterial based biopesticide production for pest management

Ardahan Eski*, Kazım Sezen, Zihni Demirbag, Ismail Demir

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Chemical pesticides had made a great contribution to the battle against pests and diseases. However, their use resulted in the development of insecticide resistance, extensive damage to the environment, and lethal effects on nontarget organisms. Biopesticides, key components of integrated pest management programs, are receiving practical attention as a means to reduce the amount of synthetic chemical products being used to control plant pests. In order to develop an effective biopesticide against coleopterans, particularly against *Agelastica alni* (Coleoptera: Chrysomelidae) which is one of the important pests of alder leaf and hazelnut, we tested 21 Bacillus isolates originated from insects against the larvae of pest at the laboratory condition. According to screening test *Bacillus thuringiensis* var. *tenebrionis* (Xd3) showed the highest insecticidal effect on *A. alni*. Then, cultivation of Xd3 was carried out in a laboratory scale fermentor and cells were harvested by centrifuge. After production, cells were mixed with other ingredients and dried by spray dryer. Spore count of the product were determined as $1,6\times10^{10}$ cfu/g. Moisture content, suspensibility and wettability of the product were determined as 8.3%, 86% and 21 s, respectively. The product showed 94% mortality on larvae at laboratory conditions at 10^9 cfu/ml concentration. LC_{50} were determined as $0,15\times10^5$ cfu/ml. Product was also yielded 88% mortality against larvae of *A. alni* at field condition at 10^9 cfu/ml concentration. LC_{50} values were determined as $0,45\times10^6$ cfu/ml at the field condition on larval stage.

Key words: *Bacillus thuringiensis* var. *tenebrionis,* Biopesticide, Spray Dryer, Insecticidal effect

Acknowledgements: This research was supported by The Scientific and Technological Research Council of Turkey (113Z904 and 2211-C)

OP-2. Bacterial isolation from *Capnodis tenebrionis* and determination of the insecticidal effects of these bacteria

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Capnodis tenebrionis is one of the pests which create critical harm on the most of fruit trees from Coleoptera order. Therefore, struggle with these harmful pests is very crucial in both in our country and in the countries of Asia and Europe. The struggle with *C. tenebrionis* is done by insecticides. However, the alternative methods have gained importance because of insecticides's the damage of both nature and all creatures. Because of this reason, in this studying which aimed to struggle with this pest, 21 bacterial isolates from larvae and adults of *C. tenebrionis*'s have been obtained and the morphological, physiological, biochemical and molecular properties have been defined. Due to this characterizationstudies, the bacterial flora of *C. tenebrionis* was defined as, *Bacillus cereus* (E-1, E-4, E-5, E-6, OL-4, L-2, L-5, L-6) *Bacillus mycoid*es (OL-1), *Bacillus pumilus* (L-7), *Paenibacillus xylanilyticus,* (L-8), *Bacillus flexus* (L-9), *Bacillus simplex* (L-10, OL-5), *Raoultella terrigena* (L-1), *Enterobacter cloacae* (L-3), *Bacillus anthracis* (L-4), *Klebsiella oxytoca* (E-2), *Bacillus safensis* (E-3), *Bacillus amyloliquefaciens* (OL-2), *Bacillus aryabhattai* (OL-3). The insecticidal activities of the bacterial isolates on the larvae of the elm leaf beetle (*Pyrrhalta luteola*), the mealworm (*Tenebrio molitor)* and the honeycomb moth (*Galleria mellonella*) were investigated. It is detected that isolates with a number of ÖL-4, E-4 and E-5 have the highest lethal effect on these harmful larvae. These isolates are considered as an effective pest control factor.

Key words: Bacterial flora, Biological control, *Capnodis tenebrionis*, Coleoptera

Acknowledgements: This research was supported by The Scientific and Technological Research Council of Turkey (2210-C) and KTU BAP (FYL-2016-5366).

OP-3. Effect of UV-stabilized BtK on *Helicoverpa armigera* larvae after exposure to sunlight

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Helicoverpa armigera (Heliothine moth**,** [*Lepidoptera: Heliothinae*]) is an insect pest, which harms field crops, vegetables, cotton and flowers in the most severe way. A good solution for control of *H. armigera* and other lepidopteran larvae, especially in organic farms, are treatments with *Bacillus thuringiensis* (Bt).

The problem of spraying with Bt is its sensitivity to UV light emanating from the sun, making the spray preparation short lived. The laboratories of BotanoCap, Israel has developed a formulation, which may be stable to radiation and might be active for longer periods of time, compared with the regular preparation.

In our experiments, we compared the efficacy of BotanoCap's encapsulated B.t. var kurstaki formulation, which is considered to be UV-resistant, to a non-encapsulated standard preparation. Both formulations were tested with and without exposure to sunlight.

In the conditions set in these experiments, exposure to sunlight of the encapsulated formulation did not cause a reduction in mortality of *H. armigera* larvae (3rd instars) in comparison with the standard (non-encapsulated) preparation. The non-encapsulated formulation was a bit less effective without exposure to the sunlight, and lost much of its efficacy after exposure to about four hours of sunlight.

It is advisable to repeat the experiments in the field, with direct spraying on the crop plants.

Key words: Lepidopteran pests, microbial control, Bt, encapsulated formulations, cotton.

OP-4. Identification of Cultivable Tsetse Gut Microbiota and Assessment of Their Probiotic Potential to Improve Fly Quality and Performance

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The control of the tsetse vector via the sterile insect technique (SIT) involves mass production of sexually sterile males. However, the irradiation procedure used to induce male sexual sterility is also suspected to cause damages the gut epithelia and the beneficial gut-inhabiting microbiota of the flies. These effects may reduce the quality (sexual performance and competitiveness) of the sterile males, thereby negatively affecting the success and operational costs of SIT. It is therefore paramount to enhance the quality of the sterile males. A potential strategy to achieve this is to exploit the beneficial traits conferred by the gut microbiota to their insect hosts. We used culture-dependent and culture-independent methods to determine the variability of tsetse gut microbiota, identify which of the bacterial species are cultivable, and assess the amelioration potential of a selection of the cultivable species to improve the quality of sterile males. Both methods were based on PCR-amplification of the bacterial *16S rRNA* gene. The microbiota diversity and abundances were performed at different fly developmental stages (larvae, pupae and adults), and on adult flies of different ages that emerged from pupae irradiated at day 22 (110 Gy) compared to flies from non-irradiated pupae. The probiotic potential of the bacteria was assessed by offering experimental flies with blood meals supplemented with a selection of candidate bacterial isolates. Amongst the identified tsetse gut microbiota were members of family *Microbacteriaceae* (genus *Microbacterium*), *Sphingobacteriaceae* (genus *Sphingobacterium*) and *Moraxellaceae* (genus *Acinetobacter*). These bacterial species have been reported in other insects, some of which are documented to confer beneficial traits in their insect hosts. These results indicate that the densities of the cultivable gut microbiota significantly decrease from six to 10 days old flies; this age is important for the release of SIT sterile males into the wild populations for vector control. Secondly, the gut microbiota community was composed of both beneficial and potentially harmful bacterial species. Consequently, in addition to exploiting the beneficial bacteria, it may be necessary to design a strategy to reduce the populations of the harmful bacterial species. In terms of SIT applications, the identified bactria species, esecially the species demonstrated to confer anti-parasitic benefits to some insects, are ideal candidates that can be targeted to enhance anti-trypanosome capabilities of SIT sterile males.

Key words: 16S rRNA gene, Vector competence, Probiosis, Trypanosomes, SIT

OP-5. Root-colonizing fluorescent pseudomonads as biocontrol agents of insects

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Abstract: Fluorescent pseudomonads are well known for their manifold plant-beneficial activity. Several strains have already been commercialized and are successfully used as plant strengthener or biocontrol agents against fungal diseases. The discovery that certain phylogenetic groups of these bacteria comprising the species *Pseudomonas protegens* and *P. chlororaphis* also have insecticidal in addition to antifungal activity opens new and exciting perspectives for biocontrol. We are currently investigating the mechanisms and traits enabling these pseudomonads to colonize and kill insects, the ability of the bacteria to persist throughout different stages of the insect life cycle and their potential to control insect pests. Our results show that pseudomonads, when taken up by larvae upon feeding can persist in insects until the adult stage. The major problem in using pseudomonads for insect control is that in order to achieve high mortality rates, insect larvae have to take up high cell numbers orally. However, the performance of insecticidal pseudomonads can be markedly improved, respectively applied cell numbers reduced, if the target insect already is weak, or if the bacteria directly get into the insect hemolymph. Therefore, we combine insecticidal pseudomonads with other entomopathogenic biocontrol agents e.g. the nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* or the fungus *Metarhizium anisopliae*. In addition to biocontrol activity, root-colonizing insecticidal pseudomonads can also have indirect beneficial effects by activating the defense mechanisms of plants against insect herbivores and plant pathogens. First pot and field trials have shown that insecticidal pseudomonads and entomopathogenic nematodes are compatible and indicate, that insect damage can be reduced, not only by direct, but also by indirect plant-mediated effects.

Keywords: Insecticidal activity, *Pseudomonas protegens*, *Pseudomonas chlororaphis*, entomopathogenic nematodes, pest control, combination of biocontrol agents

OP-6. Analysis of the bacterial community associated to different life stages of the forest pest *Lymantria dispar*

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Abstract:Gypsy moth (*Lymantria dispar,* Lepidoptera) outbreaks can cause significant damage to the forestries across Europe. Gypsy moth can furthermore be a threat to orchards. In Latvia, the first mass outbreak was recorded only in 2008 (Silins, Smits, 2010), since then we have started to investigate the possibilities to control this pest.

Insects are colonized by various microorganisms and insect-associated microbiota can play an essential role in the growth, development, health and environmental adaption of insects. Most studies of the lepidoptery on microbiota so far focused on microorganisms associated to the gut. However, a lepidopteran insect goes through four life stages and each stage has its own morphology and develops its own microbial community. Up to now, there is only little knowledge on the role the different bacteria may have during different stages of the insect life cycle. A profound knowledge on the composition of the microflora and the role it might play in insect development can be used for the development of new pest management strategies.

The objective of this study performed within the frame of the SCIEX program with ETH Zürich and the University of Daugavpils as partners was to characterize the bacterial community of *Lymantria dispar* and to monitor 1) differences between individual Lymantria dispar larvae, 2)changes during larval development from the first to the fourth Instar), and 3) bacterial community changes during the life cycle (eggs, larvae, pupae, adults). Full-length 16S rRNA genes were sequenced using PacBio technology. The Mothur software package was used to process sequenced reads.

Our results showed that the diversity and community composition substantially changes during different *Lymantria dispar* life stages. Interestingly, the microbial community in the egg masses was more diverse (34 OTU's at 0.03 cutoff level) compared to larvae, pupae and adults. In larvae, the lowest number of OTU's (14 OTU's at 0.03 cutoff level) was detected. Our findings further indicate that the community composition in the midgut of individual larvae is relatively simple and

dominated by *Enterococcus* sp. We observed differences between individual larva and structural changes of diversity in bacterial communities through larval development.

Key words: *Lymantria dispar*, lifecycle, microbial community

OP-7. Cloning, Characterization and Expression of a cry1B-type Gene of *Bacillus thuringiensis* to Combat *Capnodis tenebrionis* - a Coleopteran Pest of Stone Fruit Trees

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Conventional methods fail to control the flat-headed borers *Capnodis spp.* (Coleoptera; Buprestidae), major pests of stone fruit trees; the larvae are protected from insecticides and predation because they feed deep in the roots. A potential solution is transgenic rootstock producing in their roots toxic compounds such as *Cry* toxins of *Bacillus thuringiensis* (Bt). In the present study, a new gene, *cry1Bd*, was cloned from a field-isolate of Bt K4 that has been demonstrated toxic to 3 *Capnodis spp* (Gindin et. Al., Pest Manag Sci, 2014). The BLAST analysis of two directional sequence results confirmed that the cloned gene corresponded to *cry1Bd* group, is 3696 base pair long. *cry1Bd* was ligated into pLATE series of bacterial expression vectors and transformed in *Escherichia coli* ER 2566 under the control of T7 promoter induced by isopropyl-beta-D-thiogalactopyranoside (IPTG). In next phases, protein expression, purification and bioassay studies of recombinant strain will be performed.

Key words: *Bacillus thuringiensis*, cloning, cry proteins, coleoptera, pests, diseases, integrated control

OP-8. Recent findings on *Brevibacillus laterosporus* toxins and virulence factors

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Brevibacillus laterosporus is a spore-forming bacterium showing significant biopesticidal potential against insect species in different orders including coleoptera, lepidoptera, diptera and against nematodes and molluscs. A main morphological feature of this microbial species is the typical canoe-shaped parasporal body attached to one side of the spore. Recent genome sequencing and analysis of entompathogenic strains led to the identification of several toxins and virulence factors. Among these, some mosquitocidal toxins, vegetative insecticidal proteins, chitinases, and several polyketides and nonribosomal peptides. Besides, highly conserved proteins from the spore coat canoe-shaped parasporal body complex (SC-CSPB) of entompathogenic strains were reported to be involved in pathogenesis. Whilst different strains show varying degrees of virulence against diverse insect pests, some represent a beneficial component of the intestinal bacterial community of certain species like the honey bee *Apis mellifera*. Unexpectedly, a strain isolated from honeybees was pathogenic to the house fly *Musca domestica*. These findings, supporting the development of either mutualistic or pathogenic interactions of this bacterium with diverse insect species, as the result of a coevolutionary process, are here presented and discussed.

Key words: entomopathogens, toxicity, pathogenesis, bacteria, pests

PP. 1. Genetic Diversity of *Bacillus thuringiensis* Isolates from Cuba

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The Gram-positive bacterium *Bacillus thuringiensis* or "Bt" is the economically most important entomopathogen for insect biocontrol. Systematically, *B. thuringiensis* forms a sub-species within the *Bacillus cereus sensu lato* complex. The bacterial pathogen kills its host through the action of highly specific, crystal-forming Cry protein toxins (in addition to further toxin types). A large number of *cry* toxin encoding genes have been analyzed and organized into several groups that in part reflect host group adaptation. In particular, proteins encoded by *cry1, cry3,* and *cry4* genes are generally toxic for Lepidopteran, Coleopteran, and Dipteran insects, respectively. In the present study, the molecular taxonomy and *cry* gene diversity of a set of Bt isolates from Cuba were investigated.

Genes *pycA* and *glpF* that had previously been established as markers for molecular taxonomic studies of the *Bacillus cereus sensu lato* complex (Priest et al. 2004, J. Bacteriol 186: 7959-7970), were amplified and sequenced for the set of bacterial isolates. Phylogenetic reconstruction located all isolates within the *B. cereus* complex and unambiguously differentiated them from human pathogenic *B. cereus* and from *B. anthracis.* Moreover, Cuban isolates were shown to belong mainly to two Bt lineages, namely "Kurstaki" and "Sotto".

When a PCR-based diagnostic approach using *cry1, cry3*, and *cry4* gene specific primer sets was employed in a first assessment of *cry* gene diversity, most isolates appeared to comprise either *cry1* or *cry4* genes, whereas *cry3* copies were not detected in any of the Bt strains.

Key words: *Bacillus thuringiensis*, Multilocus sequence analysis (MLSA), *cry* genes

PP. 2. Genetic Characterization of *cry* Gene Diversity in *Bacillus thuringiensis* Isolates from Kyrgyzstan

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The Gram-positive bacterium *Bacillus thuringiensis* (i.e. a subspecies of the species *Bacillus cereus sensu lato* that is currently referred to as "*Bt*") is the economically most important entomopathogen for insect biocontrol. *Bt* kills its host through the action of highly specific, crystal-forming Cry protein toxins and further toxin types. A large number of *cry* toxin encoding genes have been analyzed and organized into several groups that in part reflect host group adaptation. In particular, proteins encoded by *cry1, cry3,* and *cry4* genes are generally toxic for Lepidopteran, Coleopteran, and Dipteran insects, respectively.

A set of *Bt* strains isolated from insect and soil samples from different environments in Kyrgyzstan was molecular taxonomically characterized using the *pycA* gene encoding bacterial pyruvate carboxylase as marker for phylogenetic reconstruction. Within a sequence space spanning the whole *Bacillus cereus sensu lato* species complex, all Kyrgyz isolates were shown to belong to the *B. cereus* subspecies *thuringiensis* and are, therefore, clearly distinct from human pathogenic *B. cereus* subspecies including *B. anthracis*. Among the lineages that further subdivide *Bt,* most isolates from Kyrgyzstan were assigned to *Bt tolworthi*, with two isolates each belonging to the lineages *Bt kurstaki* and *Bt sotto*.

