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Plenary Session: State-of-the-art in microbial control

Authorisation of biological control agents - theory and practice

Ralf-Udo Ehlers 3-7

Abstract: The legislation of the European Union regarding the placement of plant protection products on the market (Regulation (EC) No 1107/2009) and also the Directive 2009/128/EC on the sustainable use of pesticides pave the ground for increasing use of biological control agents in Europe. Both clearly give priority to the use of alternative, non-chemical control measures. However, the general practice in member states is different. Whereas member states largely seem to ignore the potential of biological control, chemical control companies have increasing interest in biological control and have acquired several biocontrol companies in order to get access to biocontrol biodiversity and know-how.

Dynamics of baculovirus as insect biocontrol agent

Just M. Vlák, Monique M. van Oers 9-10

Extended Abstract

Insect pathogenic fungi: what was obtained and where to go?

Jørgen Eilenberg 11-13

Abstract: Since its start in 1991 the IOBC working group 'Insect Pathogens and Insect Parasitic Nematodes' has held 13 meeting, plus some subgroup meetings. Papers on fungal entomopathogens have been presented at all meetings, and likewise, fungi have been part of presentations at all Annual Meetings in Society for Invertebrate Pathology (SIP). By looking into especially the most recent presentations and literature I will discuss the status of insect pathogenic fungi in biological control: what was obtained and where to go?

Fungi

Session 1: Entomopathogenic fungi in the control of soil-dwelling pests

Biological control of wireworms with entomopathogenic fungi

S. Eckard, M. A. Ansari, T. M. Butt, J. Enkerli, G. Grabenweger 17-20

Abstract: Three species of wireworms, *Agriotes lineatus*, *A. obscurus* and *A. sputator*, are economically important soil pests in arable and vegetable crops in Europe. Fungi of the genus

Metarhizium are natural pathogens of wireworms. We tested the virulence of three European *Metarhizium* strains in laboratory experiments and found a maximum mortality of up to 80% four weeks post inoculation. We further investigated stability of the virulence of the most effective strain ART2825 after ten times of subcultivation on artificial media. There was no difference in virulence compared to a treatment of larvae that were infected with freshly host-passed conidia. We conclude that *Metarhizium* strain ART2825 is a potential candidate for the control of wireworms and we will continue to validate its efficacy under field conditions.

Monitoring of the entomopathogenic fungus *Beauveria brongniartii* in cockchafer infested areas of the Euroregion Tyrol

Johanna Mayerhofer, Jürg Enkerli, Roland Zelger, Hermann Strasser 21-25

Abstract: The persistence of the entomopathogenic fungus *Beauveria brongniartii* in soils in the Euroregion Tyrol has been evaluated over a period of two decades. The fungal product Melocont® Pilzgerste was successfully applied on the fields in different concentrations and intervals between 1989 and 2012. In July and August 2012 the soil samples were drawn and analyzed on selective media to determine the occurrence of *Beauveria* spp. Preliminary results from microsatellite analysis showed that the re-isolated *Beauveria* strains from the test sites, which had been treated during the last four years, were identified as the production strain. New insights into colonisation, mobility and persistence of *B. brongniartii* in soils will be discussed in the presentation.

Susceptibility of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to entomopathogenic fungi: Laboratory assays and field trials

Hannes Rauch, Roland Zelger, Stefan Hutwimmer, Hermann Strasser 27-31

Abstract: Since the introduction of *Diabrotica virgifera virgifera* to Europe between the late 1980s and the early 2000s, the western corn rootworm subsequently has extended its presence across many parts of Europe and can currently be found in 20 European countries. Several different strategies aiming at the control of *D. v. virgifera* have at least partially limitations, making the biological control probably the most encouraging management method. Bioassays revealed two *Metarhizium anisopliae* strains and one *Beauveria bassiana* strain with the highest pathogenicity against 3rd instar larvae of *D. v. virgifera*. Although results obtained from fungal density measurements after fungal application in Hungarian croplands revealed quite displeased persistences, the potential of certain fungal strains for the control of the western corn rootworm can be considered indisputable. However, further lab assays as well as field trials are needed to confirm this high potential.

Efficacy of biological control agents for the control of western corn rootworm

Emese Balog, Bui Xuan Hung, György Turóczy, József Kiss 33-36

Abstract: The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, has been introduced to Europe more than 20 years ago, and it is a well-established maize pest in Hungary since 1995. The larvae of WCR cause damage on the maize roots. The efficacy of various biological control agents (BCAs), such as fermented cultures of various entomopathogenic toxin producing preparations of *Bacillus thuringiensis*, and some strains of the entomopathogenic conidial fungus *Metarhizium anisopliae*, was screened against the larvae of WCR but the practical application of them still needs additional research and development inputs. In *in vitro* tests, WCR larvae were treated with microbial products (fermented cell cultures or spore suspensions in various concentrations) at the second larval stage. Larvae were fed with freshly germinated maize roots and larval mortality was recorded until pupation. In greenhouse experiments maize plants were grown in pots placed in isolators. WCR eggs (20 for each plant) were put directly under the seeds. In greenhouse experiments the microbial preparations were applied at the time of sowing, in the same way as they were applied in the *in vitro* trials. One month after the planting, the root mass was measured, and the damage caused by larvae was determined based on the modified IOWA 1-6 scale. Most of the bacterial preparations and fungal strains proved to be effective both in killing WCR larvae and preventing root damage on maize

plants. Some microbial treatments almost reached the efficacy of the control treatments (Tefluthrin (FORCE 1.5 G) and *Bacillus thuringiensis* var. *tenebrionis* (NOVODOR FC)) and can be considered as promising control agents of WCR.

- Exploring synergistic effects of semiochemicals, entomopathogenic fungi and nematodes against root-herbivores
Michael A. Brandl, Mario Schumann, Stefan Vidal 37
Abstract only

Session 2: Above-ground use of entomopathogenic fungi in protected and open field crops

- Entomopathogenic fungi ecology and diversity from different Mediterranean ecosystems
María Fernández-Bravo, Enrique Quesada-Moraga, Inmaculada Garrido-Jurado 41
Abstract only

- Efficacy of two strains of *Beauveria bassiana* entomopathogenic fungus on the red palm weevil in France and in Spain
Samantha Besse, Ludovic Crabos, Karine Panchaud 42
Abstract only

- Beauveria bassiana* strain ATCC 74040 interferes with oviposition behavior of Mediterranean fruit fly
Luca Ruiu, Giovanni Falchi, Edith Ladurner 43-46
Abstract: The entomopathogenic fungus *Beauveria bassiana* is known to interact with insects in several ways. The present work reports the results of observations on the potential of *Beauveria bassiana* strain ATCC 74040 against the Mediterranean fruit fly, *Ceratitidis capitata*, with special regard to disturbance effects on oviposition behaviour. A commercial formulation (Naturalis) and different fungal preparations (pure conidia, hyphae, culture supernatants) were applied to orange fruits offered to ovipositing medflies. A significantly lower number of fly visits and oviposition punctures were recorded on fruits treated with Naturalis and with pure conidia than on control fruits. The observed effects are examined on the basis of additional proteomic and genomic observations, and the potential molecular implications of the rodlet layer of aerial conidia are discussed.

- Pathogenicity of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* on larvae and adults of the sisal weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae)
V. T. Gkouti, D. Markoyiannaki, D. C. Kontodimas 47-49
Abstract: The sisal weevil is a severe pest of both ornamental and cultivated agave species. As the use of synthetic insecticides causes undesirable effects, the evaluation of potential biological control agents is necessary. Field collected adults and larvae of *Scyphophorus acupunctatus* were used to evaluate the pathogenicity of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales). Different concentrations of spore suspensions were tested. As in some cases 100% mortality was achieved it is indicating that this strain could serve as a potential biological control agent of the sisal weevil.