Moreover, a PCR-based diagnostic approach using *cry1, cry3*, and *cry4* gene specific primer sets was employed in a first assessment of *cry* gene diversity throughout this set of Kyrgyz *Bt* isolates and revealed pronounced differences in *cry* gene frequencies. Whereas *cry1* and *cry4* genes were regularly detected, *cry3* genes were identified in only a small number of strains investigated. Several copies of *cry1* or *cry4* genes appeared to be present simultaneously in numerous isolates. Interestingly, the combination of *cry1* and *cry4* genes in the same strain was frequent, whereas the combination of *cry1* and *cry3* occurred only in a single *Bt* strain.

We conclude that a high degree of *cry* gene diversity is present within the set of *B. thuringiensis* isolates from Kyrgyzstan. The rather regular presence of several *cry* gene copies, potentially combining protein toxins of different specificities, within a single strain is of high interest with respect to the possible application of these strains for biocontrol purposes.

Key words: *Bacillus thuringiensis*, *cry* gene diversity, molecular taxonomy, pyruvate carboxylase (*pycA*)

PP. 3. Isolation and Screening of Extracellular Proteases Produced by different Entomopathogenic *Bacillus thuringiensis* isolates

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Proteases create the largest group of enzymes in bioindustry with extensive usage area. They have an unbeatable role in industrial biotechnology, especially in detergent, food and pharmaceutical arenas. *Bacillus* sp. being industrially important organisms produces a wide variety of extracellular enzymes including proteases. The purpose of this research was to appraise protease production by different local *B.thuringiensis* isolates obtained from different insect species previously. Sixty bacterial isolates exhibited proteolytic activity based on clear zone formation on skim milk agar medium. Six protease producing isolates (Eca4, Eca8, Lyd9, Lyd6, Tp7, N9) were selected on the basis of gelatin hydrolyses. The extracellular proteases were examined using fermentation production medium and it is seen that these six bacterial cultures produced proteases at varying levels from 0.76 µmol ml^{-1} min^{-1} to 1 µmol ml^{-1} min^{-1}. These isolates were selected for further studies.

Key words: Extracellular enzyme, proteases, *Bacillus thuringiensis*.

ABSTRACTS:
SECTION FUNGI

OP-9. First report of enthomopatogenic fungi on the insect vector *Hyalesthes obsoletus Signoret* in Georgia

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The polyphagous soil born plant hopper *Hyalesthes obsoletus* Signoret, 1879 - (Hemiptera: Cixiidae), is the vector of phytoplasma diseases in various crops of economic importance. Among them is grapevine bois noir (BN) disease, caused by stolbur phytoplasma (16SrXII-A group). BN disease now occurs in Europe, Israel, Libya, thouth America, Australia, Turkey and Georgia. It was reported that in Eastern Georgia on grapevine and bindweedplants. Two phytoplasma strains, are associated with grapevine yellows 'Candidatus Phytoplasma solani' (subgroups 16SrXII-A) and 'Ca. P. a convolvuli' (subgroups 16SrXII-H).

H. obsoletus were found on the grapevine (*Vitis vinifera*), sunflower (*Helianthus annuus*), liquorice (*Glycyrrhiza glabra*) and bindweed (*Convolvulus arvensis*) in Sighnaghi district of Eastern Georgia, 2014-2016. Its imagoes were met in June, July and August.

Adults were collected on sunflower crop in Sighnaghi district, village Bodbe, while I and II decades of Juley 2016. Laboratory researches shown, that some examples had visual symptoms of fungal infection. The pure culture consisted entomopathogenic fungi *Beauveria bassiana*.

The strain of *B.bassiana* from Bodbe have been used as biological control agent against *Hyalesthes obsoletus.*

Key words: *Hyalesthes obsoletus,* vector, phytoplasma diseases, *Beauveria bassiana*

OP-10. Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* – the tricky path to an efficient formulation

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Pollen beetles (*Meligethes* spp.) cause substantial yield loss in oilseed rape cultivation throughout Europe. High level insecticide resistance and narrow control options in organic oilseed rape production motivate the research for alternative control methods. Studies with the entomopathogenic fungus *Beauveria bassiana* showed high pollen beetle mortality in laboratory experiments but limited impact on yield in field trials so far (Kuske S. et al., 2011). To improve the efficacy of entomopathogenic fungi in field applications, we explored combinations of *B. bassiana* spores with vegetable oils or stone dusts that have previously been shown to reduce pollen beetle abundance (Dorn C. et al., 2013). Further, natural UV protective compounds were tested to prolong *B. bassiana* spore viability when exposed to sunlight.

The combined application of *B. bassiana* spores and 2% vegetable oil caused a pollen beetle mortality of up to 70% in laboratory experiments. Remarkably, the two active compounds exhibited a synergistic interaction that accounted for 20% of observed pollen beetle mortality.

Formulation of *B. bassiana* spores with natural UV protectants resulted in 80-100% spore survival when exposed to 40kJ/m^2 artificial UV-B irradiation, relative to untreated spores. This equals the UV-B radiation dose expected at two sunny days in April under Swiss lowland conditions. A combined treatment with *B. bassiana* conidia, vegetable oil and a natural UV-protectant was tested in a field experiment in 2016. Pollen beetle abundance in treated plots was lower than in control plots one, three and seven days after application, respectively. Unfortunately, the impact of a strong hail did not allow any interpretation of yield results. The promising biocontrol strategy will be tested in a further field experiment in spring 2017.

Key words: *Beauveria bassiana*, entomopathogenic fungi, formulation, *Meligethes* spp., vegetable oil, synergistic interaction, UV protection

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OP-11. Exoenzymes improvepenetration and colonization of potato plants by endophytic entomopathogenic *M. brunneum*

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Biocontrol of insect pests with entomopathogenic fungi is challenging because of the lower efficacy, difficult handling and limited shelf life of these microorganisms compared to synthetic pesticides. However, recent studies have provided evidence that some of these fungi can grow endophytically in plant tissues, paving the way for novel plant protection measures. Therefore, the overall aim of our investigations is to combine fine-tuned cultivation of "endophytically competent "biomass with customized formulations that support the delivery of *M. brunneum* into potato plants for a systemic protection from herbivorous insects, like wireworms and Colorado potato beetles.

Inspired by penetration mechanisms of phytopathogenic fungi, we hypothesized hat an increased expression of plant cell wall-degrading enzymes, such as pectinase, hemicellulase and cellulase will improve plant penetration and colonization. Here, we present results on the effect of different cultivation and formulation additives to induce enzyme expression in preconditioned biomass. To investigate the effect of several additives on enzyme expression during submerged cultivation, pectin, hemicellulose and cellulose derivatives were supplemented to cultivation media. In a second approach, the same biopolymers were incorporated into bead matrices to look into the substrate specific enzyme expression driven by formulation. Enzyme activity was correlated with the penetration efficacy on potato tubers as well as with the colonization success in plant roots and shoots by using real-time PCR with *M. brunneum* specific primers. To flank this data, endophytic *M. brunneum* was re-isolated from plant tissues and the fungus was identified by PCR.

First experiments on the general influence of cell wall-degrading enzymes on the colonization success on potato plants showed that the addition of cellulase and hemicellulose enhanced endophytic *M. brunneum* emergence in roots by 60% and 20%, respectively. Pectinase supplementation alone had no effect on plant colonization. We therefore focused on the induction of cellulase expression and found that after supplementation of cellulose during submerged cultivation,

cellulase activity was verified in the supernatant. When incorporated into beads, a combination of amidated pectins, starch, cellulose and mycelium led to an increased expression of pectinases, amylases and cellulases. After application of these beads on potato tubers, endophytic *M. brunneum* was detected in tubers, roots as well as in shoots after 21 days of incubation.

These results demonstrate successful induction of enzyme expression during cultivation and in a formulation. Delivery of encapsulated mycelial biomass to potato tubers enabled endophytic establishment of *M. brunneum* in tubers, roots and shoots. First results suggest a correlation between the expression of cell wall-degrading enzymes and endophytic *M. brunneum* emergence.

On-going experiments are dealing with the impact of formulation additives and fungal enzyme expression on endophytic insect virulence. In addition, further beneficial effects of the formulation, such as plant growth promotion, will be evaluated.

Key words: *Metarhizium*, endophytes, formulation, enzymes, pests, integrated control

OP-12. Possible variants of the interactions on tree-component system: entomopathogenic fungus *Beauveria bassiana* - great wax moth *Galleria mellonella* - ectoparasitoid *Habrobracon hebetor*

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Interactions in the three-component parasitoid – entomopathogen – insect-host system have attracted significant attention in studies on status of competition, host immune reactions and biological pest control. *Habrobracon hebetor Say* (Hym.: Braconidae) is an idiobiont, gregarious, polyphagous ectoparasitoid of many lepidopteran larvae. The *H. hebetor venom* causes a complete and permanent paralysis of host larvae. Venom strongly suppresses the cell and humoral immunity of the host, including suppression of phenoloxidase (PO) activity in the hemolymph and hemocytes, as was shown previously on the wax moth larvae (*Galleria mellonella*).

The vectoring of the entomopathogenic fungus *Beauveria bassiana* by ectoparasitoid females among the wax moth larvae *Galleria mellonella* was explored under laboratory conditions. Vectoring occurred both from infected parasitoids (adhesion stage) to wax moth larvae and from infected (adhesion stage) to healthy wax moth larvae by parasitoids. The efficacy of vectoring in both cases was dose-dependent. Parasitoid females were unable to recognize infected larvae in a labyrinth test. Envenomation by *H. hebetor* increased conidia germination on the cuticles of the wax moth larvae by 4.4-fold. Both envenomation and mycoses enhanced the phenoloxidase (PO) activity in the integument of *G. mellonella* and, in contrast, decreased the encapsulation rate in hemolymphs. We hypothesize that changes in the fungistatic properties of cuticle and inhibition of cellular immunity provide the highest infection efficacy of entomopathogenic fungi with *H. hebetor*.

Local fungistatic effect have been detected at the parasitoid larvae feeding places. This effect had lead us to suppose the influence of the enzymes or some gut juice components from H.hebetor larvae on the fungi development. Entomopathogenic fungus Beauveria bassiana was used in the investigations. However, in the control samples as well as in the samples in which aliquots of the gut's supernatant (0.3 mg/ml) were added no significant difference was detected. The following studies have been focused on the analysis of the parasitoid's larvae microbiota, which can suppress or prevent the growth of entomopathogenic fungus. Ten bacterial strains were isolated from the gut of the H. hebetor larvae. Analysis of the interaction

between the isolated bacteria and fungi in vitro was conducted. We showed that six strains had antagonistic activity against entomopathogenic fungus B.bassiana leading to the formation of sterile zone around the agar-agar blocks of bacteria (till 14 mm). Further investigations will be focusing on the bacterial identification.

The obtained results show that changes in the integument property and inhibition of cellular immunity by H. hebetor provide vectoring and the highest infection efficacy by entomopathogenic fungi. At the same time, some metabolites, produced by gut microflora of parasitoid larvae, are able to suppress the growth of the entomopathogenic fungi. On the one hand that uneasy equilibrium allows braconidae finish its behavior and on the other hand provide the highest infection efficacy of entomopathogenic fungi even with low doses of conidia.

Key words: Ectoparasitoid, Entomopathogenic fungi, Vectoring, Fungistatic.

OP-13. Non-target effects of *Metarhizium brunneum* on soil microorganisms

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Biological control based on entomopathogenic fungi includes application of large quantities of fungal propagules to soil, often resulting in densities of up to 10^{14} propagules per ha. Such mass applications potentially affect soil microorganisms (fungi and prokaryota) and the ecosystem functions they fulfil. The goal of the study was to test for such non-target effects in experiments to control *Agriotes obscurus* (pot and field) or *Diabrotica virgifera virgifera* larvae (pot) with the fungal entomopathogen *Metarhizium brunneum*. Four different formulations of *M. brunneum*, two co-formulations of *M. brunneum* and garlic extract, garlic extract alone as well as an insecticide and formulation controls were tested. Non-target effects of *M. brunneum* on soil fungi and prokaryota were assessed using Illumina high throughput sequencing of the ribosomal internal transcribed spacer region 2 and the variable regions V3-V4 of the small ribosomal subunit, respectively. *M. brunneum* densities and soil microbial communities were monitored over a period of four months.

Applications of *M. brunneum* led to a 10 to 100-fold increase in abundance of *Metarhizium* CFU per g soil dry weight. *M. brunneum* formulated as fungus colonized barely kernels was the most efficient treatment in controlling *A. obscurus* larvae in the pot experiment with 77% reduction of potato tuber damage compared to untreated control pots. No biocontrol effect was detected in the pot experiment to control *D. v. virgifera*. Formulated *M. brunneum* affected fungal communities only slightly and only in the *Agriotes*-pot experiment. Prokaryotic communities changed upon treatments including garlic or some of the formulation controls. All effects detected were in the range of the effects caused by formulations alone or observed in the controls over time. In the field experiment neither treatment- nor time-effects but spatial differences in fungal and prokaryotic communities were detected. Results of the study suggest that the application of *M. brunneum* have only marginal effects on soil microorganisms.

Key words: *Metarhizium brunneum*, non-target effects, soil microbial community structures, amplicon sequencing.

OP-14. Commercial production of conidia emitting granules for the soil application of *Metarhizium brunneum*

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Metarhizium brunneum Cb15 was produced in liquid culture and formulated into an extruded granule for controlling wireworms (*Agriotes sp.*) in potatoe-fields by applying the granules at planting. The production was optimized by changing the production medium for the fungus. Best conidia emission of resulting granules was achieved in media where the highest concentration of submerged conidia was produced. More submerged conidia were produced if the liquid culture was inoculated with aerial conidia than if started from a liquid preculture with mycelium. In the full-grown fermentation broth, a concentration of 5e7 submersed conidia/ml was achieved. The granules were produced directly from the fermentation broth by adding soy-flour, glucose, potassium-mono-phosphate and sodium-alginate. The resulting granules were dried at 45°C inlet - and max. 38°C outlet temperature to a residual moisture of approx. 6%. Conidia production of the resulting granules was tested on water agar. While 100% of the granules produced aerial conidia after 10 days incubation at15°C, the concentration of conidia was 4.2e9 ±7e8 conidia per g granules. The method for producing these conidia emitting granules is highly efficient and can easily be adapted to other entomopathogenic or disease suppressing fungi.

Key words: *Metarhizium brunneum*, conidia emitting granules, mass production, biocontrol agent

OP-15. Evaluation of different strategies employing entomopathogenic and soil fungi against spotted wing drosophila (*Drosophila suzukii*)

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Spotted wing drosophila (*Drosophila suzukii* [Matsumura, 1931]; SWD; Diptera: Drosophilidae) management is very challenging for the most part due to its brief generation time, polyphagy and serrated ovipositor, which allows it to infest undamaged, ripening fruit, but also because its larvae can pupate in the orchard soil and are thus protected from aerial insecticide applications. Usually there is zero tolerance for SWD larvae in fresh or processed fruit products, thus fruit producers have adopted intensive management programs, relying primarily on frequent insecticide applications, in an attempt to prevent infestation. However, many insecticides are not allowed in organic fruit production and may be disruptive to beneficial agroecosystem services and human health. Therefore, we investigated interactions between entomopathogenic (EPF) and insect-associated soil fungi and their hosts in soil and aboveground environment to offer soft fruit producers a viable biocontrol management solution. We performed several laboratory experiments evaluating different fungal deployment strategies and a field experiment utilizing uncommercialized fungal isolates and bioinsecticides against SWD in blueberries. We hypothesized that EPF and soil fungi would express different pathogenicity against SWD pupae in soil environment. Hence, in addition to known entomopathogens (*Metarhizium brunneum* (isolates H.J.S. 1154 and 1868), *Beauveriabassiana* (2121 and 2122)), also soil fungi (*Trichoderma atroviride* (1873) and *Clonostachys rosea* (1884)), were tested against pupae in conidia-spiked soil and via direct conidial applications against pupae. Further, we hypothesized that different SWD life stages would vary in their susceptibility to infection. Therefore, a selection of most pathogenic strains from pupal exposure experiments was also tested against imagos via spray application. Within these experiments also horizontal transmission of fungal infection was evaluated. Additionally, we tested an attract and infect strategy, in which the flies were exposed to EPF growing on SWD artificial food. *M. brunneum* strain H.J.S. 1154 significantly reduced fly emergence in conidia spiked soil (by 15 %), and bioinsecticide Naturalis (based on *B. bassiana*, strain ATCC 74040) in direct pupal exposure tests (by 21 %). Several strains caused significant mortality of sprayed flies: The average LT_{50} was 9.4 d for *M. brunneum* (1154), 10.9 d for *M. brunneum* (1868), 16.6 d for *B. bassiana* (2121), 24.6 d for Naturalis, 29.2 d for negative control, and 0.9 d for insecticide Laser (a.i. spinosad) treated flies. Even higher virulence was recorded in the attract and infect strategy: The average LT_{50} was 4.5 d for *M.*

brunneum (1154), 5.6 d for *M. brunneum* (1868), 2.2 d for *B. bassiana* (2121) and 21.6 d for negative control. Horizontal transmission of fungal infection was observed rarely. The field results were inconclusive, because of the very low SWD pressure in 2016 in central Slovenia. We conclude that the imagos were generally more susceptible to fungal infection than pupae. Most likely the pupal stage is too brief to allow entomopathogens to cause a significant reduction of fly emergence. On the other hand, the high virulence obtained in direct spraying and attract and infect experiments shows promise in a potential EPF-based SWD management strategy and will be further evaluated under field conditions.