- Microbial control of European red spider mite (*Panonychus ulmi*) with *Beauveria bassiana* strain ATCC 74040
Edith Ladurner, Massimo Benuzzi, Andrea Braggio, Sergio Franceschini, Veselin Zivkovic 51-55
Abstract: The European red spider mite, *Panonychus ulmi*, can cause severe damage on many

fruit crops, especially on apple. Outbreaks of mite populations usually occur in summer on warm and humid days. Natural occurring predator populations may not always be able to keep the pest under control, especially because of the likely occurrence of a lag in time in build-up of prey and predator populations and due to the use of non-selective chemical pesticides. The efficacy of the microbial control agent *Beauveria bassiana* strain ATCC 74040 (Naturalis®) against *P. ulmi* was tested in open trials on apple. In one of the trials, also observations on the potential side effects of the formulated product on natural occurring predator populations (*Phytoseiulus* spp. and *Stethorus punctillum*) were made. The microbial control agent showed high efficacy against *P. ulmi* in all field trials, and did not adversely affect predator populations. *Beauveria bassiana* strain ATCC 74040 can be considered a valuable tool to be integrated into *P. ulmi* control strategies.

Mycopathogens of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch.)
infesting wheat plants at Assiut, Egypt
Ahmed Y. Abdel-Mallek, Mohamed A. A. Abdel-Rahman, Gamal H. A. Hamam 56
Abstract only

Session 3: New strategies for delivering and monitoring of entomopathogenic fungi

Exploiting vine weevil behaviour to disseminate an entomopathogenic fungus
*Tom Pope, Charlotte Arbona, Harriet Roberts, Jude Bennison,
John Buxton, Gill Prince and Dave Chandler* 59-62

Abstract: Control of adult vine weevil (*Otiorhynchus sulcatus*) is currently reliant on the use of insecticides. However, using insecticide applications targeted against this pest is difficult, as they need to be applied at dusk, and are often incompatible with integrated pest management programmes. This study investigated the potential of a novel control strategy that uses artificial refuges containing spores of an entomopathogenic fungus and exploits vine weevil behaviour to disseminate the pathogen throughout weevil populations.

Preliminary experiments identified a simple plastic crawling insect trap as a suitable artificial vine weevil refuge. Subsequent semi-field experiments using fluorescent powders in place of an entomopathogenic fungus spore formulation showed that vine weevil aggregation behaviour and movement between refuges effectively disseminated the powders throughout weevil populations.

Field persistence of *Metarhizium* spp. strains applied as biocontrol agents
against ticks (*Ixodes ricinus*)
Maria Mitteregger, Sarah Sonderegger, Hermann Strasser 63-67

Abstract: In two semi-field trials the persistence of three *Metarhizium* strains (BIPESCO 5, ARSEF 3297, ARSEF 4556) after foliar spray application was monitored and bioassays with *Tenebrio molitor* larvae were made to preclude a negative effect on germination and vitality of conidia by using the adhesive agent Neo-wett™ and the antifoaming agent Antischiuma Schaumstop™. Three different outdoor conditions (i.e. unprotected, rainfall protected, rainfall protected and fully covered) were tested and all strains showed an adequate persistence after 25 days (20-50%). BIPESCO 5 conidia were re-isolated from foliage even after heavy rain showers in open sites after 44 days. Conidia showed high virulence in bioassay with and without Neo-wett™ and Antischiuma Schaumstop™: 90% of all larvae were killed after 5 to 20 days. No negative effects of the adhesive- and antifoaming agent on the germination ability and vitality of *Metarhizium* conidia were observed.

Vertical transmission of an endophytic strain of *Beauveria bassiana*
(Ascomycota; Hypocreales) colonizing opium poppy *Papaver somniferum*
Enrique Quesada-Moraga, Blanca B. Landa del Castillo, Cristina López-Díaz 68
Abstract only

Development of a novel fermentation and formulation process
for an endophytic *Beauveria bassiana* strain
Rieke Lohse, Desiree Jakobs-Schönwandt, Anant Patel 69-73

Abstract: There is an increasing demand for alternative or complementary crop protection strategies. A novel approach could be the use of the entomopathogenic and endophytic fungus *Beauveria bassiana* isolate ATP-04. To use the endophyte as a commercial biocontrol agent, the fungus has to be mass-produced. *B. bassiana* was raised in shake flask cultures to produce submerged conidiospores (SCS) which are reported to show a higher shelf life than mycelium and blastospores (BS). It was found that in mineral media with 5% sugar beet molasses *B. bassiana* produced 0.1×10^{10} SCS g^{-1} sucrose in 192 h. By adding $50 g l^{-1}$ NaCl 48 h after inoculation the SCS yield increased to 1.4×10^{10} SCS g^{-1} sucrose. The scale-up to a 2 l stirred tank reactor was carried out at 25°C, 200-600 rpm and 1 vvm at pH 5.5. A total spore yield of 5.2×10^{10} spores g^{-1} sucrose corresponding to a SCS yield of 0.2×10^{10} SCS g^{-1} sucrose was obtained after 216 h. Also the yield of SCS increased to 1.1×10^{10} SCS g^{-1} sucrose by the addition of NaCl. After fermentation the *B. bassiana* was formulated in a novel spray formulation that delivers the fungus on oilseed rape leaves, increases persistence, germination and growth on leaves as well as penetration, colonization and efficacy in bioassays with *Plutella xylostella*.

Development of analytical tools to monitor the fate
of *Metarhizium anisopliae* metabolites in the environment
Judith Taibon, Sonja Sturm, Christoph Seger, Hermann Strasser,
Hermann Stuppner 75-78

Abstract: Destruxins (dtxs) are structurally closely related cyclic hexadepsipeptides secreted as relevant metabolites by the entomopathogenic fungus *Metarhizium anisopliae*. To monitor dtxs in fungal culture broth, plant derived matrices and cell cultures, a fast and selective off-line SPE UHPLC-DAD/MS method was established. Sample preparation was carried out by a solid phase extraction (SPE) on a reversed phase material. Optimal purification was achieved by a washing step with 40% (v/v) methanol, removing most of the polar components. The highest amounts of dtxs were obtained by using 85% (v/v) methanol for elution. An UHPLC-DAD system hyphenated to a Q-TOF mass spectrometer was utilized to separate and detect the dtx congeners. A sub-2 μ m particle size column was used as stationary phase, with a water/acetonitrile solvent gradient at a flow rate of $0.3 ml min^{-1}$ serving as mobile phase. A total analysis time of 12 min was achieved with the UHPLC-DAD assay with the dtx congeners eluting from 1 min to 8 min with a higher resolution of the peaks compared to previous HPLC-DAD assays. Besides the available reference compounds dtxA, dtxB, dtxE, dtxE-diol 18 dtx derivatives were tentatively identified by analyzing TOF-MS data.

Cross-species transferability of 41 microsatellite markers for *Metarhizium* spp.
Andy Lutz, Franco Widmer, Adrian Leuchtman, Jürg Enkerli 79-83

Abstract: The genus *Metarhizium* includes insect pathogenic fungal species, which are used as biological control agents (BCAs). Genetic tools for identification and monitoring of such BCAs are important. A genotyping tool based on 41 simple sequence repeat (SSR) markers has been developed for *M. anisopliae s.l.* However, detailed phylogenetic analyses based on a multilocus approach revealed that *M. anisopliae s.l.* is a cryptic species complex of nine different *Metarhizium* species. According to this new taxonomy, the 41 SSR markers were isolated from *M. brunneum*, *M. robertsii* or *M. anisopliae s.s.* The goal of this study was to assess the transferability of the 41 SSR markers to individual species of the former *M. anisopliae* species complex. Successful PCR-amplification of SSR markers was observed in all species but the number of loci yielding PCR products varied among species. Amplification of individual SSR

loci did not always yield products for all strains of a particular species and not all were polymorphic. The study revealed that SSR markers can be transferred to different species of the former *M. anisopliae* species complex. However, the number of available SSR markers strongly depends on the species to be analyzed. The markers will provide a valuable tool for identification and monitoring of *Metarhizium* BCAs and they will allow investigation of genetic diversity and population structure of seven species of the former *M. anisopliae* species complex.

Posters

A review of the use of entomopathogenic fungi for the control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the UK
 Andrew G. S. Cuthbertson 87-90

No abstract

Effect of entomopathogenic fungi against *Trialeurodes vaporariorum* and its parasitoid *Encarsia formosa*: preliminary laboratory assays
 Monica Oreste, Michele Polisenio, Eustachio Tarasco 91

Abstract only

Laboratory *Beauveria bassiana* (Bals.) Vuill. bioassays on spruce bark beetle (*Ips typographus* L.)
 Ana-Maria Andrei, Daniela Lupăștean, Constantin Ciornei, Ana-Cristina Fătu, Mihaela Monica Dinu 93-96

Abstract: The massive damages caused by *Ips typographus* in spruce forests in Romania, the severe restrictions on the use of chemical insecticides, the identification of natural *Beauveria bassiana* outbreaks in the Romanian forests infested with bark beetle and the isolation of a new *B. bassiana* strain, led to the development of some researches on the possibility to use this entomopathogenic fungi for reducing the damage caused by bark beetles. In laboratory conditions, the susceptibility of *I. typographus* to infection by a naturally occurring *B. bassiana* strain was tested. Utilization of a *B. bassiana* conidial suspension (3.31×10^{11} conidia ml⁻¹) induced beetle mortality, length of mother galleries reduction and larval galleries number reduction.

Effect of local strains of *Beauveria bassiana* (Bb024) and *Metarhizium anisopliae* (M7/2) against the fallweb worm *Hyphantria cunea* (Lepidoptera: Arctiidae) in Georgia
 Medea Burjanadze, Elena Nakaidze, Mariam Arjevanidze, Tea Abramishvili 97-101

Abstract: The indigenous isolates of the entomopathogenic fungi *Beauveria bassiana* (Bb-024) and *Metarhizium anisopliae* (M7/2) against fifth to seventh instars larvae of *Hyphantria cunea* in two concentrations of 1×10^7 and 1×10^8 conidia ml⁻¹ were tested in the laboratory. Maximum mortality of larvae was observed 4-9 d after treatment. Both isolates were pathogenic to *H. cunea* larvae. The mean mortality caused by *B. bassiana* ranged from 59.8% to 84.3% and that of *M. anisopliae* ranged from 52% to 68%. The living larvae hidden under leaves and cordon made cocoons and transformed into pupae. The adult moths appeared from the overwintering pupae. They began to emerge massively and mated. The emergence of adults of *B. bassiana* was 69.6%, that of *M. anisopliae* was 60%, and that of the control was 55.7%. The larvae hatched 7 to 10 d later (the hatching rates were: *B. bassiana* – 76.3%, *M. anisopliae* – 70%, control – 89.5%). In case of *B. bassiana* treatment, dimorphic males emerged from pupae often showing undeveloped wings.

Highly effective *Beauveria pseudobassiana* strain (Dm-5)
against the great spruce bark beetle, *Dendroctonus micans* (Kugelann)
(Coleoptera: Scolytidae)
Ismail Demir, Seda Kocacevik, Ali Sevim, Mahmut Eroglu, Zihni Demirbag 102
Abstract only

Laboratory testing of insect associated fungi
for the control of wireworms (*Agriotes* sp. L.)
Jaka Razinger, Matthias Lutz, Hans-Josef Schroers,
Gregor Urek, Jürg Grunder 103-107

Abstract: The aim of the study was to assess entomopathogenic potential of 7 isolates from 6 entomopathogenic fungal species (EPF) isolated from various substrats in Slovenia against larvae of *Agriotes* sp. The fungal isolates tested were *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae* (2 isolates), *M. robertsii*, *Purpureocillium lilacinum* and *Clonostachys solani*. Conidia of these species were incorporated into the test substrate as a water suspension to reach a final concentration of 3.85×10^6 conidia g^{-1} air-dried soil. The larval mortality was observed on a weekly basis for a total of 90 days. The mortalities observed exhibited a linear trend with slopes ranging from 0.20 to 1.23 for the fungal treatments and 0.08 to 0.18 for the control treatments. Abbott's corrected mortality at day 90 ranged from 20.7 to 76.9%. The most promising candidate biological control agent was *Metarhizium anisopliae* isolate 1154.

Laboratory and semi-field trials on the effects of *Beauveria bassiana*
(JW-1, ATCC 74040) against soil-dwelling stages
of *Frankliniella occidentalis* (Thysanoptera: Thripidae)
Andrea Boaria, Alberto Pozzebon, Mauro Pesce, Mauro Lorenzon,
Carlo Duso 109-112

Abstract: *Beauveria bassiana* (Balsamo) Vuill. is an entomopathogenic fungus used in controlling various pests. Previous research showed that *B. bassiana*, applied to the plant canopy, could exert a significant control of thrips populations, in particular of *Frankliniella occidentalis* Pergande. However, some stages (e.g., prepupae and pupae) of this species develop in the soil being less affected by control treatments applied to the canopy. The identification of biological control agents active against soil-dwelling stages of *F. occidentalis* is an important issue for the implementation of IPM. Here we present laboratory and greenhouse experiments carried out to evaluate the potential of *B. bassiana* (JW-1 ATCC 74040) in controlling soil-dwelling stages of *F. occidentalis*. In laboratory bioassays *B. bassiana* reduced significantly the emergence of *F. occidentalis* adults. In the greenhouse experiment, a significant control of thrips population was obtained on cyclamen potted plants.

Prevalence of the species *Beauveria pseudobassiana*
among tick-associated fungal isolates from the Republic of Moldova
Polina V. Mitkovets, Natalia V. Munteanu, Galina V. Mitina, Yuri S. Tokarev,
Alexandr A. Movila, Ion Toderas, Regina G. Kleespies, Andreas Leclerque 113-117

Abstract: Fungal strains isolated from ixodid ticks in the Republic of Moldova were genetically characterized using the ribosomal RNA operon internal transcribed spacer (ITS) regions together with the 5.8S rRNA gene as well as the nuclear genes *efl α* encoding the alpha subunit of eukaryotic translation elongation factor 1 α as phylogenetic markers. On the basis of the sequence data obtained, eight out of ten isolates were assigned to the *Beauveria* species *B. pseudobassiana*. The two remaining isolates were consistently characterized as *Isaria farinosa* and as an *Aspergillus* species, respectively. Further work to elucidate if the prevalence of the species *B. pseudobassiana* in ticks is or not a regional phenomenon is in progress.