Key words: biological control, entomopathogenic fungi, insect-associated soil fungi, IPM, insect, organic, pathogenicity, pest, soft fruit, virulence.

OP-16. Biological Control of Adult *Diabrotica*- Spray experiments with *Metarhizium brunneum* strain BIPESCO 5 under real farm conditions

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The Western Corn rootworm *Diabrotica virgifera virgifera* (WCR) is causing economic damages in maize production in parts of the European Union (EU), so in Austria. The control of adults is becoming increasingly cumbersome as the WCR has a history of adapting to several management practices. The European Union has directed Member States to consider Integrated Pest Management as a strategy for controlling WCR. Entomopathogenic fungi *Metarhizium brunneum* (BIOPESCO 5) have been found to have a real potential also for adult control. First studies conducted in Styria (Austria) showed the potential of adult spray application using safe, water dispersible *Metarhizium brunneum* formulations (Hypocreales: Clavicipitaceae), under real farm conditions as an innovative direct biological control method for WCR in maize production.

OP-17. *Metarhizium brunneum*BIPESCO 5: a sustainable and preventive biological control agent to control *Diabrotica virgifera virgifera* larvae

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A long term field study was carried out to demonstrate how the preventive application of GRANMET GR™ (biological control agent based on *Metarhizium brunneum* BIPESCO 5) affects the mortality of *Diabrotica virgifera virgifera* larvae (western corn rootworm) for a sustainable population control in maize fields in Styria and Tyrol (Austria). We will report on results from a 1-year and a 5-year field study examining the effects of (i) *Metarhizium* abundance after a single (in Tyrol) or periodically inundative mass application (in Styria) of GRANMET GR™; (ii) determine the efficacy in suppressing *Diabrotica* pest populations by monitoring the swarming activity of the adults using a novel emergence trap system, and to consider parameters such as (iii) damage assessment of maize plants. All relevant data will be discussed in this presentation.

OP-18. Potential of entomopathogenic fungi and nematodes against *Drosophila suzukii* in laboratory assays

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Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a polyphagous pest that causes considerable damages on economically important crops, including grapes and cherry. Currently, broad-spectrum insecticides are used several times during the harvest season to prevent the fruits loss. This approach has multiple challenges such as affecting natural enemies, increasing the risk of other pest outbreaks, managing pre-harvest and restricted entry intervals (PHI and REI), and the high risk of resistance developing. Nevertheless, few means are available for organic production. Entomopathogenic fungi and nematodes have been found to be effective against a large number of dipteran species. Furthermore, the possible use of *Beauveria bassiana*as deterrent of Tephritidae ovipositionis well known. The effect of several EPFs and EPNs autochthonous strains against *D. suzukii* individuals (larvae, pupae and adults) was evaluated in laboratory assays. Results showed a moderate effect against *D. suzukii* larvae and adults and a higher virulence against pupae. Furthermore, the deterrent effect on *D. suzukii* oviposition activity of several EPF strains was evaluated in laboratory conditions (no-choice and double-choice test), in comparison with other mineral compounds (potassium silicate and kaolin). Results reveals promising perspectives for the biological and integrated management of this pest.

Key words: virulence, deterrent of oviposition, microbial control

OP-19. Entomopathogenic fungi and natural avermectins are interact as a synergists

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The entomopathogens and insecticides are the important agents of pest management in agrocenoses. As a rule, interaction between them causes additive or synergistic effects. The combination of fungal entomopathogens with the toxicants allowing to reduce the consumption of the components, gets a stable insecticidal effect, reduces latent period and minimizes the toxic pressure on the environment. As a result, such combinations are of great interest to biological control of economically important insect.

Both in laboratory and in the field, the stabile synergetic effect towards the larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) was provided by the combined treatment in half lethal doses with complex of the avermectins (produsedby *Streptomyces avermitilis*) and entomopathogenic fungus *Metarhizium robertsii.* That effect was registered even in the last larval stage when insects are least susceptible to the action of fungi.

More than 2.5-fold food consumption decrease and significant larvae development delay were registered under the influence of avermectins. Moreover, disturbance of antifungal immune response and intensification of mycosis have been registered in presence of avermectins.

We have shown that avermectins inhibit cell-mediated immunity in the hemolymph. Specifically, avermectins dramatically decreased granulocytes count in infected and uninfected with fungus larvae. In addition, avermectins enhanced phenoloxidases activity in cuticle and the haemolymph and increased glutation-S-transferases activity in fat body and the haemolymph of infected and uninfected larvae. It is important that combined treatment (avermectins+fungus) led to more rapid colonization of the hemolymph with fungus as compared to treatment with fungus alone.

Synergy between fungi and avermectins has been shown in the field conditions under sharp daily variation of temperature and humidity (potato fields in steppe zone of Western Siberia). The half-lethal time (LT_{50}) reduced 2.8-fold after combined treatment as compared to the treatment with *M. robertsii* alone. Thereby, combined treatment by *M. robertsii* with natural avermectins against *L. decemlineata* larvae allows to achieve high insecticidal effect even under climate conditions that is unfavorable for the mycoses development. This approach may be promising for the development of multi-bioinsecticides.

Keywords: *Metarhizium robertsii*, avermectins, Colorado potato beetle, synergy, insect'simmunity, biological control.

OP-20. Entomopathogenic fungi *Metarhizium* spp. from Russia and neighbouring territories, ecological preferences and activity against Colorado potato beetle larvae

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The M*etarhizium* species in Russia and neighboring territories are little known. Thirty-four isolates of the *Metarhizium* spp. from Russian collections was identified based on 5' EF-1α gene sequence analysis. Most frequent species were *M. robertsii* and *M. brunneum,* whereas *M. anisopliae* and *M. pemphigi* were singular. *M. robertsii* cultures were isolated predominantly from sun-opened habitat (agro-landscapes, steppes and meadows), but *M. brunneum* were predominately isolated from forests. Radial growth studies in the temperature range of 10-37.5°C revealed that *M. robertsii* were highly thermotolerant in comparison to *M. brunneum*. The optimum temperature for the *M. brunneum* isolates was 25 or 30°C. All cultures of this species exhibited an absence of mycelial growth at high temperatures (35-37.5°C) but were more active at low temperatures (10–20°C). In contrast, *M. robertsii* cultures were thermotolerant i.e., were able to grow at high temperatures (35–37.5°C). The optimum temperature for all cultures of this species was 30°C. The virulence against Colorado potato beetle larvae *Leptinotarsa decemlineata* Say was evaluated under two regimes: humid (21°C, 80% RH) and arid (31°C, 55% RH). *M. brunneum* isolates were less virulent compared to *M. robertsii* under both regimes. *M. robertsii* activity was the same under both conditions, but *M. brunneum* was less virulent under the arid regime in comparison with the humid one. Field experiment in condition of daily range 10–43°C and 13–98% RH showed that *M. robertsii* was significantly more active than *M. brunneum* toward Colorado potato beetle larvae.

Taking into account the results we can summarize that *M. robertsii* isolates are significantly more effective to control the Colorado potato beetle larvae under continental climate conditions. Specifically, these isolates exhibited the highest levels of thermotolerance and virulence under the conditions of potato fields.

Key words: pests, *Metarhizium*, thermotolerance, continental climate, *Leptinotarsa decemlineata*, cryptic species.

PP. 4. Potential of anamorphic entomopatogenic fungi and biological products on their base in the control of greenhouse whitefly *Trialeurodes vaporariorum* West. (Homoptera: Aleyrodidae) in Belarus

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Among the phytophages of the family Aleyrodidae greenhouse whitefly *Trialeurodes vaporariorum* West. is one of the most dangerous pests of greenhouse agrobiocenoses. In the Republic of Belarus every year there is a significant damage of greenhouse crops by this phytophage bringing yield decrease for 10-15% and the vegetable product degeneration. Carrying out the protective measures is complicated by the ability of the pest to a rapid recovery and high population number capacity, high level and the speed of resistance development to chemical pesticides, as well as the increased hygiene requirements for working conditions and product quality in greenhouse industry. These factors require the development of alternative environmentally friendly phytophage control, in particular, limiting the pest number and severity at the cost of using the anamorphic entomopathogenic fungi and products based on them in IPM of greenhouse crops.

The Institute of Plant Protection (Belarus) has a collection of anamorphic entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill, *Isaria fumosorosea* Wize, *Lecanicillium lecanii* (Zimm.) Zare & W. Gams strains. The results of long-term laboratory, vegetative and field research of entomocidal activity of the mycopathogens against *T. vaporariorum* indicate their availability for application in biological control of Aleyrodidae family phytophages. Depending on the pest population number the biological efficiency of entomopathogenic fungi has made 22.6-100%. Using these entomopathogens at the initial stage of greenhouse whitefly population development allows a long-term limit of the population number. The application of the studied mycopathogens during the population number increase is also highly effective.

On the basis of high-active and technological strains of entomopathogens the biological preparations Pecilomicin-B (based on *Paecilomyces fumosoroseus*), Boverin-BL and *Melobass*® (both on different strains of *Beauveria bassiana*), Entolek (based on *Lecanicillium lecanii*) have been developed. The studies have covered all the stages of activities from search and selection of strains on the basis of the target activity, studying their morphological, physiological and biochemical properties to develop the technologies for industrial production and application of the final

product have been realized during the biological preparations development at the Institute of Plant Protection. These products have passed through the state registration procedure and have been approved for use in agriculture.

In most cases, the range of insecticidal activity of anamorphic entomopathogenic fungi is not limited to a single insect species. There is also a positive correlation between the level of susceptibility of closely related host species. In addition, recently, there are numerous references to the other whitefly species (*Bemisia tabaci, B. argentifolii*) susceptibility in respect of *T. vaporariorum*, identified in the investigated strains and biological preparations, suggesting the possibility of their effective use against other phytophages from the Aleyrodidae family.

Key words: *Trialeurodes vaporariorum*, anamorphic entomopatogenic fungi, strains, biological preparations, biological efficiency

PP. 5. Application of Zeolite formulations in combination with *Beauveria bassiana* and *Trichoderma asperellum* against *Tribolium confusum*

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Zeolites are microporous crystalline aluminosilicates derived from the reaction of volcanic rocks, ash layers and an alkaline groundwater. Their use against stored product pests may contribute future Integrated Pest Management (IPM) and organic farming in respect to long term depot conditions. Current study was performed with single entomopathogenic fungi, *Trichoderma asperellum* and *Beauveria bassiana*, and zeolite combinations. The bioassays were conducted at 20°C, 25°C, and 30°C temperatures and 55% relative humidity on stored wheat. Single characters containing each fungus and zeolite formulation (Zeolite 4A-Ph8, Zeolite 800MSC, ECOZEO Project®) were applied at 400 ppm dosage alone, aside from binary combinations (FMC-008=*T. asperellum*+Zeolite 4A-Ph8; FMC-009=*B. bassiana* + Zeolite 4A-Ph8, 6x10^{9}UFC/gr). Mortality was measured after 7, 14, 21, and 28 d of exposure. F1 progeny was recorded after 8 weeks. The mean mortality of *T.confusum* adults after 21 d of exposure considering all single and binary combinations was less than 50%, 80%, and 80% at the 20°C, 25°C, and 30°C temperatures respectively. The highest efficacy reached at the longest exposure time (28 d) with *B. bassiana*+Zeolite (91% at 20°C, 95%? at 25°C, 95.66% at 30°C) in all temperatures. In case of single characters Zeolite, 4A-Ph8 showed better efficiency at 20°C and 25°C, while Zeolite 800MSC showed efficacy at 30°C. *T. asperellum* + Zeolite combination was not exceeding 80-85% efficiency at 20 and 30°C in comparison with 96% at 25°C. According to our results, FMC-009 (*B. bassiana* + Zeolite 4A-Ph8) was the most efficient binary combination of mortal and progeny suppression (~10%), yet no synergistic effect achieved with any of the combination against adult *T. confusum*. However, both zeolite formulations unveil high potential to use at various temperatures even without any formulation of entomopathogenic fungi.

Key words: Zeolites, *Beauveria bassiana*, T*richoderma asperellum, Tribolium confusum.*

PP. 6. Isolation and characterization of entomopathogenic fungi from *Pristiphora abietina* Christ. (Hymenoptera: Tenthredinidae)

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The lesser spruce sawfly, *Pristiphora abietina* Christ. is a serious pest of spruce forests all over the world. It started to damage the forest areas around Artvin, Turkey since 2007. In order to investigate fungal pathogens and determine the fungal control agents against this pest, we collected larvae of *P. abietina* from different areas in 2014-2015. Thirteen fungal isolates were purified from these larvae. After morphological (infection contour, colony morphology, spore contour) and molecular characterizations [the internal transcribed spacer (ITS)and elongation factor 1-alpha (*EF1-α*)sequences], isolates were identified as *Lecanicillium muscarium* (Pa-3, Pa-8, Pa-9 and Pa-11*)*, *Beauveria bassiana* (Pa-4,Pa-5,Pa-6,Pa-13) and *B.pseudobassiana* (Pa-1,Pa-2,Pa-7,Pa-10,Pa-12). Screening studies showed thatPa-4 (*B. bassiana*) had the highest mortality with 93,33% at 1×10^6 conidia ml^{-1}within 14 days on *P. abietina* larvae. Our results indicate that *B. bassiana* speciesis a promising biocontrol agent against *P. abietina*, and can be improved as a fungal biological control agent in the future.

Keywords: *Pristiphora abietina, Lecanicillium muscarium, Beauveria bassiana,* biocontrol, entomopathogenic fungi

PP. 7. Entomopathogenic fungus *Entomophaga aulicae* as agents in classic biological control of browntail moth in some broadlief forest in Serbia

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The browntail moth, *Euproctis chrysorrhoea* is well known defoliator pest of broad-leaved forests and orchards in central and southern Europe, and often occurs in the outbreak. Entomopathogenic fungi are an important regulatory factor for bio-control in agriculture and forestry. *Entomophaga aulicae* is widespread Holarctic species, with many hosts of the order Lepidoptera, including the browntail moth. It has been reported as the causal agent of epizooticsamong lepidopterous pests in Central Europe for many years.

In some forest areas of Central Serbia, this fungus was first discovered in 2015. In the literature, this species is referred as entomopathogen of browntail moth larvae, and there is no data about its pathogenicity for other development stages, primarily for pupae. Since this is a very strong allergen, not recommended any activity with a manual tracked browntail caterpillar nests, and they have not been the subject of laboratory researches. The studies were conducted during the growing season in the period 2015-2016, in some broadleaved forests in Novi Pazar region in which *E.chrysorrhoe a* increases in number and *E.aulicae*was found in the host populations. Browntail mothnewly litters (40) were collected in oak and beech stands. A detailed quantitative and qualitative analysis of sampled litters was conducted at the laboratory of the Institute of Forestry. Two or more months after, microscopic analyses of dead pupae from sampled litters, were conducted. The evaluation of *E. aulicae* infections was recorded as positive when hyphal bodies, primary conidia, or resting spores were detected on the surface of pup aria or in the pupal tissues. The species identification was based on the size, shape and structural characteristics of different life forms of the fungus. In the investigation period, at the end of June, in the newly litters, there were an average of 9.7 pupae, of which 26% were alive, 23% were parasites by species from order Diptera, and 51% dead (causing ocular invisible). By the microscopical studies of the causes of the mortality of the browntail moth pupae, the presence of hyphal bodies, primary conidia and resting spores of the entomopathogenic fungus *E. aulicae* were confirmed in them. As entomopathogenic fungus on two development stages of the host, larvae and pupae, presented results indicate that *E.aulicae* is a promising microbial control agent against the browntail moth in some broadlief forests and orchards in central part of Serbia.