Evaluation of indigenous *Beauveria* isolates as potential agents for emerald ash borer management and the development of a diagnostic marker to monitor a post-release isolate

George Kyei-Poku, Shajahan Johny 119-124

Abstract: To search for effective and safe indigenous biocontrol agents to manage emerald ash borer (EAB), we conducted a survey in 2008-2009 of entomopathogenic fungi (EPF) infecting EAB in outbreak sites in southwestern Ontario, Canada. Many *Beauveria* spp. isolates were recovered from dead and mycosed EAB cadavers residing in the phloem tissues of dead ash barks, larval frass extracted from feeding galleries under the bark of dead trees. Molecular characterization using sequences of the ITS, 5' end of elongation factor 1 alpha (EF1- α) and intergenic Bloc region fragments revealed that *Beauveria bassiana* and *B. pseudobassiana* were commonly associated with EAB in the sampled sites. Initial virulence screening against EAB adults of 23 isolates representing the different clades yielded 8 isolates that produced more than 90% mortality in a single concentration assay. These isolates differed in virulence based on LC₅₀ values estimated from multiple concentration bioassay and based on mean survival times at a conidia concentration of 2×10^6 conidia ml⁻¹. *B. bassiana* isolate L49-1AA was significantly more virulent and produced more conidia on EAB cadavers compared to the other indigenous isolates and the commercial strain *B. bassiana* GHA suggesting that L49-1AA may have potential as a control agent against EAB. Studies have been developed to use auto-contamination trapping system to disseminate L49-1AA to manage EAB field populations. We targeted the EF1- α gene sequence from L49-1AA to develop an allele/strain specific primer set that will be used to monitor the introduced L49-1AA in terms of its establishment, persistence and virulence in the environment.

Isolation and identification of endophytic entomopathogenic fungi from dent corn

Daigo Aiuchi, Tatsumi Takanami, Sayaka Toba, Minehiro Ishii, Shin-ichiro Asano, Masanori Koike 125-128

Abstract: In this study, we sought endophytic entomopathogenic fungi from dent corn, which have potential to be developed as endophytic biopesticide with multiple roles. Dent corn samples applied to this study were collected at three locations of East Hokkaido island. Each plant sample was divided into root, stem, leaf and kernel, and then these were surface-sterilized by 70% ethanol and 0.5% sodium hypochlorite. Pieces of each tissue were placed on entomopathogenic fungi selective medium. All fungal isolates growing on this plate were transferred onto potato dextrose agar. Morphological identification to genus level was conducted by slide culture method by observing under light microscope (x100). In total, 2252 fungal isolates (greater part of isolates were *Penicillium* spp. and *Cladosporium* spp.) were detected on selective medium, and among them, 168 isolates were entomogenous fungi. Five genera of entomopathogenic fungi including *Beauveria*, *Lecanicillium*, *Isaria*, *Metarhizium* and *Simplicillium* were detected in this study. In this study, only five plant samples were applied, but entomopathogenic fungi were detected from all locations and at all parts of plant tissue. Moreover, it is indicated that endophytic entomopathogenic fungi multiply colonize in the plant body. Although, *Beauveria*, *Lecanicillium*, *Isaria* and *Metarhizium* showed tendency to localize to some plant part, *Simplicillium* tended to be ubiquitous presence in plant body. Our result can indicate that entomopathogenic fungi universally colonize into dent corn.

Endophytic establishment of the entomopathogen *Beauveria bassiana* in *Vitis vinifera* plants

Yvonne Rondot, Annette Reineke 129

Abstract only

Effect of temperature, water activity and UV-B radiation on conidia germination and colony growth of <i>Beauveria bassiana</i> isolates from soil and phylloplane <i>María Fernández-Bravo, Inmaculada Garrido-Jurado, Monica Oreste, Enrique Quesada-Moraga</i>	130
Abstract only	

Viruses

Session 1

Deletion genotypes influence occlusion body potency and production in insects infected by a <i>Spodoptera frugiperda</i> nucleopolyhedrovirus isolate from Colombia <i>Gloria Barrera, Trevor Williams, Laura Villamizar, Primitivo Caballero, Oihane Simón</i>	133-136
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Abstract: The Colombian field isolate (SfCOL-wt) of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) is a mixture of different genotypes. To evaluate the insecticidal properties of the different variants in SfCOL-wt a plaque assay was performed and ten distinct genotypes were identified. Genotype SfCOL-A was the most prevalent (71%) and showed a *Pst*I restriction profile identical to that of SfCOL-wt. The remaining nine genotypes presented genomic deletions of 3.8-21.8 Kb that affected the region between open reading frames (ORFs) *sf20* and *sf33*. The potency of SfCOL-A occlusion bodies (OBs) was approximately 4-fold higher than SfCOL-wt OBs, whereas the speed of kill of SfCOL-A was similar to that of SfCOL-wt. Deletion genotype OBs were similarly or less potent than SfCOL-wt, but six deletion genotypes were faster killing than SfCOL-wt. The potency of mixtures of OBs and co-occluded mixed genotype OBs were consistently reduced in two-genotype mixtures involving equal proportions of SfCOL-A and one of three deletion genotypes (SfCOL-C, -D or -F). Speed of kill and OB production were improved only when certain genotype mixtures were co-occluded, although OB production was higher in the SfCOL-wt isolate than in any of the genotypes or genotype mixtures that we tested. The SfCOL-wt population appears to be structured to maximize the production of OBs in each infected host suggesting this to be the principal limitation to transmission.

On the role of baculovirus photolyases in DNA repair upon UV damage of occlusion bodies <i>Magdalena A. Biernat, Primitivo Caballero, Just M. Vlak, Monique M. van Oers</i>	137-142
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Abstract: The use of baculoviruses in insect biocontrol is hampered by their sensitivity to ultraviolet (UV) light. This irradiation induces cyclobutane pyrimidine dimers (CPDs) in DNA. CPD-photolyases repair CPDs using visible light. Plusiine baculoviruses encode photolyases, which could potentially repair UV-damage prior to infection of larvae. Whether the photolyases encoded by *Chrysodeixis chalcites* nucleopolyhedrovirus are involved in UV damage repair was tested by infecting larvae with UV-irradiated viral occlusion bodies (OBs) that were subsequently treated with visible light or kept in the dark. The observed mortality was the same for both treatments. We postulate that photolyases are not active as DNA repair enzymes in OBs, but may play a role in other aspects of baculovirus pathogenesis.

Effect of top spray drying and freeze drying on the photostability and insecticidal activity of a <i>Spodoptera frugiperda</i> nucleopolyhedrovirus (SfMNPV 003) formulation <i>Mauricio Cruz, Martha Liliana Chaparro, Laura Fernanda Villamizar, Martha Isabel Gomez</i>	143-147
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Abstract: The nucleopolyhedroviruses are considered as an effective biopesticide against the fall armyworm *Spodoptera frugiperda*. Top spray and freeze drying methods were used to prepare a

wettable powder formulation based on nucleopolyhedrovirus of *S. frugiperda* (SfMNPV 003) and its photostability and virulence were assessed. Top spray drying method was more efficient for removing moisture content than freeze drying. No obvious differences in the insecticidal activities were observed for both drying methods although a higher photostability (88.54%) was observed in the formulation prepared with top spray drying method compared to freeze drying (77.77%) and unformulated virus (15.62%) after 6 hours of UV radiation exposure. Top spray drying method was selected as the most favorable process for being implemented in a manufacture process.

Variations in the susceptibility to CpGV in populations of the codling moth,

Cydia pomonella

Benoît Graillot, Christine Blachere, Samantha Besse, Myriam Siegwart,

Miguel López-Ferber 149-153

Abstract: Failure in codling moth populations control with CpGV in apple orchards has been attributed to the action of a single allele located in the Z chromosome. However, differences in the mortality patterns between genetically homogeneous susceptible and resistant insects in the laboratory strongly suggest that other mechanisms are responsible of variation in the susceptibility of insect to CpGV isolates.

Characterisation of novel CrleGV isolates for false codling moth control - lessons learnt from codling moth resistance to CpGV

John Opoku-Debrah, Sean Moore, Martin Hill, Caroline Knox 155-159

Abstract: Recently some codling moth, *Cydia pomonella*, populations in Europe developed resistance to CpGV. In order to prepare for the possibility of a similar occurrence with the false codling moth, *Thaumatotibia leucotreta*, in South Africa, a search was conducted for novel CrleGV isolates. Through overcrowding, outbreaks of novel isolates were recorded from laboratory populations of five geographically distinct host populations. The genetic novelty of these and two commercially available isolates was confirmed through restriction enzyme analysis and sequence analysis of the *granulin* and *egt* genes. Phylogenetic analysis showed the existence of two CrleGV-SA genome types. Significant differences in virulence were also shown between certain isolates against certain host populations.

Elucidation of a novel mode of resistance of codling moth

against *Cydia pomonella* granulovirus by homogenization experiments

Annette J. Sauer, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle 161-165

Abstract: Since 2005, codling moth (CM, *Cydia pomonella*) populations with a reduced susceptibility to *Cydia pomonella* granulovirus (CpGV, *Baculoviridae*) products have been reported from about 40 European orchards. The resistance could be traced back to a single, dominant, sex-linked gene. Currently, resistance management strategies are based on the application of improved CpGV products containing resistance-overcoming CpGV isolates. Recently, two CM field populations (NRW-WE and SA-GO) with a reduced susceptibility to even these improved CpGV products were found. First single pair crossing experiments between individuals of these resistant field colonies and a susceptible laboratory CM strain (CpS) indicated that the inheritance of resistance of these populations did not follow the previously described pattern of Z-linked, dominant resistance. In single-dose bioassays the susceptibility of neonates of the resistant CM colonies NRW-WE and SA-GO to different CpGV isolates (CpGV-M, -S, -V15 and -E2) was estimated. The aim of the current study was the genetic homogenization of the genetically heterogeneous field populations NRW-WE and SA-GO by two different methods: (i) repeated single pair crossings followed by family selection and (ii) successive mass crossing experiments under virus pressure. The resulting homogenous strains of NRW-WE and SA-GO with fixed resistance will be used for backcrossing experiments with CpS to elucidate the mode of inheritance of their resistance.

Session 2

- Biological control of the box tree moth *Cydalima perspectalis* with *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV)
Jana Rose, Johannes A. Jehle, Regina G. Kleespies 169-172

Abstract: The box tree moth *Cydalima perspectalis* originated from East Asia. Since several years, it is a novel invasive insect pest in many European countries, causing widespread damage on box tree plants. The potential of the baculovirus *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV) as a potential biological control agent for the control of *C. perspectalis* was investigated in this study. Two AnfaNPV isolates, termed Dn10 and BI-235, were used. The infectivity of AnfaNPV Dn10 and BI-235 to *C. perspectalis* was evaluated by leaf disc bioassays and the median lethal concentration (LC₅₀) was determined for both isolates. In addition, light and electron microscopic analyses were performed to study the infection process. In conclusion, larvae of *C. perspectalis* were shown to be susceptible to both AnfaNPV isolates.

- Interactions between structural proteins of *Chilo iridescent* virus
Emine Özşahin, Remziye Nalcacioglu, Just M. Vlak, Monique M. van Oers, Zihni Demirbağ 173
- Abstract only**

- Natural populations of *Spodoptera exigua* are infected by multiple viruses: implications for the production and use of virus insecticides
Cristina Virto, David Navarro, M. Mar Tellez, Salvador Herrero, Trevor Williams, Rosa Murillo, Primitivo Caballero 175-177

Abstract: Covert infections of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) have been detected in laboratory or field populations of the homologous host, *S. exigua*. Two RNA viruses belonging to the *Iflaviridae* family (SeIV-1, SeIV-2) were identified in transcriptome studies from different laboratory colonies of *S. exigua*. The three viruses are vertically transmitted and establish persistent infections. For this reason, coinfection of individual insects by these viruses is considered likely. In this study, we determined the prevalence of covert infections caused by iflaviruses and SeMNPV in order to identify virus associations in natural *S. exigua* populations. SeMNPV was detected in the 54% of field-caught adults, whereas 13% and 8% of insects were infected by SeIV-1 and SeIV-2, respectively. The prevalence of SeIV-1 and SeIV-2 in F₁ adults obtained in laboratory showed higher levels than in the parental generation, whereas the prevalence of SeMNPV decreased from parents to their offspring. These findings have important implications for the *in vivo* production of virus based insecticides using mass-reared insects and the efficacy of these products in controlling pest populations that may be coinfecting by iflaviruses.

- Estimating the importance of maternal and paternal contributions to the vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV)
Cristina Virto, Carlos A. Zárate, Rosa Murillo, Primitivo Caballero, Trevor Williams 179-181

Abstract: Vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) is believed to be a common feature in field populations of *S. exigua*. To assess whether gender affects trans-generational virus transmission, four mating groups were performed using healthy and sublethally infected insects: i) healthy males (H♂) × healthy females (H♀); ii) infected males (I♂) × healthy females (H♀); iii) healthy males (H♂) × infected females (I♀) and iv) infected males (I♂) × infected females (I♀). These adults and their offspring were analyzed by qPCR to detect SeMNPV infection. Both males and females were able to transmit the infection to the next generation, although females infected a higher percentage of the offspring and female-mediated transmission was more consistent than that of males. Venereal transmission appeared to be half as effective as maternally-mediated transmission, and the main route of transmission is likely

transovarial rather than transovum. The prevalence of the infection in the offspring did not vary according to gender, therefore both males and females can be infected by their parents in similar proportions. Incorporating vertically-transmitted genotypes in biological insecticides might have the potential for reducing pest densities and extending periods between virus applications.

Baculoviruses for the biological control of cutworms (*Agrotis* spp.)

Jörg T. Wennmann, Gianpiero Gueli Alletti, Johannes A. Jehle 183-186

Abstract: Caterpillars of the common cutworm *Agrotis segetum* and black cutworm *A. ipsilon* (Lepidoptera: Noctuidae) are wasteful feeders of various crops in agriculture. These cutworms are mainly controlled by chemical pesticides but recent attempts are aimed to control these soil pests by the application of baculoviruses. Four different baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *Agrotis segetum* nucleopolyhedrovirus B (AgseNPV-B), *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV) and *Agrotis segetum* granulovirus (AgseGV), were isolated from larvae of *A. segetum* and *A. ipsilon* and are considered as potential biocontrol agents. In natural infections, larvae of both hosts are susceptible to all four viruses and individual caterpillars of the common cutworm were observed to become infected simultaneously by AgseNPV-B and AgseGV. Co-infections may be advantageous in terms of virulence and resistance management, although the level of interaction is critical. To test for a mutualistic, neutral or antagonistic interaction and to evaluate a combined application of *Agrotis*-specific baculoviruses, we exemplarily performed mixed infection experiments of *A. segetum* larvae that were exposed to AgseNPV-B and AgseGV at different concentrations. For quantitative analysis of the outcome of mixed infections as well as for quality control in virus production a reliable method for detection and discriminative quantification for *Agrotis*-specific baculoviruses is required. We established a multiplex PCR analysis based on highly specific oligonucleotides which also permit quantification by quantitative PCR. As a prerequisite of these studies the genome of AgseNPV-B was completely sequenced by 454 sequencing technique. Comparative genome sequence analyses gave a detailed insight into the molecular setup of the three *Agrotis*-specific NPVs and confirmed that they can be regarded as three different but close related species. Our results will help to develop and evaluate *Agrotis*-specific baculoviruses as biocontrol agents and to understand the evolutionary co-existence of viruses that are highly adapted to the same hosts.