Key words: *Euproctis chrysorrhoea,* pupae, *Entomophaga aulicae* development stages, microbial control agent.

PP. 8. Group-I intron based strain-specific diagnosis of entomopathogenic *Metarhizium* fungi from Cuba

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Anamorphic fungal entomopathogens of the genus *Metarhizium* (Ascomycota: Hypocreales) are of particular interest as biological control agents of numerous pest insects. Bioprospection for these fungi in Cuba has given rise to a set of isolates from a wide range of hosts. Internal transcribed spacer (ITS) based molecular taxonomic analysis locates the isolates within the *Metarhizium anisopliae* complex.

In order to facilitate further research and the development of isolate M41 into a myco-insecticide, a PCR-based diagnostic tool allowing the reliable and fast differentiation of this strain from the other Cuban isolates was highly solicited. Screening of this set of fungal strains 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 comprising two group-I introns in insertion positions 1 and 4, for the 28S rRNA gene of strain M41.

These findings were exploited in the development of an identification assay for this isolate. Primer pairs hybridizing against the 28S rRNA intron sequences were designed and used to amplify partial rRNA gene sequences in a strain specific manner. The approach was shown to unambiguously identify strain M41 across the full set of isolates investigated.

In conclusion, the feasibility of strain-specific identification based on group-I intron sequences has been demonstrated for a potential *Metarhizium* biocontrol strain from Cuba.

Key words: *Metarhizium*, internal transcribed spacer (ITS), group-I intron, diagnostic PCR.

PP. 9. Preliminary study of selected entomopathogenic fungi forcorn (maize) aphid, *Rhopalosiphum maidis*, control

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Rhopalosiphum maidis, corn (maize) leaf aphid is a pest of maize and other related crops with worldwide distribution. This insect can infest all aboveground parts of the corn plant and cause serious damage to the yield. The aim of this study was to determine the pathogenicity of selected entomopathogenic fungi (*Beauveria bassiana*, *Isaria fumosorosea* and three different strains of *Metarhizium anisopliae* against *R. maidis* in the second nymphal stage under controlled conditions (23°C temperature, 60% humidity). Georgian local corn variety "Adjametistetri" was used for the assay. Corn seeds were grown in separate pots containing growth substrate mix. Two weeks old corn seedlings` stem and leaves were sprayed with suspension of entomopathogenic fungi with concentration 10^6sp/ml. Control versions were sprayed with distilled water. Ten minutes later, ten *R. maidis* aphids were confined on each corn seedling using micro-perforated polypropylene bags. Aphid mortality and the progeny product ion were studied 7 days later. Four replicates have been done per treatment. Significantly highaphids' mortality rate has been shown by *B. bassiana* and *I. fumosorosea* and relatively low influence was caused by *M. anisopliae* strains. Almost all studied fungi caused suppression in aphid progeny production - the highest was caused by *B. bassiana* and strain MA2 and lowest by strain MA3 but results were not significantly different from the control samples. Number of aphids' new generation after *I. fumosorosea* and MA1 treatment was almost the same.

Key words: Biocontrol, *Rhopalosiphum maidis*, entomopathogenic fungi, *Beauveria-bassiana*, *Isaria fumosorosea, Metarhiziu manisopliae*

PP. 10. Isolation and identification of fungal community of alfalfa pest weevils (Coleoptera: Curculionidae) in the Republic of Moldova

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Alfalfa (*Medicago sativa*) is attacked by different pests among which *Sitonalineatus*, *Hyperapostica* and *Protapionapricans* (Coleoptera, Curculionidae) are one of the most dangerous, being widespread in Europe, Africa, Asia and North America. An alternative to chemical pesticides to control this pest, with reduced effects on human health and environment, is microbial control agents, especially entomopathogenic fungi. Little is known about the fungal communities associated with *Sitona lineatus*, *Hypera postica* and *Protapion apricans*.

The main objective was to investigate the fungal community of alfalfa pest weevils *Sitona lineatus*, *Hypera postica* and *Protapion apricans*in the Republic of Moldova and highlight strains with potential for biological control development. Traditional culturing, morphological, physiological and molecular techniques (18S rRNA gene fragments and internal transcribed spacer (ITS) regions together with the 5.8S rRNA gene sequences) were used to identify the fungal flora of these insects. All together 42 fungal strains were revealed belonging to 23 species from 20 genera included in Ascomycota (19 species), Basidiomycota (2) and Zygomycota (2). Fungal flora of *Sitona lineatus* and *Hypera postica* was represented by 13 species, while the *Protapion apricans* flora consisted by 5only. Species *Torula caligans*, *Aureobasidium pullulans* and *Alternaria alternata* were characteristic for both *Sitona lineatus* and *Hypera postica* host insects, *Cladosporium cladosporoide*s and *Candida deformans* for *H. postica* and *Protapion apricans,* while *Penicillium polonicum* for *S. lineatus* and *P. apricans*. None of the identified fungal species were found in the flora of all three insect pests. Among isolated strains 4 were identified as entomopathogenic fungi *Beauveria bassiana* (3) and *Paecilomyces fumosoroseus* (1). This is the first study of fungal flora from alfalfa pests *Sitona lineatus*, *Hypera postica* and *Protapion apricans* in the Republic of Moldova. Our data can offer useful information for future investigations on microbial control agents development.

Key words: weevils, fungal flora, alfalfa pests, microbial control

PP. 11. Enhanced colonization of tomato plants by encapsulation of endophytic entomopathogenic *Metarhizium brunneum*

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Entomopathogenic fungi like *M. brunneum* are promising biocontrol agents since they are pathogenic to many different insect orders and are able to endophytically colonize different plant species and parts which has recently opened the door for a new plant protection measure. However, biocontrol of insect pests with these fungi is challenging because of the low efficacy, difficult application and limited drying stability of the usually insufficiently formulated “active ingredient”. Therefore, the aim of our investigations was to encapsulate *M. brunneum* strain BIPESCO5 mycelial biomass to deliver the fungus to tomato plants to support plant colonization via the roots for a systemic protection from herbivorous insects, such as whiteflies.

We hypothesized that *M. brunneum* would establish endophytically in plants after application of mycelial biomass to roots and that encapsulation of active biomass would substantially increase colonization because of the beneficial microenvironment provided by the bead. In addition, we looked into the impact of drying on plant colonization. We therefore encapsulated mycelial biomass in Ca-alginate corn starch beads and conducted drying of beads at 30 °C for 3 d to an $a_W \leq 0.2$. Tomato plant roots were inoculated with (1) unformulated moist mycelial biomass, (2) moist beads and (3) dry beads containing mycelial biomass. Endophytic emergence in the stem was assessed by a biphasic approach using light microscopy and real-time PCR after 21 days of incubation.

We successfully demonstrated for the first time that tomato plants were colonized by *M. brunneum* after application of mycelial biomass. With light microscopy, endophytic hyphae were found in 98% of all analyzed plants. In contrast, real-time PCR verified endophytic *M. brunneum* in 40% of plants after application of unformulated moist mycelial biomass, in 52% of plants after application of dry beads and in 64% of plants after application of moist beads. Most notably, encapsulation significantly enhanced colonization efficacy 4-7 fold.

In conclusion, our study demonstrates that *M. brunneum* establishes endophytically in tomato plants after delivery of mycelial biomass to roots. Furthermore, we

provided exciting evidence that encapsulation substantially enhances plant colonization. These findings provide a valuable basis for the development of a new plant protection strategy with endophytes and encourage the development of further customized formulations that support plant penetration and colonization for systemic protection from herbivorous insect pests.

Key words: *Metarhizium*, endophytes, formulation, tomato, colonization.

PP. 12. The effects of natural substrates and artificial media on the production of conidiospores and blastospores of entomopathogenic fungus *Isaria fumosorosea*, strain CCM 8367

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Entomopathogenic fungus *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) (WIZE) Brown & Smith (Hypocreales: Cordycipitaceae) is among the most common species used in biological control. The aim of this study was to find the most suitable media for both surface and submerged productions of spores of the strain CCM 8367 of *I. fumosorosea* isolated in the Czech Republic from the horsechestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae). For the surface cultivation we used the following commercial agar media: Sabourad dextrose agar (SDA), V8 juice agar (V8JA) Corn meal agar (CMA), Czapek dox agar (CDA), Agar malt extract (MEA) and Potato dextrose agar (PDA) and also natural substrates (hulled barley, rice and oatmeal) were tested. For submerged cultivation three standard liquid media were used: Sabourad dextrose broth (SDB), Czapek dox broth (CDB) and Potato dextrose broth (PDB) and two modified media (A and B). The radial growth of the strain and the production of conidiospores was evaluated after 7, 14, and 21 days. Production of blastospores was evaluated daily for 7 days. Best growth of the strain CCM 8367 was observed when two agar nutrient media CDA and SDA were used, while the highest production of spores was observed on medium V8JA when fungus produced after 21 days of cultivation 6.28×10^5 conidiospores per mm^2 of culture. Production of conidiospores on natural substrates was highest on the rice. After 21 days, the production of coniodiospores was 2.58×10^9 per 1 gram of rice. Blastospores production of strain CCM 8367 was the highest in the modified medium of our own recipe (medium A). After 4 days of cultivation, amount of blastospores per 1 ml in this medium was 3.34×10^8. The best medium producing the highest amount of blastospores among the commercially available artificial media was SDB. The effect of C:N ratio (glucose:peptone) on agarized and liquid media is currently evaluated.

Key words: entomopathogenic fungus, *Isaria fumosorosea*, surface cultivation, submerged cultivation, production of spores

PP. 13. Soil application of entomopathogenic fungi as approach to control the seabuckthorn fruit fly?

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In the year 2012, the seabuckthorn fruit fly (*Rhagoletis batava* HER.) was detected in Germany in the federal land Brandenburg for the first time. Since then the abundance increased and high yield losses occurred regularly.

In Germany,up to 90 % of the production of seabuckthorn (*Hippophae rhamnoides* L.) is done by organic farming. At present and depending on the production standard, non or only one active substance is authorized at present. Therefore, there is a need for additional biological control strategies.

The use of entomopathogenic fungi is considered to be one of them. The fungi could be applied against the adult stage as well as against the pupae. Therefore, a preliminary test was done to evaluate *Lecanicillium muscarium* (Petch.) ZARE & GAMS, *Isaria fumosorosea* (Wize), *Metarhizium anisopliae* (Metschn.) SOROKIN 1883, one strain each fungus.

For this, pupae of *R. batava,* obtained from an organic farm, were incubated in hatching cages in soil (10 pupae each repetition, 5 repetitions each strain). The inoculation was done by placing 5 wheat kennels overgrown with mycelium on the soil of each cage. The hatching rate was evaluated 4 weeks after the fly hatching period came to an end. A statistically significant reduced hatching rate compared to the untreated control (58%) was obtained in the variants with *I. fumosoroseus* (14%) and *M. anisoplieae* (32%). On the other hand, *L. muscarium* had no effect (56%).

Because of its preliminary character of the test, comparisons between the fungi are not possible (lack of standardized titer of inoculum). Furthermore, an evaluation of long-term effects on the flies has not been conducted either. Nonetheless, the results are promising und the research on this topic will continue.

Key words: *Rhagoletis batava, Hippophae rhamnoides*, biological control, *Metarhizium, Lecanicillium, Isaria*

PP. 14. First report of *Beauveria pseudobassianana* isolates ecosystems of Georgia

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Eentomopathogenic fungi *Beauveria spp* from various ecoregions of Georgia have been studies morphologicaly and on the genome level. Samples were obtained in 2007-2009 from 2 different geographical sites at different altitudes (600-1700 m a.s.l.) and climate zones, each representing a unique agricultural and forest ecosystem. 6 strains of local isolation of *Beauveria spp*: - Bb001; Bb006; Bb007; Bb009, Bb010; Bb011) were selected for further morphological and molecular investigations.

Morphological analysis and BLAST analysis of sequences strains of monocultures, two species of *Beauveria* were identified: (1) *Beuveria bassiana* (Bb001; Bb006) and (2) *Beauveria pseudobassiana* (Bb007; Bb009, Bb010; Bb011).

For molecular study several DNA fragments of isolates, ITS region (the rRNA gene cluster), EF1 (the Elongation Factor 1-alpha) and (the intergenic) BLOC region were amplified and sequenced which were compared to data other strains of GenBank.

Georgian strains belong to multiple clades, with the greatest homology in samples belonging to the A (*Beauveria bassiana*) or C (*Beauveria pseudobassia*) clade. Samples collected in this study were identified as *Beauveria bassiana* (99-100% coincidence) through the comparison of sequences obtained from the ITS region of the ribosomal gene with those published in GenBank. The regional differences between strains were identified through phylogenetic tree. Base on this research, it was confirmed that the samples taken from the same region were located at the same species on phylogenic tree.

Key words: *Beauveria bassiana, Beauveria pseudobassiana*, morphological and molecular study, ITS, EF1, BLOC region

PP. 15. Effect of Ultraviolet Radiation and screening tolerance native isolates of Beauveria spp.

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Solar radiation, particularly the UV-B component, cause negative effect on conidia of entomopathogenic fungi in the field. In an effort to identify Beauveria spp. isolates with promise for use in biological control settings with high insolation, we examined local isolates of Beauveria spp. The objective of this study was to explore diverse habitats as potential sources of local strains of the Beauveria, their relative distribution in various geographical areas and their potential application as biological control agents against the target insect. The origin 10 isolates were obtained from 3 different geographical sites at different altitudes and climate zones: (1) South slope of the Great Caucasus Mountain (1700 m a.s.l), (2) South Caucasian Mountain (1200 m a.s.l), (3) Colchis Lowland (214 m a.s.l).

In experimental trial, selected *B. bassiana* isolates (Bb002, Bb007, Bb010, Bb017, Bb026, Bb027, Bb029, Bb070, Bb114, Bb Geometridae), were weighted by UV-B irradiance at dosages 850 mW /cm^2, while 1- 6 h, with 1 h interval. As a control not exposed to UV radiation were used.

As a result, after 6 h irradiation, achieved in 50% growth inhibition of selected isolates compared to control. Also, conducted to experiments it was determined that, isolates from the different ecosystems and sea level, are characterized with different tolerance, in particular, high-sensitive isolates were more adapted to the UV-B radiation. This phenomenon can be explained as B.b-'s tolerance, as well as his post-irradiation recovery functions of features. In order to establish, two different isolates - B.b 029 (214 m above sea level) and B.b 070 (1200 m above sea level) were evaluated. By provide to analysis of the relative increase of the intensity, this two isolates are differed only by individual tolerance, but restorative processes, in both cases were going equally. Getting results, can be used as a guide for the production of high-efficiency biopestitsidebis.

Key words: *Beauveria bassiana*, UV-B radiation, tolerance

PP. 16. Variability of Wheat Cultivars to Host Endophytic *Beauveria* and *Metarhizium*

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In 2013-2014 I unexpectedly encountered *Beauveria pseudobassiana*, *B. amorpha* and *Metarhizium pemphigi* infections in diapausing larvae of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), which is a very important, stem-dwelling pest of wheat in the north central U.S. Given the biology of the insect, totally isolated within the wheat pith, it is highly likely that these infections arose from endophytic fungi.

The nature of these infections immediately led to the possibility of managing this insect using an endophytic *Beauveria* or *Metarhizium*. Toward this end we evaluated the endophytic potential of 2 sawfly-derived *B. pseudobassiana* isolates, a *B. amorpha* and 2 *M. pemphigi* isolates in 15 varieties of spring, durum and winter wheat, using foliar application of conidia at stem elongation stage, seed treatment, or injection of blastospores into the bottom internode (as a control). We also examined the infectivity of the five fungi for larval sawfly.

Satisfactory surface sterilization of wheat plants necessitated validation of a decontamination protocol; many of the published methods were inadequate. A final quality control step involved leaf imprints or rolling stems onto potato dextrose agar (PDA), with tissues then being explanted onto a dodine-based selective agar. If *Beauveria* or *Metarhizium* was present on the PDA that plant sample was discarded from further analysis, but less than 0.1% of samples were insufficiently decontaminated.