Posters

Insecticidal activity of a spray dried formulation based on a

Colombian *Spodoptera frugiperda* nucleopolyhedrovirus

Judith Elena Camacho, Martha Isabel Gómez, Mauricio Cruz,

Laura Fernanda Villamizar 189-193

Abstract: A Colombian *Spodoptera frugiperda* nucleopolyhedrovirus (SfMNPV 003) with high potential for the development of an efficient biopesticide was microencapsulated by top spray drying with a pH dependent polymer (Eudragit®S100) and its insecticidal activity was evaluated under laboratory and greenhouse conditions. Significant differences between LC₅₀ values of the microencapsulated virus, the dried virus and the virus without any treatment were not detected under laboratory conditions, suggesting that microencapsulation by top spray drying did not affect the virus insecticidal activity. Three different microencapsulated batches showed the same efficacy under greenhouse conditions and significant differences between formulated and unformulated virus were not detected ($p > 0.05$). In conclusion, SfMNPV003 insecticidal activity was not affected by formulation process and developed biopesticide demonstrated its potential for *S. frugiperda* control and could be included in programs of integrated pest management (IPM).

Cydia pomonella granulovirus knockout mutants: The potential role of *pe38*
in overcoming codling moth resistance
Manuela Gebhardt, Karolin E. Eberle, Johannes A. Jehle 194
Abstract only

Sequence analysis of CpGV-R5 isolate, able to efficiently control
CpGV-M resistant insects: relation between biological activity and genome
*Benoît Graillot, Samantha Besse, Christine Blachère-Lopez,
Jérôme Olivares, Myriam Siegwart, Miguel López-Ferber* 195-199
Abstract: The CpGV-R5 isolate is able to overcome resistant populations of codling moth to the CpGV-M isolate. The complete sequences of CpGV-R5 and the CpGV-M used for industrial production at Natural Plant Protection have been determined. Among the differences found, some are specific to the R5 isolate, others are common to various isolates able to overcome the resistance, like modification on the p38 gene product.

Functional characterization of serine/threonine protein kinase gene (AMV197)
of *Amsacta moorei* entomopoxvirus
*Hacer Muratoglu, Remziye Nalcacioglu, Srin Perera, Basil Arif,
Zihni Demirbag* 201
Abstract only

Transcriptional analysis of CpGV isolates in *Cydia molesta*
Dönüs Toy, Diana Schneider, Zihni Demirbag, Johannes A. Jehle 202
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An examination of stress-related activation of SeMNPV
in covertly infected *Spodoptera exigua*
*Cristina Virto, David Navarro, María Mar Tellez, Rosa Murillo,
Trevor Williams, Primitivo Caballero* 203-205
Abstract: The aim of this study was to evaluate the effect of different stress factors on covertly infected *Spodoptera exigua* larvae in terms of nucleopolyhedrovirus (NPV) activation. For this, adult survivors that had ingested occlusion bodies of *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) were mated and the subsequent generation (F₁) tested for virus activation in the second instar in both laboratory and field conditions. In the laboratory, a number of treatments were tested including chemical stressors, inoculation with heterologous NPV species and *Bacillus thuringiensis* spores and crystals. Both, parental and F₁ adults were confirmed to harbor the infection by qPCR. Virus activation was observed in insects treated with 0.1% copper sulphate, 1% iron sulphate, and 1 ppm sodium selenite, resulting in 12%, 15%, and 41% mortality due to SeMNPV, respectively, whereas no larvae with symptoms of viral infection were registered in virus-free controls. No effect on NPV-induced mortality was detected after inoculation with heterologous virus. Field trials were carried out by artificial infestation of pepper crops in experimental greenhouses using sublethally infected *S. exigua* larvae to evaluate copper sulfate and sodium selenite as activation factors. Very little NPV-induced mortality (< 5%) was observed in those larvae treated in field conditions.

Functional analysis of *Chilo* iridescent virus zinc-binding
matrix metalloproteinase gene
Aydın Yesilyurt, Hacer Muratoglu, Zihni Demirbag, Remziye Nalcacioglu 206
Abstract only

Soil pests

Latest field results on the biological control of *Diabrotica virgifera virgifera*
with nematodes

Ralf-Udo Ehlers 209

Abstract only

Development of new formulations for soil pest control

Miriam Hanitzsch, Michael Przyklenk, Bianca Pelzer, Anant Patel 211-215

Abstract: In formulation science, there are few systematic investigations on encapsulation of agro-biologicals with regard to materials, methods and technology for mass production. The aim of this work was to develop novel mechanically stable capsule systems with increased persistence in soil. To this end, we tested different methods with several biopolymers, combinations of biopolymers and lignin as capsule additive. Capsule systems were prepared by ionic gelation, thermal gelation, complex coacervation and additional bead coating. In selected capsules, lignin was incorporated. Capsules based on single biopolymers were able to form stable spherical capsules, e.g. alginate, pectin derivatives and gelatin. Capsules based on combinations of polymers also showed stable capsule formation, e.g. alginate/gelatin, alginate/lignin and SEC/PDADMAC. Additionally, lignin was used successfully as additive in SEC hollow beads. First experiments indicate significant differences in biological degradability and thus persistence in soil with different capsule systems. These novel capsule systems with increased persistence are suitable for delivery of BCAs into the soil.

Click beetles disperse widely across farmland: what else do we need to know?

Rod Blackshaw, Robert S. Vernon 217-220

Abstract: For many years wireworm research focussed on the distribution of larvae and their control in field crops. The development of sex pheromone lures has facilitated the study of adult male distributions over greater spatial scales but the assumption that these would provide a simpler monitoring technique has not been fulfilled and it is now clear that we cannot be confident about what the trap counts actually mean. This secondary focus on adult males neglects the critical issue of female behaviours in agricultural landscapes. Understanding these is essential to the development of new management strategies in an era of declining insecticide availability. In this paper we briefly review what is known about the movement of click beetles across farmland and identify research gaps that need to be filled if we are to develop area-wide management strategies.

Distribution and abundance of *Agriotes ustulatus* L. adults
on pheromone traps in four regions in Croatia

Antonela Kozina, Maja Čačija, Renata Bažok 221-225

Abstract: During several years of investigations, the distribution and the abundance of *Agriotes ustulatus* in four different regions of Croatia were researched with the aim to correlate the abundance with the prevailed climatic conditions in each region. *Agriotes ustulatus* was captured by pheromone traps (Csalomon) on 17 fields distributed at seven localities in four different regions in Croatia according to the climatic data. The highest dominance indices of *A. ustulatus* were recorded in the warmest county, County of Vukovar-Srijem and species was classified as eudominant. *Agriotes ustulatus* was subdominant at locality Čazma where the average temperature was the lowest comparing to the other localities.

Efforts to develop female-targeted attractants for click beetles – a summary
Miklós Tóth, Lorenzo Furlan, József Vuts, Éva Bálintné Csonka, István Szarukán, Teodora B. Toshova, Mitko Subchev, Dimitar I. Velchev, Christine M. Woodcock, John C. Caulfield, Patrick Mayon, John A. Pickett, Michael A. Birkett 221-225

Abstract: An overview is given on recent research efforts to develop attractant combinations capable of attracting female click beetles.

New perspectives for wireworm control based on an improved understanding of their feeding ecology
Michael Traugott, Karin Staudacher, Nikolaus Schallhart, Corinna Wallinger 231
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Exploratory use of geometric morphometrics in the identification of wireworm species
Darija Lemić, Katarina Mikac, Hugo A. Benitez, Maja Čačija, Antonela Kozina, Renata Bažok 235
Abstract only

Development of novel biocontrol encapsulation techniques for garlic extracts: first results
Bianca Pelzer, Miriam Hanitzsch, Anant Patel 237-240
Abstract: The aim of the work presented here is to develop novel eco-efficient encapsulation techniques for bioactive ingredients used in biological pest control for the EU project INBIOSOIL. A process for production of small-scale alginate beads containing garlic extract by air atomization with self-constructed technical encapsulation equipment was developed. The encapsulation protects the active ingredients against oxygen and other outside influences, thus enhancing shelf life and allows a slow release effect. The capsule size produced with this technology can be varied – depending on the desired product – between 4 and 600 µm. Particles are stable and spherical.