Wheat varieties varied considerably in receptiveness to endophytic establishment by these fungi via foliar application. There was also variability in endophytic capability among the strains for any given wheat variety, as well as in suitability of different application methods (foliar application, seed treatment). Seed treatment was generally unsuccessful. The general inferiority of foliar sprays compared to 20-min dips of entire plants into conidial suspensions indicated that simple aqueous sparys are inadequate to establish substantial levels of endophytism; formulations have to be devised to make foliar applications practical.

All the strains were highly pathogenic for larval sawfly in a “contaminated substrate” bioassay with doses spanning 1-100 conidia mm^{-2} of surface. Attempts are currently underway to demonstrate capability of one or more of these strains to attack the sawfly *in planta*. If successful, a biological management tool for wheat stem sawfly may be feasible. Research was supported by grants from the Montana Wheat and Barley Committee.

Key words: wheat, biocontrol, endophytism, *Beauveria*, *Metarhizium*

PP. 17. Side Effects Of Fungicides And Insecticides On Entomopathogenic Fungi

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The products based on different strains of the entomopathogenic fungi are now being used in IPM programs. Compatibility studies of chemical and biological control agents are necessary to be able to give proper recommendations for their integrated use. The effect of three insecticides: Confidor 200 SL (imidacloprid), Biospin 120 SC (spinosad), Vertimec 018 EC (abamectin) and three fungicides: Bravo 500 SC (chlorotalonil), Amistar 250 SC (azoksystrobina), Topsin M 500 SC (tiophanat methyl) on activity of entomopathogenic fungi: *Metarhizium anisopliae* (Metsch.), *Beauveria bassiana* (Balsamo) Vuillemin, *Acremonium* sp. was tested in laboratory conditions. Tests of the influence of the pesticides on growth and production of conidia were examined. From this study, we conclude that the insecticides abamectin, imidacloprid, acetamipid and spinosad can be used together with the fungus *B. bassiana* products in IPM programs. In case of use fungicides chlorotalonil, azoksystrobina and tiophanat methyl were reduced growth and production of conidia this fungus. The fungus *Acremonium* sp. was susceptible for all using pesticides. These pesticides reduced production of conidia in different doses. In case of the fungus *M. anisopliae* only abamectin using in half doses increased production of conidia. Other insecticides inhibited the growth and production of conidia of the fungus *M. anisopliae.* This fungus wasn't susceptible for all using fungicides.

Key words: side effects of pesticides, entomopathogenic fungi

ABSTRACTS:
SECTION NEMATODE

OP-21. Potential of entomopathogenic fungi and nematodes against the two cryptic species *Parahypopta caestrum* and *Cossus cossus* in laboratory assays

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Pests residing in cryptic habitats, as insects which bore into the plant tissue (wood-boring insects) or under the bark (bark beetles) or in the soil (wireworms) are very difficult to control because chemical pesticides are not able to reach the target. Usually, the only option to reduce infestations is the removal and destruction of infested or injured plants. One potential alternative to chemical insecticides for the control of cryptic insects can be the use of microbial control agents, as entomopathogenic nematodes (EPNs) and fungi (EPFs), because they may be able to penetrate into cryptic habitats and to be horizontally transmitted within the pest populations. *Parahypopta caestrum* (Hübner) and *Cossus cossus* (L.) (Lepidoptera, Cossidae) are highly-destructive cryptic pests in Europe. *Parahypopta caestrum*can be considered the key pest of *Asparagus* spp. in Italy, due to its high destructiveness and the lack of effective control options available. The soil-borne larvae bore mines into the roots and the shoots, causing the total destruction of plantations after 2-3 years. The goat moth *C. cossus* (L.) is a wood-boring pest whose larvae bore large galleries under the bark and even deeply into trunks and branches of fruit and forest trees, reducing plant growth and vigour, and causing limbs and branches to fall. Preliminary assays were performed in laboratory conditions in order to evaluate the infectivity of several EPF and EPN autochthonous strains against *P. caestrum* and *C. cossus* larvae. Results revealed the efficacy of these microbial control agents in killing the larvae, although a wide inter- and intra-specific variability in virulence was detected among different microbial strains. Considering the lack of effective chemical control means, the microbial control of the Asparagus moth and the goat moth by EPNs and EPFs reveals promising perspectives and needs further investigations.

Key words: *Cossus cossus, Parahypopta caestrum*, microbial control

OP-22. Exon-intron structure and sequence variation of the hsp-90 gene in the entomopathogenic nematode *Heterorhabditis bacteriophora*

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The *hsp*-90 represents one of the most abundant and evolutionarily conserved proteins that are present in a wide range of organisms from bacteria to humans, functioning both as molecular chaperones and stress proteins. Nematodes are among the most successful organisms in withstanding stress conditions. Little is known about biochemical and molecular events underpinning entomopathogenic nematode responses to environmental stresses, in particular cold and heat stresses. In this study, we report the isolation and molecular characterization of the full-length cDNA of *hsp*-90 and its corresponding gene from the entomopathogenic nematodes *Heterorhabditis bacteriophora*. Sequencing analyses of several cDNA and genomic clones revealed the presence of different transcripts and genes. The unexpected finding of intron presence-absence in the *hsp*-90 genes of *H. bacteriophora* opens some questions about their function and their evolutionary significance. Further investigations are ongoing in order to assess the function of the different transcripts in response to different temperature stresses.

Key words: *Heterorhabditis bacteriophora*, *hsp*-90, PCR, PCR, sequencing

OP-23. Response of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to physical stimuli by Red Palm Weevil: Behavioral and molecular analysis

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Entomopathogenic nematodes (EPNs) reciprocate to an array of external stimuli from insect hosts. We showed that *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* are attracted to the different developmental stages of the Red Palm Weevil (RPW). In the present study we evaluated the effect of chewing voice generated by RPW larvae on EPNs. Choice assay between RPW larvae and its recorded voice also showed the preferential movement of EPNs towards the RPW larva. *S. carpocapsae* was more sensitive the attraction or repulsive movement than *H. bacteriophora*. We further investigated the molecular mechanism caused by this stimuli using RNA-seq to determine the changes in gene expression as a response to the different stimuli. The data set suggests that more than 60% of the differentially regulated genes were unknown with no similarity to *C. elegans* and no functional classifications but they shared homologs with parasitic nematodes like *Anglystoma, Ascaris;* and those genes denotes their relation to parasitic phenotype. Strong expression of locomotive, nematode larval development, reproduction, determination of adult lifespan, locomotion, metabolic process, oxidation-reduction process, transport, lipid storage genes, suggests the presence of insects, IJs undergoes many growth and developmental functional processes by the differential expression of sensory perceptions to detect insect and its voice. More specifically, the changes in the expression pattern of locomotion genes denote they have special sensory system to sense the false positive signal for the presence of target insect by exhibiting self-propelled movement away from the voice source. Dramatic differential expression of transcripts confined to the different cellular compartments such as membrane, cytoplasm, ribosome, mitochondria, endoplasmic reticulum, nucleus, neuronal bodies, also denote the external sensing of RPW larvae or its voice by the EPNs. Overall the results of this study will lead to improve the ability of the EPN to allocate and infect burrowing insects.

OP-24. Susceptibility of the cutworm, *Agrotis segetum* (Lepidoptera: Noctuidae) to entomopathogenic nematodes

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The cutworm, *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae) is a species widely spread in Europe that damages the cultivated plants belonging to more than 15 families and including host plants such as okra, cabbage, cauliflower, rutabaga, bell pepper, tomato, potato, maize and cotton. The effect of entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* and a new species from Georgia, *Steinernema tbilisiensis* was investigated in the last instar of cutworm larvae and pupae in the laboratory conditions. The nematodes were used in the following doses: 50 and 100 infective juveniles (IJs) per insect. The mortality of tested insects was estimated on the third day after the EPN application. The last instar of larvae turned out to be more susceptible in the course of laboratory experiments. No significant differences were observed between *H. bacteriophora* and *S. tbilisiensis* at a low concentration of 50 IJs per insect during all exposure times, whereas *S. tbilisiensis* was more pathogenic against larvae at a high concentration of 100 IJs/per insect as compared with *H. bacteriophora* and the mortality reached 97.2 - 100%. The emerging IJs were harvested and counted throughout the interval of 11-15 days. The experiments were carried out in the laboratory conditions at a temperature of 22°C and 80% RH**.**

Key words: Entomopathogenic nematodes, *Heterorhabditis, Steinernema,* cutworms, *Agrotis segetum*

OP-25. Biological Control of *Naupactus godmani* (Curculionidae), Fuller Rose Beetle, with Entomopathogenic Nematodes

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The Fuller rose beetle (FRB), *Naupactus godmani*, is a flightless weevil species that is commonly found in California citrus orchards. Neither the adults nor the larvae cause economically important direct damage to the citrus plants or fruit. However, The Republic of Korea, which is California's most important export market for citrus, currently fumigates imported navel oranges with methyl bromide to kill any FRB eggs that may be attached to the fruits. But, due to the high risks of worker exposure and environmental concerns associated with methyl bromide, Korea plans to eliminate this material from use within the next 2 years. There has never been a pest management program developed for this insect in citrus. Thus, different integrated biological approaches are in development for use. We tested efficacy of entomopathogenic nematodes against larvae and adults of FRB. Nematodes in the experiments were either laboratory cultures of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *S. riobrave* or commercial product of *H. bacteriophora* and *S. carpocapsae named as* Grub Gaurd.

After experiments, laboratory culture of *S. carpocapsae* killed all FRB adults in petri dish assays in 3 days. In the greenhouse, no evidence of infected FRB adults with *S. carpocapsae* were recorded. However nematode mixture, Grub Gaurd, could infect *14% of FRB adults after 2 days and 70% after 4 days on filter paper respectively.* In the field, two rates (25 IJ/cm^2 and 50 IJ/cm^2) of *H. bacteriophora* and *S. riobrave* were applied against larvae to supress adult emergence. And Tedder's traps were used to evaluate emergence of FRB adults. The lowest number of adults were found in Control, *H. bacteriophora* (50 IJ/cm^2) and, *H. bacteriophora* (25 IJ/cm^2). Higher number of FRB were in *S. riobrave* (25 IJ/cm^2) and *S. riobrave* (50 IJ/cm^2) respectively. In addition to Tedder's traps, leaf damage were also measured and the damage was much lower in the *S. riobrave* (50 IJ/cm^2) treatment compared to the control. In the Grub Gaurd experiments, levels of FRB damage were similar early in the season, and there were no differences in leaf damage between treatment and control trees before application. After three applications of Grub Gaurd, leaf area lost to damage was 44% lower in treated trees compared to controls.

Key words: *Naupactus godmani*, biological control, *Heterorhabditis*, *Steinernema,* Grub Gaurd

OP-26. Challenges toward implementation of microbial pesticide with emphasis on entomopathogenic nematodes as biocontrol agents in Iran

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In Iran, agriculture is pesticides dependent and pests control is often done using chemical products. This huge pesticide use, bring concerns about possible links between the growing levels of several concerns while market demand for organic products is increasing. Besides, sometimes chemical control of certain pests is inefficiency. Therefore, there is tendency within Iranian farmers for using IPM approach by emphasis on biological control application in recent years. Biological control against agrarian pest in the country is a practice that has initiated in 1930s.Currently indigenous work on microbial biopesticides has increased rapidly and a list of about 20 biopesticide products are producing by companies (Table 1). In the case of entomopathogenic nematodes, no registered product from them are available. All we did here are research based works. Here I will discuss about the current status of the works in term of research issue. Moreover, main challenges toward progress of microbial pesticides as biocontrol in the country will be another part of the presentation. Finally, there are some suggest for possible improvement for nematodes as suitable element for biological control of insect pest in IPM.

Key words: Microbial control, Entomopathogenic nematodes, progress, IPM.

OP-27. Complex of endoparasitic nematodes of four-eyed fir bark beetle Po*lygraphus proximus*, and their impact on its immunity

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Invasion of four-eyed fir bark beetle *Polygraphus proximus* Blandford (FFBB) – forest pest of Far Eastern origin is the unique phenomenon due to its large scale ecological and economic impact on Siberian fir stands. The complex of parasites and pathogens of FFBB in the boundaries of its native habitats due to low economic significance and relatively recent finding in region of invasion is remained poorly known. In the recent studies, we have showed that the main fungal pathogens of this bark beetle in Siberia are *Beauveria pseudobassiana* S.A. Rehner & Humber 2011 and *Isaria farinosa* (Holmsk.) Fr. The aim of this study was to determine the complex of endoparasitic nematodes and determine the impact of the most abundant among them in the state of immunity in a specially infected laboratory cultures of *P. proximus*.

To create a culture of bark beetles infested by nematodes, the substrate with feeding on it larvae of FFBB was injected by juvenile nematodes from the hemolymph from infected beetles of a natural population at the rate of 15-20 nematodes per 20-30 larvae. Success of infection was tested on 60th day of insects' development on 15 randomly selected beetles. Number of larvae in the body cavities of bark beetles totaled 18.2 ± 6.4 pc. To analyze the enzyme activity used hemolymph of adults.

It was found that FFBB is infected by several species of nematodes: *Protorhabditis sp.* (Rhabditidae) *Rhabditolaimus sp, Pristionchus sp.* (Diplogasteridae) *Sychnotylenchus* (Anguinidae). The influence of most numerous endoparasite nematodes – *Sychnotylenchus sp.* (Nematoda: Anguinidae) on the immune response of the host is revealed. In particular, it was detected the inhibition of the host insect basic immune defense mechanisms such as the PO-cascade in 4.7 and activity of non-specific esterases in 3.02 times in compare with control.

This study not only expands knowledge on the number of natural enemies attacking this invasive insect, and also shows that nematodes can act as an ancillary factor of reducing of bark beetles immunity, that can be useful in the development of technologies of integrated pest management.

This study was supported by the Russian Science Foundation (grant № 15-14-10014)

Key words: bark beetle, *Polygraphus proximus*, nematodes, parasites, immunity

OP-28. Efficacy of entomopathogenic nematodes against European cherry fruit fly *Rhagoletis cerasi* (L.) in laboratory and field conditions

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European cherry fruit fly *Rhagoletis cerasi* (L.) is the most damaging pest of sweet and tart cherries. Methods to control it are mostly based on insecticides. The phase-out of old insecticides challenges management of this pest, since there is still lack of environmentally sound products. Entomopathogenic nematodes have shown high efficacy against soil dwelling stages of some pests. Since *R. cerasi* spends majority of its life cycle in soil it is potentially good target to entomopatogenic nematodes.

The objective of this study was to determinate efficacy of *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora* against adult stage of *R. cerasi*. In laboratory conditions 25, 50, 100, 200 and 400 nematodes per pupa were released in cell wells (24 cells filled with sterilized silver sand) in time before anticipated adult emergence. Number of death adults infested with the nematodes was assessed. In field conditions tests were performed in 2015 and 2016 in an orchard with high infestation with *R. cerasi*.12 cherry trees cultivar Regina were covered with insect proof net before estimated start of adult emergence to create a tunnel. The tunnel was divided on four compartments. Treatments included commercial products of *S. carpocapsae*, *S. feltiae*, Neemazaland non-treated control. The nematodes were applied on rate of 0.5 million/m^2. First application of nematodes was at time before anticipated start of adult emergence and second one followed 15 days after. In 2016 in each compartment additionally 150 cocoons were buried to soil depth of 2-5 cm. In laboratory conditions the highest adult mortality caused by nematodes was in treatments with *S. carpocapsae* (up to 83.6%). In field conditions the lowest percentage of infested fruits was with the same nematode in bought years (0-0.3%). This study reveals that entomopathogenic nematode *S. carpocapsae* have potential as control agents of the key pest of cherry *R. cerasi.*

Key words: biological control, *Steinernema feltiae*, cherry pests

OP-29. New insights in biocontrol strategy against *Cephalcia tannourinensis*, the principal insect defoliator of cedars in Lebanon

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The Tannourine forest is characterized by the presence of cedars (*Cedrus libani*, Richard) that suffer from the attacks of *Cephalcia tannourinensis* (Chevin). In this study, we investigated the presence of endemic entomopathogenic nematodes (EPNs) in the forest for their potential use as biocontrol agents in an integrated pest management (IPM) to control *C. tannourinensis*. We used *Galleria mellonella* baits in fifteen selected sites taking into consideration the cedars' different habitats. All the EPNs found belonged to the same strain of *Steinernemae feltiae* determined by morphometric and molecular analyzes. The pathogenicity of the endemic strain along with a commercial strain of *S. feltiae* (ENTONEM) was tested *in vitro* under different concentration rates. Both *S. feltiae* strains showed similar insect mortality rates but were significantly less virulent than the *Heterorhabditis bacteriophora* strain previously tested *in vitro* by Noujeim et al. (2015). This led to the choice of the commercial strain *H. bacteriophora* (HbCom) rather than the endemic *S. feltiae* strains for a controlled *in situ* experiment aiming to reduce the *C. tanourinensis* outbreaks. The *in situ* pathogenicity of HbCom was evaluated by inundative treatments on 1 x 1 m plots with jars buried in the soil containing either *C. tannourinensis* or *G. mellonella* larvae. Under natural conditions, a concentration rate of 625,000 Infective Juveniles (IJs)/m^2 of *H. bacteriophora* caused a mortality rate of 85.0 and 86.7% on *C. tannourinensis* and *G. mellonella* larvae, respectively. This experiment resulted in an unexpected emergence of opportunistic nematodes from the *C. tannourinensis* cadavers.