The project ATTRACT: Protection of crops from soil-borne insect pests with a novel attract and kill strategy
Marina Vemmer, Wilhelm Beitzel-Heineke, Hubertus Kleeberg, Edmund Hummel, Stefan Vidal, Anant Patel 241-242
Abstract: The project ATTRACT targets the development of a novel attract-and-kill strategy for the protection of crops from soil-borne insect pests. The aim is the design of a plant protection product with an innovative formulation based on CO₂ emitting sources as an attractive compound and environmentally friendly insecticidal compounds.

IPM (Fungi/Bacteria)

IPM microbial control based strategies

Combined use of entomopathogenic fungi and their extracts to improve the control of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)
Inmaculada Garrido-Jurado, Gloria Resquín-Romero, Enrique Quesada-Moraga 245
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Insecticidal activity of a semi-purified extract from *Metarhizium brunneum* (Ascomycota: Clavicipitaceae) against the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)
Inmaculada Garrido-Jurado, Óscar Dembilio, Josep Anton Jacas, Lola Ortega, Carlos Campos, Enrique Quesada-Moraga 246

Abstract only

Subterranean control of an arboreal pest: EPNs and EPFs for FCM
Sean Moore, Candice Coombes, Aruna Manrakhan, Wayne Kirkman, Martin Hill, Ralf-Udo Ehlers, John-Henry Daneel, Jeanne de Waal, Jo Dames, Antoinette Malan 247-250

Abstract: Control measures against the false codling moth (FCM), *Thaumatotibia leucotreta*, have traditionally ignored the soil-borne pupal stage. Recent trials with entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPFs) have targeted this life-stage. Application of *Heterorhabditis bacteriophora* to a citrus orchard floor, reduced *T. leucotreta* infestation of fruit by up to 81%. Conservation of *H. zealandica* through non-usage of a nematicide also resulted in dramatically lower fruit infestation. Dose-response and exposure time-response bioassays identified the three most promising fungal isolates against pupating *T. leucotreta*. Orchard trials showed persistence of these fungi in orchard soil for at least six months.

Do plant-associated insect toxin producing pseudomonads have the potential for the biocontrol of insect pests?
M. Maurhofer, B. Ruffner, P. Flury, M. Péchy-Tarr, E. Fischer, P. Kupferschmied, C. Keel 251

Abstract only

Untangling insect pathogenicity in plant-associated pseudomonads by a combination of comparative genomics and bioassays
P. Flury, B. Ruffner, M. Péchy-Tarr, P. Kupferschmied, C. Keel, M. Maurhofer 252

Abstract only

Colorado potato beetle (*Leptinotarsa decemlineata* Say) – control strategies in organic farming using biological insecticides (azadirachtin, *Bacillus thuringiensis* var. *tenebrionis*, pyrethrum and spinosad)
Stefan Kühne, Uta Priegnitz, Benjamin Hummel, Frank Ellmer 253-256

Abstract: Field experiments using different control agents for Colorado potato beetle control showed that all tested application strategies were effective. Time-shifted application of neem (NeemAzal T/S) and *Bacillus thuringiensis* var. *tenebrionis* (*B.t.t.*) (Novodor FC) as well as double treatment with *B.t.t.* achieved under optimal weather conditions gave an effectiveness level of over 80% and increased yields. Surprisingly, a single application of spinosad (SpinTor) also proved to be very effective (> 80%) in the three years studied in spite of the difficult study conditions in 2009. Due to lower cost and a high efficacy of Spinosad it is likely farmers would prefer this plant protection product. Considering resistance of the Colorado potato beetle it is recommended to change the insecticides every year.

Nematodes

Update on life cycle of entomopathogenic nematodes
Ralf-Udo Ehlers 259-260

Extended abstract

Aiming to eradicate small hive beetle *Aethina tumida*

using entomopathogenic nematodes

Andrew G. S. Cuthbertson, James J. Mathers, Lisa F. Blackburn,

Gay Marris, Mike A. Brown, Giles E. Budge 261-265

Abstract: The small hive beetle (*Aethina tumida*) is an endemic parasitic pest and scavenger of colonies of social bees indigenous to sub-Saharan Africa. In this region the beetles rarely inflict severe damage on strong colonies since the bees have developed strategies to combat them. However, *A. tumida* has since 'escaped' from its native home and has recently invaded areas such as North America and Australia where its economic impact on the apiculture industry has been significant. Commercially available entomopathogenic nematodes were screened for their potential to control beetle larvae. The nematodes *Steinernema kraussei* and *S. carpocapsae* provided excellent control with 100% mortality of larvae being obtained. Delayed applications of the nematodes following larvae entering sand to pupate also provided excellent control for up to 3 weeks. The information gained supports the development of contingency plans to deal with *A. tumida* should it occur in the UK or Europe.

The development of mollusc-parasitic nematode *Phasmarhabditis hermaphrodita*

(Nematoda: Rhabditidae) in different substrates

Jiří Nermuť, Vladimír Půža 267-270

Abstract: The effect of different growing substrates on the development of the facultative slug parasite *Phasmarhabditis hermaphrodita* has been studied in a series of laboratory experiments. Wild, laboratory and Nemaslug strains of *P. hermaphrodita* were reared in agar plates on homogenized pig kidney, the homogenized bodies of *Deroceras reticulatum*, *Arion lusitanicus*, and *Galleria mellonella*, the faeces of *D. reticulatum* and *A. lusitanicus*, or leaf compost. Development time, yield, lipid reserves, and the body length of females and dauer larvae were assessed. All *P. hermaphrodita* strains were able to grow and reproduce on all tested substrates. However, yields were markedly higher on animal substrates. Lipid content and body size varied across the substrates, however, even plant tissue produced normal sized individuals with normal lipid content. It thus seems that the quality of the substrate is expressed mainly in yield. High and less variable yields and faster development of the wild and Nemaslug strains, in comparison with the laboratory strain, were probably due to different bacterial associates. The dramatic differences in yields on animal substrates, in comparison to those on plant tissue, illustrate the evolutionary advantage of the association of nematodes with invertebrates.

New nematodes associated to *Rhynchophorus ferrugineus*

(Coleoptera: Curculionidae): preliminary description

Monica Oreste, Francesca De Luca, Elena Fanelli,

Alberto Troccoli, Eustachio Tarasco 271

Abstract only

The role of bacterial symbionts in the competition

of entomopathogenic nematode species

Vladimír Půža, Jiří Nermuť, Zdeněk Mráček 273-276

Abstract: Competition between entomopathogenic nematode (EPN) species is still a largely neglected topic. Previous research has shown that in the competition within one insect host, nematode *Steinernema affine* strongly dominates over *S. kraussei* and suggested a possible role of symbiotic bacteria in the competition. In present study, *S. affine* and *S. kraussei* and their symbionts were reared in different combinations on Wouts agar plates, and nematode development was observed. Resulting progeny from these combinations was harvested and body size and lipid content of infective juveniles (IJs) were assessed. *S. affine* was able to develop, mature and produce viable progeny on the symbiont of *S. kraussei*. Interestingly, there was no difference in the duration of the cycle or reproduction potential, IJ size and lipid content between *S. affine* reared on their own symbiont and symbiont of *S. kraussei*. On the other hand, *S. kraussei* developed and reproduced well only on its own symbiont. These experiments explained the

previously observed dominance of *S. affine* over *S. kraussei*. Research with more EPN species is planned to further clarify the topic.

Research and development for a nematode-based biological control solution
for western corn rootworm in maize

Stefan Toepfer, Ulrich Kuhlman 277-282

Abstract: 10 years of joint efforts in research and development have led to a nematode-based biological control solution for one of the most destructive maize pests, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae).