Keywords: *Cedrus libani, Cephalcia tannourinensis*, entomopathogenic nematodes (EPNs), natural environmental conditions

OP-30. Microbial and nematode control of the Colorado potato beetle

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The Colorado potato beetle, *Leptinotarsa decemlineata*, is the most widespread and best-known insect pest that causes great economic losses especially on potatoes. As a result of the intensive use of insecticides this species has gradually developed resistance to most pesticides and its regulation is thus currently very difficult. In addition to the use of disputable genetically modified crops as a promising solution to the problem of pest resistance development of biocontrol methods using natural pathogens might be solution for sustainable potato production. The aim of our study was to assess the efficacy of entomopathogenic fungus *Isaria fumosorosea* strain CCM 8367 and entomopathogenic nematode *Steinernema feltiae* strain Ustinov against *L. decemlineata* under laboratory conditions. Petri dish trials revealed the highest susceptibility in the last-instar larvae followed by pre-pupae and pupae. The median lethal concentration (LC50) of *I. fumosorosea* was estimated to be 1.03×10^6 blastospores/ml. Simultaneous application of the fungus with the nematodes increased the mortality of *L. decemlineata* larvae up to 98% and shortened the median lethal time to two days while no obvious changes in development of nematodes in cadavers were found. When, however, nematodes were applied more than 24 hours after fungus treatment, their development was negatively affected and adults were smaller in comparison to control. In soil application experiments, standard soil substrate was inoculated by either *I. fumosorosea*, *S. feltiae* or both before last instar larvae which finished feeding and searched for place to pupate were placed individually into the pots. The mortality of *L. decemlineata* was 44% and 45% when 1×10^8 blastospores of *I. fumosorosea* and 1000 IJ of *S. feltiae* were applied to the pot, respectively. Combined application of the fungus and the nematode at the same doses increased mortality to 84%. We can conclude that both entomopathogens are prospective biocontrol agents against *L. decemlineata* and that they could be applied together for higher efficacy.

Key words: entomopagogenic fungi and nematodes, *Leptinotarsa decemlineata*, synergistic effects, soil application

OP-31. The use of *Daldinia concentrica*, an endophytic fungus, and its bioactive volatiles against the plant parasitic nematode *Meloidogyne javanica*

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Endophytic organism is an organism located most of its live cycle inside the plant tissue without causing any visible damage or plant defense. Many endophytes secrete specialized metabolites that possess biological activity. Recently, we demonstrated that the endophytic fungus *Daldinia concentrica* secretes biologically active volatile organic compounds (VOCs). The plant pathogenic root-knot nematode *Meloidogyne javanica* is an obligatory parasite and a major concern to solanaceous cropping, including tomato and pepper. In the present study, we examined the ability of *D. concentrica* and its VOCs to control *M. javanica* both *in vitro* and in greenhouse experiments. To this aim, we exposed, without any direct contact, the nematode to the fungus culture or to an artificial mixture composed from four of its emitted volatiles. We found that exposure of J2 larvae to *D. concentrica* live culture caused 67% mortality of the larvae with nematocidic activity. We found that a mixture of the VOCs: 3-methyl-1-butanol, (±)-2-methyl-1-butanol, 4-heptanone, and isoamyl acetate at a ratio of 1:1:2:1, elevated J2 larvae mortality up to 99%. We demonstrated that although each of the four VOCs significantly increased the mortality of the larvae relative to the control, only the compound 4-heptanone exhibited the same mortality level as the whole mixture, and this compound had a nematocidic activity. Interestingly, only the mixture, but not the fungus, affected the hatching ability of *M. javanica* eggs. Finally, application of the mixture to inoculated soil in the presence of nematode suscepotible tomato seedlings resulted in significantly lower galling index and smaller number of eggs per gram soil with no effect on root weight. On the basis of these results we suggest the use of *D. concentrica* and/or an artificial mixture of its VOCs as an alternative approach, from a natural origin, to control the root-knot nematode *M. javanica*.

PP. 18. The assessment of the effect of entomopathogenic nematodes on bulb mites, pests of garlic and onion

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Bulb mites of the genus *Rhizoglyphus* are among the most serious pests of garlic and onion but also tulips, hyacinths and other plants. Females of these soil dwelling mites lay their eggs into the underground parts of many plants and their larvae altogether with the adult stages cause damage to plant tissues that are, consequently, more sensitive to bacterial and fungal pathogens. What more these mites also transmit many of plant pathogens, e.g. *Fusarium* sp. The damage mostly occurs in the fields, but under favorable conditions, these mites can continue with their harmful activity also in stored products As the females of *Rhizoglyphusechinopus* and less common *R. robini* are relatively large organisms that spend the whole life in the soil environment, the management by using entomopathogenic nematodes could be very promising.

In the present study, the screening of entomopathogenic nematodes in the fields of onion and garlic specialized farms in the Czech Republic and Israel was performed and more than thirty strains of *Steinernema* and *Heterorhabditis* nematodes were isolated. Selected strains were tested for infectivity and pathogenicity against garlic and onion pests, both insect (*Ephestia*) and mites (*Rhizoglyphus*) and the potential of the use of EPNs against bulb mites of the genus Rhizoglyphus was assessed.

Collection of soil samples in an organic onion field in Israel.

Key words: *Rhizoglyphus*, biological control, *Steinernema*, *Heterorhabditis*, Allium

PP. 19. Diversity of mollusc-parasitic nematodes of the genus *Phasmarhabditis*. How to identify them?

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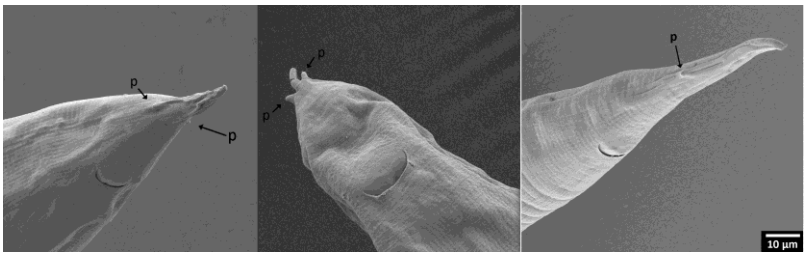
As the mollusc pests cause more and more damages in agriculture and horticulture parasites of molluscs are getting much attention. *Phasmarhabditis* nematodes as one of the most common slug and snail parasites do not fall behind. *Phasmarhabditis hermaphrodita* is the best known mollusc-parasitic nematode of the family Rhabditidae (Nematoda). However, during the last few years several new species from different parts of the world have been discovered and described. Only during the last year four descriptions were published with two species originating from the Czech Republic, one from Italy and one from USA. Up to these days (January 2017) we know nine species: *P. bonaquaense* (2017), *P. bohemica* (2016), *P. apuliae* (2016), *P. californica* (2016), *P. huizhouensis* (2015), *P. tawfiki* (2003) and older species *P. hermaphrodita*, *P. papillosa* and *P. neopapillosa*. Other new species will probably accrue in the close future.

In this study, we would like to show the diversity of these amazing nematodes and present the most apparent morphological, morphometrical and ecological characteristics that allow correct identification of all known species of the genus *Phasmarhabditis*.



***Phasmarhabditis* females tails with phasmids: *P. bohemica* (left), *P. bonaquaense* (center) and *P. apuliae* (right)**

Key words: *Phasmarhabditis*, morphology, identification, diagnostic key

PP. 20. Entomopathogenic nematodes to replace soil insecticides in western corn rootworm control

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In an attempt to replace insecticides against the maize-root feeding larvae of the western corn rootworm (*Diabrotica virgifera virgifera*, Coleoptera: Chrysomelidae), biological control solutionshave been developed. For example, commercially mass-produced *Steinernematid* and *Heterorhabditis* species of beneficial entomopathogenic nematodes are available in most world regions. However, their use on a larger scale such as in field crops like maize is still limited. Recent studies aimed at developing optimal application techniques for nematodes in field crops, i.e. being practical and effective at such a scale at reasonable costs. Field studies revealed that nematodes can be applied into the soil at sowing as well as into the soil along rows of young plants depending on the crop. As for the sowing period, fluid and micro-granular applications as well as seed coating with nematodes are, in principle, possible. However, the easiest and currently most promising technique is the fluid stream spray of a nematode-water suspension into soil at the moment of sowing or during mechanical weed control. This method requires a relatively low amount of water (200-400 l per hectare) compared with onto-soil applications. Against rootworms in maize for example, sowing machines are used that have simple fluid applicators that spray nematodes behind the sowing or press wheel into the furrow prior the soil-closing wheels. Farmers may adapt their equipment for fluid soil insecticides, or may use the nematode-specific application tools recently developed for common sowing machines. This allows reducing the required nematode dose to between 2 and 3 x 10^{9} nematodes per hectare, and thus the costs of this control technique. When comparing nematodes with soil insecticides, numerous field trials in central Europe have shown that nematodes as well as insecticides are of medium efficacy in reducing rootworms and preventing root damage. Variability of the control efficacy of the different agents appears high, likely due to the long hatching period of the rootworm larvae, as well as the variable soil environment. However, in general, beneficial nematodes are at least as effective as Tefluthrin -based soil granules, nearly as effective as clothianidin-based seed coatings, and more effective than chlorpyrifos-based or cypermethrin-based soil granules. In conclusion, beneficial nematodes are ready to be used for the biological control of western corn rootworms.

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PP. 21. Entomopathogenic nematodes to control *Sciarid* flies in mushroom-cropping systems of Georgia

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In recent few years the soil habitant harmful insects - *Lycoriella* and *Bradysia* spp. (*Diptera: Sciaridae)* were spread at hothouse champignon, *Agaricus bisporus* industries of Georgia. *Sciarid* flies reproduce extensively in organic substrate; the larvae feed by mushroom mycelium and cause great damage to the national economy. To obtain ecologically pure mushroom production, it is strongly recommended to use environmentally safe means for pest control. The hothouse conditions - high moisture content of the mushroom growing substrate, conducive to dipteran pest development offers a unique potential for exploiting entomopathogenic nematodes (EPNs). The assays have been carried out on 600 sq. m territory ofLTD Mushpro Company (Shida Kartli Region, Kaspi, Georgia). The commercial nematode, NEMYCEL® 300 *Steinernema feltiae* was imported from Germany (*e-nema* GmbH). Nematode suspension (2×10^5) was applied with every 14 days interval by the irrigation system, when larvae reached to the second or third instars. Biological efficacy reached to 90-95%. The longevity of infested pests has been reduced by approximately three days. Nematode juveniles actively search the pests; destroy the insect fat body and decrease copulation and oviposition. Additionally, nematodes, liberated as second instars, search and infect new hosts. As the results of investigations, the pests density been brought below to the economic threshold on the experimental plot. The perspectives planned to extensive use of EPNs as an optimal way to protect mushroom-cropping system from *Sciarid* flies in Georgia.

Key words: *Sciarid* flies, Entomopathogenic nematodes (EPNs),Biological efficacy

PP. 22. Are entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) compatible with acaricides?

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Entomopathogenic nematodes (EPNs) are organisms that can be used in biological control programs. To expand our knowledge about the compatibility of EPNs to pesticides, we studied the compatibility of EPNs infective juveniles (IJs) to five acaricides under laboratory conditions. The direct exposure of acaricides to EPNs was studied in Petri dishes at 15, 20, and 25 °C. EPNs were exposed to acaricides for 1, 4, and 24 hours. Four EPNs species were included in our investigation: *Steinernema feltiae*, *S. carpocapsae*, *S. kraussei*, and *Heterorhabditis bacteriophora*. The results of our laboratory investigation showed that *H. bacteriophora* was the most tolerant EPN species. On the other hand, the most sensitive EPN species was *S. feltiae*. Our observations showed that *S. feltiae* can be mixed with only two acaricides. The active ingredient fenpyroximate proved to be the most suitable for mixing with EPNs. Our results showed that fenpyroximate was only lethal to *S. feltiae* (44 % mortality) at 25 °C. The mortality of EPNs was highest in the active ingredients abamectin and pyrethrin. Our results confirmed that the compatibility of EPNs to acaricides is a species-specific characteristic. The mortality of EPNs was also influenced by the exposure time, active ingredient, and temperature. The combined use of EPNs and acaricides could represent an advantage in integrated plant protection programmes. Combinations of EPNs and acaricides could save time and money in the simultaneous control of various pests (insects, mites) in glasshouses.

Key words: acaricides, temperature, *Steinernema*, *Heterorhabditis*, compatibility

PP. 23. Management of Leaf Curl Plum Aphid, *Brachycaudus helichrysi* (Hemiptera:Aphididae) by entomopathogenic nematodes

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Aphids are small, soft-bodied insects that suck sap directly from plant tissue. Although many are unprotected and flightless, a few aphids on fruit trees exude a waxy coating, an example being the leaf curl plum aphid (LCPA) (*Brachycaudus helichrysi* Kaltenbach, 1843), a pest of plum trees in Georgia. These insects feed on the undersides of leaves, as well as on bark-covered areas, shoots and succulent growth. The most obvious sign of these aphids on plum trees is the curled leaves.

The efficacy of three entomopathogenic nematode species, *Steinernema carpocapsae*, *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the adult stage of LCPA was evaluated under laboratory conditions. The experiments were conducted in 10 cm Petri dishes lined with a moistened filter paper. One infested plum leaf containing 100-120 LCPA adults was placed in each Petri dish and the nematodes were applied as 50, 100 or 150 infective juveniles (IJs)/ml per cm^2. Plates were incubated at 15, 20 and 25°C and the insect mortality was checked 24, 48, 72 and 96 hours after the treatment. Ten Petri dishes were used for each nematode concentration and temperature. The results showed that entomopathogenic nematodes were quite effective against LCPA and the mortality was related to nematode concentration and temperature. At 15°C, *S.carpocapsae, S. feltiae* and *H. bacteriophora* exhibited 59, 24 and 23% mortality, respectively. *S. carpocapsae* showed the highest mortality (79%) following *S. feltiae* (58%) and *H. bacteriophora* (32%). For all nematode species, the highest virulence was observed 85%, 69% and 58% on the temperature 25°C and 150 IJs/ml cm^2 concentration for *S. carpocapsae, S. feltiae and H. bacteriophora, respectively*.

In conclusion, it was determined that LCPA can be controlled by *S. carpocapsae,* but further studies should be conducted under greenhouse and field conditions.

Key words: *Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis bacteriophora*

PP. 24. Compatibility of bio-nematicide and plant stimulant of microbial origin with *Heterorhabditis bacteriophora*

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Over the past decades, farmers became more and more dependent on agrochemicals as an important tool to protect crops. Due to environmental impact and health issues, consumers rather choose pesticide-free food. Biocontrol agents are often applied with inorganic and organic plant fertilizers, and many of these amendments have nematicidal effect. Previous studies mostly evaluated the tank-mix compatibility of chemical pesticides with entomopathogenic nematodes. We evaluated the compatibility of bio-nematicide and plant stimulant of microbial origin on viability of *Heterorhabditis bacteriophora* infective juveniles (IJs) under laboratory conditions. The motility of the IJs of *H. bacteriophora* was determined after 1, 3,24 and 72 hours of direct exposure to bio-nematicide and plant stimulant used in their commercial formulations and recommended concentrations. Later, to assess infectivity IJs were tested against *Achroia grisella* larvae for 96 h. We found that bio-nematicide was highly toxic to *H. bacteriophora*. IJs were paralyzed after 3 h of direct exposure to bio-nematicide, and 24 h later, we observed 100% mortality. The plant stimulant did not affect nematode motility or infectivity. Our results demonstrate that *H. bacteriophora* is highly susceptible, intolerant to bio-nematicide, and compatible with the plant stimulant with no loss in viability and infectivity up to 72 h of exposure.

Key words: biocontrol, fertilizer**,** infective juveniles, motility, infectivity

ABSTRACTS:
SECTION IPM

OP-32. IPM in practical wireworm control; struggle or challenge?

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Wireworm control in mainstream farming to date consists of applications of chemical compounds. Despite many years of research and development targeting at non-chemical alternatives, mainly including entomopathogenic fungi (EPF) and plant extracts, and some market introductions, there is much room for increase of their market share. Although research has made a huge effort in working towards improvement, market penetration of non-chemical wireworm control is hindered by product properties as difficulty to handle/apply, less than optimal efficacy, and the farming community's unfamiliarity with the product(s), all of these being interconnected too. Following the EU Directive 2009/128/EC, all EU Member States have to comply with stricter guidelines regarding IPM before 2023. In many countries also retail parties play a role in this force field, following from public awareness on crop protection issues. All of these factors imply increased (re-)registration pressure on the current chemical compounds and open new opportunities for introduction of non-chemical alternatives. Use of these alternatives may need a change in approach, and close collaboration, of research, extension services and farmers. The result can be a system innovation with a recognized position of both adequate and reliable monitoring and non-chemical wireworm control. Examples are provided of possible ways to achieve the goals described.

Key words: IPM, wireworms, system innovation, entomopathogenic fungi, plant extract

OP-33. Comparison of pathogen abundance between high and low level populations of *Ips typographus* in Croatia after an ice storm disaster

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A heavy ice storm, specifically, ice rain struck the territory of Slovenia and a part of Croatia on February 2014, and created an ice coat on branches and on tree tops, which consequently had catastrophic consequences on forest ecosystems. In Croatia, the ice-break has in the most part struck the area of Gorski Kotar, where around 1.65 million m3 of timber volume on the area of 56 000 ha had been destroyed or damaged. As a consequence of this natural disaster an exponential growth of bark beetle populations have started to build-up from 2015 and are still on outbreak level. Especially spruce forests are under attack of the two spruce bark beetles, *Ips typographus* and *Pityogenes chalcographus*. In 2016 about 100000 m3 bark beetle attacked timber were cut in order to suppress the population.

It is expected that natural enemies will respond with higher population level in the coming years and break the population. Already high population of predators and parasitoids are recorded, but nothing is known about the pathogen level.

The objective of the current study was to estimate how natural enemies, especially pathogens response to such a rapid grow of a pest population (*I. typographus)*. For this reason, two population of *I. typographus* were compared. During the winter in 2017 beetles were collected from infested spruce of the outbreak population, and likewise in Lika where no outbreak of *I. typographus* was recorded since more than 10 years. A total of 1000 mature and callow beetles from both regions were examined with a light microscope. Species spectrum and abundance will be discussed.

PP. 25. Natural microbial antagonists and model based risk assessment of oak processionary moth (*Thaumetopoea processionea*) in climate change

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The proliferation of oak processionary moth (OPM), *Thaumetopoea processionea* L. (Lepidoptera: Notodontidae), is increasingly alarming in Central Europe since the 1990s. Possibly, this gradation is linked to the current climate change. Besides the risk of defoliation of host trees by the caterpillars, their dangerous hairs (setae) that may severely affect human health are particularly considered as relevant threat. Therefore, the basic elements of an early warning system comprising regional data on the development and density of OPM-populations as well as on the associated risk for forests and human health should be developed in the course of the present project, based on five research institutional partners. The combination of basics in phenology and population dynamics of OPM with aerial spread of setae dependent on spatial distance and weather (forecast) will contribute to make current and future threat of setae pollution and defoliation by the caterpillars predictable. Hence, the envisaged early warning system - which will be applicable to the whole range of OPM - should enable a timely and effective application of measures against this pest in favor of oak tree and human health protection.

As parasites, parasitoids and predators as well as microbial antagonists including viruses are important natural regulation factors for arthropod gradations, the whole spectrum of natural antagonists of OPM is intensively monitored and examined within this project. Their impact on densities and dynamics of OPM-populations under different climatic conditions in selected regions of Germany is studied. Various developmental stages of the OPM are considered. The spectra of parasites and parasitoids as well as the infection rates of pathogens will be determined. Furthermore, the virulence of the pathogens will be tested against young developmental stages of OPM. First results of these investigations will be presented.

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PP. 26. Neem oil inhibits larval and pupal development of *Drosophila suzukii* in feeding trials under laboratory conditions

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The spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura, is native in South East Asia and Japan but has spread to Europe and North America during the last decade. Today it can be found in many regions in Europe where it causes severe damages to commercially grown crop plants like stone fruits, berries and grapes. Because of the infestation occurs on ripening fruits just before harvest, the application of chemical insecticides is difficult for residue reasons. Therefore, effective biological control strategies would be highly desirable. The oil extract from seeds of the Neem tree (*Azadirachta* spp.) is widely used as biological control agent against leaf mining, sucking and biting pest insects like blackflies, thrips, spider mites and white flies. The secondary metabolite Azadirachtin has an insecticidal effect as it inhibits moulting of insects. In this study "Naturen Bio Schädlingsfrei (Germany)" was tested against SWD larvae with promising results. In bioassays the Neem oil product caused up to 98% mortality of larvae and pupae until 12 dpi with an applied concentration that was ten times higher than recommended for black flies and other target insects.

Keywords: spotted wing drosophila, Neem oil, bioassays

ABSTRACTS:
SECTION VIRUS

OP-34. Functional Analysis of Putative glycosyl transferase gene (AMV248) of Amsacta moorei entomopoxvirus

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Amsacta moorei entomopoxvirus (AMEV) is the type member of Betaentomopoxvirus genus that infects Lepidopterans and Orthopterans, which has potential for using as microbiological control agent. AMEV is easily grew and manipulated in cell culture. The virus has 232kb dsDNA genome including 256 open reading frames (ORF). AMV248 is one of the ORFs which is suggested to encode a putative glycosyltransferase that may have role in binding host cellular glycosaminoglycans (GAGs). According to sequence information; AMV248 is the only conserved protein in AMEV genome responsible from attachment. The current study focused on functional characterization of AMV248. In order to understand the function of this protein, recombinant amv248 protein was expressed in bacterial and Baculovirus expression vector systems. Monoclonal antibody against AMEV248 protein (antigen) was produced, and this antibody was used as neutralizing agent for AMEV. Sequence analysis of AMV248 suggested that protein binds to heparin. So, heparin binding capacity of recombinant protein and virus investigated in silico and in vitro. Our results showed that recombinant 30 kDa amv248 protein was successfully expressed. This protein was used for monoclonal antibody production. AMEV infectivity was significantly reduced after neutralization with the monoclonal antibody in neutralization assay. It was shown that both produced recombinant protein and AMEV virus bind to heparin. Also, 3D structure of AM248 protein predicted, and docking experiments with heparin molecule shown that AMV248 binds to heparin in-silico. Consequently, we have found that AMV248 gene encodes a viral glycosyl transfer protein that is important for viral attachment, also neutralization and blocking of this protein cause significant decrease in viral infection.

Key words: *Amsacta moorei entomopoxvirus*, AMV248, protein production, glycosyl transferase, virus neutralization

Acknowledgements: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No. 113Z219).

OP-35. Protein-Protein Interactions Between Attachment (AMV248) and Entry Fusion Complex Proteins of Amsacta moorei entomopoxvirus

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Amsacta moorei entomopoxvirus (AMEV) isolated originally from the larvae of *A. moorei* moth that infects Lepidopterans and Orthopterans, is the most studied member of Betaentomopoxvirus genus (subfamily Entomopoxvirinae). Although most entomopoxviruses are not; AMEV can be easily grew and manipulated in cell culture. The control of host range and infectivity initiate via virus attachment, fusion and entry. Therefore, interactions between proteins which takes place significant role in the early steps of infection are important to understand the initiation of virus replication. AMV248 is the only ORF which encodes a glycosyltransferase that may have role in binding host cellular glycosaminoglycans (GAGs). According to sequence information; AMV248 is the only conserved attachment protein in AMEV genome too. Viral replication process starts with attachment to the host cell and after that a protein complex called "Entry Fusion Complex (EFC)" activated to continue replication. Early studies show that 10 viral proteins (AMV35, AMVV83, AMV118, AMV127, AMV138, AMV186, AMV217, AMV232, AMV243, AMV249) function in EFC. This study was focused on determining protein-protein interactions between attachment (AMV248) and EFC proteins of AMEV.

To investigate interactions between attachment and fusion proteins yeast-two-hybrid method was used. AMV248 gene was cloned into pGBKT7 DNA-BD (bait vector) and all entry-fusion complex proteins and amv248 gene were cloned to the pGADT7-AD (prey vector). Then protein-protein interactions of these proteins were screened with transformation into competent cells. Y2H results showed that AMV248 interacts with only AM186 protein that is required for syncytium formation. The results also confirmed with pull down assays.

Key words: *Amsacta moorei entomopoxvirus*, AMV248, protein-protein interactions, entry fusion complex, viral attachment, Y2H

Acknowledgements:This study was financially supported by Karadeniz Technical University (Project No: BAP01 FBA-2015-5187).

OP-36. Baculovirus synergism: investigating mixed alphabaculovirus and betabaculovirus infections in the false codling moth, *Thaumatotibia leucotreta*, for improved pest control

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Baculovirus based biopesticides have long been shown to be an effective and environmentally friendly approach for the control of agriculturally important insect pests. The false codling moth (FCM), *Thaumatotibia leucotreta*, is a major pest of citrus crops in southern Africa, posing a risk to exports and thus creating a need for improved control methods. The *Cryptophlebia leucotreta* granulovirus (CrleGV) has been commercially formulated into the product Cryptogran and others and used as part of an integrated pest management programme in South Africa for over a decade with much success. A concern is the possibility of resistance developing towards CrleGV, as was seen in Europe with field populations of the codling moth, *Cydia pomonella*, to the Mexican isolate of the *Cydia pomonella* granulovirus (CpGV-M). In order to prevent such a scenario occurring in South Africa in the case of CrleGV, there is a need to identify and isolate additional baculovirus variants which can be implemented as new biopesticides. In this study, a novel nucleopolyhedrovirus in FCM larval homogenates was genetically characterised and identified as *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV). The NPV was purified from a GV-NPV (alpha-betabaculovirus) mixture using *C. pomonella* fifth instar larvae. A multiplex PCR assay was developed for the rapid screening of samples. Purified NPV and CrleGV were evaluated using surface dose bioassays both individually and in various combinations against FCM neonate larvae. A synergistic effect was observed which may lead to the development of improved biopesticides for the control of FCM in the field. Furthermore, these dual infections are currently being studied by serial passage of the viruses through multiple iterations of FCM larvae. Genomic DNA extracted from recovered occlusion bodies after each passage will be examined by multiplex and quantitative PCR to examine shifts in GV to NPV ratios. Lastly, OBs recovered from the final iteration will be passaged through codling moth larvae in order to isolate the NPV from the GV. Genomic DNA extracted from these NPV OBs will be used to determine the complete genome sequence enabling the identification of potential recombination events which may have occurred during the dual GV and NPV infection.

Key words: False codling moth, Baculoviruses, Biological control, Synergism

OP-37. Measuring the fitness cost of type I resistance breaking by CpGV

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In a previous work, we have measured virus productivity and pathogenicity of two virus isolates: CpGV-M, and CpGV-R5 (a type I resistance breaking isolate). We were not able to found significate differences between them. Another way of testing fitness differences is to analyses genotype replacement in mixed genotype experimental populations over successive generations. We constructed five experimental populations with various proportions of each isolate and followed them through six successive passages on insects (Table 1).

Table1

CL50	CpNPP		R_{GV}	
Mix	**Passage 0**	**Passage 6**	**Passage 0**	**Passage 6**
M100:0R	13.1 (6.6–23.2)†		2.2 10^6 (1.2·10^6–5.7·10^6) †	
M99: 01R	28.8 (4.2-55.9)	13.9 (6.2-26.4)	4160 (1980-7610)	40.9 (7.43-109.2)
M95:05R	19.4 (10.0-33.1)	8.2 (4.9-14.4)	615.7 (142.8-1460)	34.2 (15.9-63.4)
M90:10R	12.4 (4.9-25.4) †	10.5 (6.0-16.5)	201.8 (136.0-80.8)†	30.3 (15.8-50.4)
M50:50R	10.7 (6.7-16.1) †	17.6 (10.6-27.5)	16.5 (8.4-29.1)†	38.7 (24.6-56.4)
M10:90R	7.0 (3.2-12.3)	29.9 (8.3-66.2)	31.6 (20.5-44.4)	28.2 (19.3-38.3)
M0:100R	6.8 (2.6–13.4) †		22.4 (13.7–34.4) †	

†: data from Graillot etal., 2016. Viruses 8:147, for reference

In absence of selection, that is when replicating in an insect colony susceptible to both virus isolates, the ability to kill resistant larvae is not lost. After 6 passages, the pathogenicities of the 5 experimental virus populations on the resistant insect colony R_{GV} converge to a common level (χ^2=13.35, 8 dof, P>0.05).In addition, markers specific to both isolates are detected. Our results suggest again that, at least in laboratory conditions, the cost for the virus to break type I resistance-if it exists- is under the level of detection. Putting together all these observations on the virus and on the host, they suggest the existence of multiple choices with no cost for the players (virus and insect).

Lethal concentration 50 (95% CI) on susceptible (CpNPP) or resistant (R_{GV}) insect colonies of experimental virus populations established by mixing OBs in the indicated proportions of CpGV-M and CpGV-R5, at P0 (that is the mixed OBs) and after 6 passages on CpNPP.

Key words: codling moth, *Cydia pomonella* granulovirus, resistance, genetic diversity

OP-38. The isolation of a novel alphabaculovirus and its potential for microbial control of key tortricid moth pests

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A laboratory culture of *Cryptophlebia peltastica*, a major pest of litchis in southern Africa, was established for the first time. During the rearing of *C. peltastica*, larvae showing symptoms of baculovirus infection were collected and analyzed. Occlusion bodies (OBs) were purified from the symptomatic larvae and observed by transmission electron microscopy to be a nucleopolyhedrovirus. Genetic characterization through creating restriction profiles and sequencing the whole genome, revealed that this was a novel alphabaculovirus, with no close relative, and was thus named the *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV). This is the first known record of an NPV naturally infecting any species within the Grapholitini tribe of the Tortricidae. Bioassays were used to determine the virulence of CrpeNPV against *C. peltastica*, *Thaumatotibia leucotreta* (false codling moth) and *Cydia pomonella* (codling moth). The LC_{90} of CrpeNPV for neonate larvae of these three species was respectively, 3.33×10^5, 9.97×10^4 and 1.26×10^4 OBs/ml. The average speed of kill to cause 90% mortality of each species was 5 days. As the bioassay results indicated superior virulence against *T. leucotreta* and *C. pomonella* than against the homologous host, *C. peltastica*, field trials were conducted against *T. leucotreta* in citrus and *C. pomonella* in apples at rates of between 5×10^{11} and 5×10^{13} OBs/ha. Very promising results were achieved. Consequently, CrpeNPVis considered to have significant potential for effective biological control of these, and potentially other, key tortricid pests of agriculture.

Key words: *Cryptophlebia peltastica,* nucleopolyhedrovirus, genome sequencing, bioassays, field trials, *Thaumatotibia leucotreta*, *Cydia pomonella*

OP-39. **Integratet Pest and Resistance Management of *Helicoverpa zea* (Lepidoptera: Noctuidae) with a Helicoverpa armigera nucleopolyhedrovirus**

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Helicoverpa zea (Lepidoptera: Noctuidae) the corn earworm (CEW) is a key pest in sweet corn, soy, sorghum and cotton in the USA. Since there are known resistances to common chemical-synthetic pesticides the use of Baculoviruses (BV) offers new options for integrated pest management. Helicovex® (HearNPV) is a highly specific virus strain to control *H.zea*. Thanks to the high selectivity it does not harm beneficials.

In close collaboration with researchers and distributors, Andermatt Biocontrol is developing strategies to use Helicovex® for the control of *H.zea* in sweet corn. Field trials are performed in the USA in different concentrations, stand-alone and in combination with grower standard products.

Helicovex® showed significant better protection than the pyrethroid based product Warrior II®, indicating the presence of pyrethroid resistant larvae. Even though pest pressure was exceptionally high, Helicovex® could almost double the amount of undamaged ears. For resistance management, Helicovex® can also be applied in rotation with pyrethroids, indicated in the second trial. There is no significant difference between pyrethroid (Mustang Maxx®) and the rotation of the pyrethroid and Helicovex®. In a third trial the Bta based product Xentari® and Helicovex® were compared. Helicovex® had significant less unmarketable Ears (23.2%) than Xentari® (50%). All these results indicate that with prevalent resistances to pyretroids and other insecticides, Helicovex® and other BV products are an essential tool for resistance management in organic and IPM production systems.

Key words: pests, diseases, integrated control, HearNPV, *Helicoverpa zea*, Baculovirus

PP. 27. Nucleopolyhedroviruses from the lackey moth, *Malacosoma neustria* (Lepidoptera: Lasiocampidae) in Turkey: A promising biological control agent

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The lackey moth, *Malacosoma neustria* (Linnaeus, 1758), a worldwide pest, causes extensive economic losses on especially hazelnut, prunus, quercus, populus and salix trees. In this study, *Malacosoma neustria* larvae, collected from different localities (Gümüşhane and Samsun) in Turkey, were examined for the presence of inclusion bodies under light and electron microscopes. Inclusion bodies of infected larval samples were subjected to polymerase chain reaction (PCR) using the conserved primers for *late expression factor* 8 (*lef*-8) gene. Sequence analysis, confirming microscopy results, showed that larval samples from two different localities contain multiple nucleopolyhedrosis viruses (MNPVs). These isolates were designated as ManeNPV-T2 and ManeNPV-T3. Furthermore, restriction endonuclease analysis of both geographic isolates with *Hind*III enzyme generated no differences between isolates. The mean sizes estimated for the complete genome of both isolates (ManeNPV-T2 and ManeNPV-T3) was calculated to be approximately 101.298 kb according to the *Hind*III profile. The insecticidal activities of these isolates were determined using third instar *M.neustria* larvae. Dose responds experiments showed viral isolates caused 100% mortalities at 14 days post infection for both isolates. LC_{50} value of ManeNPV-T2 and ManeNPV-T3 were determined as $0.19x10^4$ OBs/ml and $0.24x10^3$ OBs/ml respectively. Results indicated that these two nucleopolyhedrosis virus isolates are promising biological control agents against the *M. neustria* larvae. However, further studies are needed to test the insecticidal activities of these isolates against more Lepidopteran insects in more detail.

Key words: *Malacosoma neustria*, nucleopolyhedrovirus, isolation, phylogeny, virulence, microscopy

Acknowledgement: This study was supported financially by The Scientific and Research Council of Turkey (TUBITAK, 2211-A).

PP. 28. The First Detection of a Baculovirus in *Heliothis peltigera* (Lepidoptera: Noctuidae) (Bordered Straw) Larvae

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Baculoviruses are double-stranded DNA viruses which are highly selective for several insect groups. Starting in the 1940s, they have been studied and used widely as biopesticides in crop fields. We isolated a new nucleopolyhedrosis virus (HepeNPV) from *Heliothis peltigera* in safflower field, Çukurova region of Turkey. This is the first baculovirus isolate which has been detected in safflower green worm in the world up to now. The presences of inclusion bodies were examined under dark field microscope, scanning and transmission electron microscope. The sizes of inclusion bodies were measured as 0.121 µm ± 0,045 µm. The length of a rod-shaped nucleocapsid was 123 nm with a width of 25 nm. The larval samples containing inclusion bodies were subjected to polymerase chain reaction using the conserved primers for polyhedrin (*polh*), late expression factor 8 (*lef* 8) and late expression factor 9 (*lef* 9) genes. The amplified fragments were sequenced, and the sequences were compared to known nucleopolyhedrosis virus sequences from order Lepidoptera. The results showed that HepeNPV located in a position near *H. zea*, *H. armigera* and *Busseola fusca* NPV isolates in phylogenetic tree. The genome size of HepeNPV was determined approximately 131kbp. The isolated virus was propagated in healthy *Heliothis* larvae. Bioassay of four different virus concentrations (10^3-10^6 OBs/ml) against third instar larvae of *H. peltigera* and *H. armiger a*rranged from48% to 98% and 48% to 93% mortalities, respectively, within 14 days. LC_{50} of viral isolate was determined as 1.4×10^3 and 1.2×10^3 OBs/ml, respectively. Our findings indicate that HepeNPV is a promising biological control agent against *Heliothis* species.

Key words: Baculovirus, *Heliothis peltigera*, HepeNPV, nucleopolyhedrosis virus.

Acknowledgements: This study was financially supported by Karadeniz Technical University (Project No: BAP06 FDK-2016-5468).

PP. 29. Determination of Protein-Protein Interactions among Protein Kinases of *Amsacta* moorei entomopoxvirus (AMEV) and Protein Kinases with the other Proteins of AMEV

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Entomopoxviruses are an important group of virus, which infect only insects. These are from poxviruses group which can, as a family, infect both invertebrates and vertebrates, including humans. Protein kinases were known to have roles at virus morphogenesis, host selectivity, the regulation of cell division and apoptosis in some vertebrate poxviruses. In this study, interactions between 2 protein kinases and 230 virus genomic proteins of *Amsacta moorei entomopoxvirus* have been investigated by yeast-two-hybrid system. The protein kinases genes were cloned in *bait* and virus genomic genes were cloned in *prey* vectors. *Bait* vector was introduced into *Saccharomyces cerevisiae* AH109. Expression of the *bait* genes was confirmed by western blot hybridisation analysis. After confirmation, *prey* vectors were transformed into yeast cells, which carry the bait vectors. Yeast cells contain *bait* and *prey* vectors were grown on a selective medium (minimal synthetic defined) to determine the protein-protein interactions between BAIT and PREY proteins. The colonies grown on this medium indicate positive interactions between respective proteins. Five and 11 interactions were determined among AMV153 and AMV197 protein kinase proteins, respectively. The interacted proteins of AMEV protein kinases and their roles at virus replication will be identified.

Key words: *Amsacta moorei entomopoxvirus*, serin/threonin protein kinases, yeast two hybrid system, protein-protein interactions.

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PP. 30. Effect of temperature on long-term storage of Cydia pomonella granulovirus (CpGV-M)

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Codling moth (CM) (*Cydia pomonella* L.) is the major pest in apple and pear production worldwide. The application of Cydia pomonella granulovirus (CpGV) products is an ecologically safe alternative to chemical treatments to control CM. In this study, the long-term activity of occlusion bodies (OB) of the commonly used CpGV-M isolate, stored at room temperature (RT), +4 °C, -20 °C, and -80 °C for five years was assessed. Median lethal concentration (LC_{50}) of the tested OB suspensions was elucidated in full-range bioassays with neonates of the susceptible CM strain CpS after seven and 14 days. The LC_{50} values at +4 °C, -20 °C and -80 °C storage temperature was between 225 OB/ml (4 °C) and 7991 OB/ml (-20 °C) after seven days and comparable to the LC_{50} determined at the beginning of the storage test (LC_{50} 1871 OB/ml). Only the virus-induced mortality of OBs stored at RT decreased that the LC_{50} could not be determined. This research indicates that the storage of OBs of CpGV-M at 4 °C or colder will ensure the bioactivity for at least five years.

Keywords: codling moth, granulovirus, stability, median lethal concentration.

PP. 31. Genetic stability of the *Cryptophlebia leucotreta* granulovirus (CrleGV-SA), after 15 years of commercial use as a biopesticide

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Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) or otherwise commonly known as the false codling moth (FCM) is an indigenous pest of the citrus industry in southern Africa. Since FCM does not occur in most countries that South Africa exports to, the pest poses a phytosanitary concern. To control FCM in South Africa, an integrated pest management (IPM) programme incorporating the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) as a biopesticide has been implemented. This study investigated the genetic stability of CrleGV-SA used in commercial products produced in 2000 and 2015, by PCR amplification and sequencing of selected viral genes and restriction endonuclease (REN) analysis of genomic DNA.The results showed that selected gene sequences were 100% identical in both CrleGV-SA 2000 and 2015 strains but varied in percentage identity when compared to the Cape Verde isolate for which a complete genome sequence is available. In addition, genomic DNA profiles were similar to each other but differed from that of the Cape Verde isolate generated *in silico*. Dose-response bioassays also showed no significant change in virulence to neonate FCM larvae over the 15-year period. These results imply that the genome CrleGV-SA has remained stable over many years with implications for its use as a biopesticide in the field. Considering its success in the field, new approaches are being investigated to improve the efficacy of the virus. One approach is yeast-virus synergism, as it has been reported that there is a mutualistic association between codling moth, *Cydia pomonella*, and epiphytic yeasts in the genus *Metschnikowia*, leading to significantly improved efficacy in laboratory assays and field trials. Our research is looking to determine which species of yeast occur naturally in the digestive tract, frass and on the epidermis of FCM larvae and to examine whether any of these yeasts, when combined with CrleGV-SA, have a synergistic effect in increasing mortality of neonate FCM larvae and improving efficacy in field trials on citrus.
Key words: Betabaculovirus, false codling moth, biopesticide, genome stability, yeast-virus synergism

PP. 32. New method for granuloviruses differentiation based on Multitemperature Single Stranded Conformational Polymorphism

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Baculoviruses have been used as biopesticides for decades. Recently, due to the excessive use of chemical pesticides there is a need for finding new agents that may be useful in biological protection – very specific, selective and safe for humans, that do not accumulate in the environment and possess high virulence against insect pests. Sometimes, few isolates or species are discovered in one host. In the past few years, many new baculovirus species have been isolated from environmental samples, thoroughly characterized and thanks to rapid development of next generation sequencing methods, their genomes are being deposited in GenBank database. In general, NGS methodology is the most certain way of detection, but it is time-consuming, expensive and qualified staff are necessary to process raw data. Taking into account these limitations, we have searched for rapid and affordable methods for finding new isolates/species of baculoviruses from the Betabaculovirus genus. During our studies, we have developed a method which allows detection and differentiation of new granuloviruses. The method based on Polymerase Chain Reaction (PCR) followed by Multitemperature Single Stranded Conformational Polymorphism (MSSCP)allows for distinguishing new granulovirus isolates in only a few hours and at low-cost.

Species from every clade have been chosen on the basis of phylogenetic analysis of betabaculoviruses available in GenBank database. The alignment of highly conserved genes - *granulin* and *lef-9* was performed and the degenerate primers were designed to amplify the most variable, short DNA fragments flanked with the most conserved sequences. Afterwards, products of the PCR reaction were analyzed by the MSSCP technique, where short single stranded DNA fragments are electrophoresed in polyacrylamide gel followed by silver staining of the gel. The difference of even one nucleotide between sequences may be easily detected. The comparison of band profiles allows for species differentiation. In our opinion, the proposed method may be used for screening of new isolates derived from environmental samples.

Key words: pests, granulovirus, fast detection, MSSCP

PP. 33. Isolation and molecular characterization of the *heat shock protein*-90 gene (*hsp*-90) in three *Steinernema* species from Georgia

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A portion of the *hsp*-90 gene was amplified, using degenerate primers, in *Steinernema thesami*, *S. tbilisiensis* and *Steinernema* sp. isolated in Georgia. Two amplified products were obtained for each species and both fragments were cloned and sequenced. Blast search at NCBI revealed that both fragments for each species coded for *hsp*-90. Sequence analyses revealed that all three *Steinernema* species contained two different *hsp*-90 genes and the main difference was the intron presence-absence between *hsp*-90 genes. The *hsp*-90 genes containing introns showed conserved intron position between them with high sequence variation. This study showed, for the first time, the presence of two different isoforms for the *hsp*-90 in nematodes and the conserved position of the intron between the three *Steinernema* species. Further investigations are ongoing in order to characterize thermotolerant strains.

Key words: *Steinernema*, *hsp*-90, PCR, sequencing

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PP. 34. Differential susceptibility of *Popillia japonica* 3rd instars to *Heterorhabditis bacteriophora* (Italian strain) at three different seasons

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Popillia japonica Newman (Coleoptera: Scarabaeidae), a pest native to northern Japan, was recently found in northern Italy, in the Ticino valley, where the spread of this beetle has become a severe biological invasion. Entomopathogenic nematodes are useful for biological control of this invasive insect. Previous work showed that 1st and 2nd larval stages are more susceptible to nematodes than 3rd instars. In the present work we tested the effectiveness of an Italian strain of *Heterorhabditis bacteriophora* Poinar in the laboratory against *P. japonica* 3rd instars. Experiments were conducted with larvae collected in the sandy soil of a hay-field, located at Oleggio (Novara, Piedmont), in the fall, winter and spring.

Three separate tests were carried out. Thirty plastic containers, each containing one 3rd instar larva and 100 cc of native sandy soil, were used as the experimental units; each test had its control (n = 30). Larvae were acclimated at 20 °C for a week before nematode inoculation. The nematodes were tested at concentration of 1145 nematodes/insect (50 IJs/cm^2), and were applied to the soil surface of each container. The containers were incubated at 20 ± 1 °C and 90 ± 5% RH in the dark. Insect mortality was carefully checked every two days for four weeks and dead larvae were placed on modified White traps to recover Infective juveniles.

The results showed a significant decrease of the *P. japonica* susceptibility to this *H. bacteriophora* strain over the three collection periods. The corrected mortality efficacy ranged from 96.3% in October–November, to 85.7% in January–February, down to 65.5% in April - May. Our results leaded to the suggestion that, if additional applications after summer are necessary, the best period to perform additional treatments would be the fall, rather than the spring.

Key words: biological control, white grub, Japanese beetle, pest management, entomopathogenic nematode

PP. 35. Novel attract-and-kill formulation for biological wireworm control

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Wireworms, the polyphagous soil-dwelling larvae of click beetles (Coleoptera: Elateridae), are a major insect pest of worldwide relevance causing tremendous yield losses in several crop production systems, like potatoes. The entomopathogenic fungus *Metarhizium brunneum* is already kown as an efficient biocontrol agent against wireworms. However, applications of unformulated *M. brunneum* are too expensive due to a required high dose per ha. Besides, the fungus is poorly storable for months and has a low rhizosphere competence. Therefore, an innovative attract and kill formulation based on solely biological components was developed: ATTRACAP®. The granulate contains baker's yeast as an attract component, an isolate of *M. brunneum* as a kill component and a substrate as a nturient source and drying aid. The granulate emits CO_2 after contact with soil humidity over a period of 5 weeks to attract the wireworms. Simultaneously, *M. brunneum* is growing out of the beads and forming new conidia infecting the attracted wireworms. The attract and kill formulation enables a lower application dose per hectare, thus, making the granulate cost-effective and eco-friendly. In 2017, ATTRACAP® obtained for the second time the emergency registration for 9000 ha in Germany and Austria. The future aims are to improve the efficacy of ATTRACAP® and to transfer this technology to other pests.

Key words: wireworms, entomopathogenic fungi, biological control, attract and kill

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F

G

H

I

J

K

L

M

N

O

P

R

S

T

U

V

W

Y

Z

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FOR NOTES

IOBC/WPRS

Working Group "Insect Pathogens and Insect Parasitic Nematodes"

- ☑ 1st meeting - 1987, Versailles, France
- ☑ 2nd meeting - 1989 Rome, Italy
- ☑ 3rd meeting - 1991, Wageningen, The Netherlands
- ☑ 4th meeting - 1993, Zürich, Switzerland
- ☑ 5th meeting - 1995, Poznan, Poland
- ☑ 6th meeting - 1997, Copenhagen, Denmark
- ☑ 7th meeting - 1999, Vienna, Austria
- ☑ 8th meeting - 2001, Athens , Greece
- ☑ 9th meeting - 2003, Kiel, Germany
- ☑ 10th meeting - 2005, Locorotondo, Bari, Italy
- ☑ 11thmeeting - 2007, Ales, France
- ☑ 12thmeeting - 2009, Pamplona, Spain
- ☑ 13thmeeting - 2011, Innsbruck, Austria
- ☑ 14th meeting - 2013, Zagreb, Croatia
- ☑ 15th meeting - 2015, Riga, Latvia

Working Group "Microbial and Nematode Control of Invertebrate Pests"

- ☑ 16th meeting -2017 , Tbilisi Georgia

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