Posters

Development of a method to establish entomopathogenic nematodes (EPN)
in arable soils by using farm-suitable field equipment

Wolfgang Büchs 285

Abstract only

Biosafety analysis of the *Bacillus pumilus* 15.1 strain
through a *Caenorhabditis elegans* pathogenicity assay

*Juan F. Caña Roca, Diana C. García, Juan I. Vilchez-Morillas,
Maximino Manzanera, Tania Domínguez, Antonio Osuna, Susana Vilchez* 287-290

Abstract: Using a *Caenorhabditis elegans* pathogenicity assay we evaluated the biosafety of the *B. pumilus* 15.1 strain, a recently isolated bacteria active against larvae of the Mediterranean fruit fly *Ceratitidis capitata*. In the study we evaluated the toxicity of this strain toward the nematode together with other *B. pumilus* strains and compared its toxicity with a non pathogenic strain (*Escherichia coli* OP50) and a pathogenic one (*Burkholderia cepacia*). After this study, we concluded that *B. pumilus* 15.1 is a safe strain and could not represent a problem to be used as a biological control agent.

The indigenous entomopathogenic nematode searching results
at different agroecosystems of Georgia

M. Chubinishvili, Ts. Chkhubianishvili, M. Kakhadze, I. Malania, I. Rijamadze 291

Abstract only

Field evaluation of entomopathogenic nematodes for controlling
fall webworm *Hyphantria cunea* (Lepidoptera: Arctiidae) in West Georgia

*Oleg Gorgadze, Manana Lortkipanidze, Patrick Tailliez, Medea Burjanadze,
Madona Kuchava* 293-296

Abstract: The present work deals with results of application of entomopathogenic nematodes of the genus *Steinernema* (*S. carpocapsae*, *S. thesami* and *Steinernema* sp.) against the harmful pest of the forest and agricultural crops *Hyphantria cunea* (Lepidoptera: Arctiidae) distributed in Georgia. Field experiments were carried out in August of 2012 on private plots of Guria region of the West Georgia in hazelnut plantations diseased with pest's larvae. A high percentage of mortality ranging from 93.6% to 98.3% was observed in all experiments as a result of entomopathogenic nematode application. Among the species used, the efficiency of a new *Steinernema* species was specially noticed. High efficiency of the treatment was also promoted by optimum climatic conditions (Temperature = 28 °C and hygrometry = 99%).

Feeding activity and survival of slug *Arion lusitanicus* (Gastropoda: Arionidae) exposed to the rhabditid nematode, *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae)

Dinka Grubišić, Tina Hamel, Tanja Gotlin Čuljak, Ana Loparić, Mirjana Brmež

297-300

Abstract: Slugs are important pests of cultivated plants in Croatia. In many sites slug species *Arion lusitanicus* (Mabille, 1868) (Gastropoda: Arionidae) has become the most frequent species, which is very hard to control by chemical molluscicides. Since 1996 a biological molluscicide based on nematode *Phasmarhabditis hermaphrodita* (Schneider, 1859) (Nematoda: Rhabditidae) has been formulated as an effective product for slug control. In order to establish feeding activity and survival of adult *A. lusitanicus* specimens exposed to parasitic nematode *P. hermaphrodita* and to compare its efficiency to efficiency of chemical molluscicides, a laboratory experiment was performed. Adult specimens of *A. lusitanicus* (fed on lettuce leaves in flower pots) were exposed to *P. hermaphrodita*, metaldehyde and methiocarb treatments. Food consumption of slug specimens was measured daily. Survival of slugs was observed to their death, up to 30th day. In the first week of investigation, chemical molluscicide treatments were found to differ significantly from the biological product and control. At the treatments treated by nematodes, daily leaf area consumption was also reduced and was significantly different from the control treatment. Food consumption was reduced on both chemical and biological treatments but the most of adult specimens of *A. lusitanicus* survived and continued to feed. To the end of the second week of investigation, food consumption decreased on all treatments and was mainly uniform with no significant differences between treatments. During the experiment, the slugs were dying within the period of 3 to 30 d at the treatments treated by nematode *P. hermaphrodita* or in the period of 9 to 24 d at the treatments treated by metaldehyde and methiocarb. Because the tolerance level to slug damages in lettuce market is effectively zero, these results indicate a failure of biological product based on *P. hermaphrodita* in control of adult specimens of *A. lusitanicus* as well as a failure of chemical molluscicides. These data point at a great need for integrated control of slug damage which must include cultural and different alternative control measures, not only chemical control.

New insights to insect response to the infection by nematobacterial complex

Pavel Hryšl, Pavel Dobeš, Badrul Arefin, Lucie Kučerová,

Robert Markus, Zhi Wang, Michal Žurovec, Ulrich Theopold

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Compatibility of five different entomopathogenic nematode (Nematoda: Rhabditida) species with registered insecticides and fungicides under laboratory conditions

Žiga Laznik, Stanislav Trdan

303-308

Abstract: To increase our knowledge on the susceptibility of entomopathogenic nematode (EPN) species to agrochemicals, the compatibility of the infective juveniles (IJs) of the *Steinernema feltiae*, *S. carpocapsae*, *S. kraussei*, *Heterorhabditis bacteriophora* and *H. downesi* with 6 chemical, one plant-based and one bio-insecticide, and 13 synthetic organic and two inorganic fungicides were investigated under laboratory conditions. The effect of direct IJ exposure to insecticides for 6 and 24 hours was tested in Petri dishes at 15, 20 and 25 °C. In our experiment we determined the best compatibility of *S. feltiae* with active ingredients azoxystrobin, azadirachtin, *Bacillus thuringiensis* var. *kurstaki* and imidacloprid. The present study showed that *S. carpocapsae* and *S. kraussei* are sensitive to all tested insecticides, while *H. bacteriophora* is sensitive only to abamectin and lufenuron. Nematode *H. downesi* significantly suffered the highest mortality when infective juveniles were mixed with active ingredients (a. i.) tebuconazole, spiroxamine, and triadimenol. Based on our research, we conclude that compatibility is not only a species-specific but also a strain-specific characteristic.

Susceptibility of *Phytodecta fornicata* (Coleoptera: Chrysomelidae)

to *Heterorhabditis bacteriophora*

Ivana Majić, Emilija Raspudić, Marija Ivezić, Mirjana Brmež,

Ankica Sarajlić, Andrea Mirković 309-312

Abstract: The infectivity of *Heterorhabditis bacteriophora* for adult stage of *Phytodecta fornicata* was evaluated in the laboratory. Two different nematode concentrations (1000 and 2000 infective juveniles (IJs) per adult) were tested at temperatures of 22 °C and 30 °C. Mortality of insects was checked at 3 d post infection (p.i.); emergence of IJs from cadavers was noted at 8 and 11 d p.i.. For the first time, the experiment demonstrated that *H. bacteriophora* applied in rate of 1000 IJs per beetle is causing 100% mortality of *P. fornicata*, and that IJs developed inside adult insect cadavers. The effects of temperature and concentration of nematode preparation were of less importance. The harvest of IJs from cadavers was obtained but with low population level. The time of emergence of IJs was temperature dependent. In order to develop cost effective and sustainable control measure, further mortality studies on individuals, with other species and strains, and assays with more realistic environments are needed.

The susceptibility of mulberry moth to infection by entomopathogenic nematodes,

Heterorhabditis bacteriophora and *Steinernema carpocapsae*

Nona Mikaia 313-316

Abstract: The mulberry moth, *Glyphodes pyloalis*, is considered as an urban pest and therefore the application of environmentally safe means for mulberry trees protection is recommended. Among the entomopathogenic nematode (EPN) species *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* are important as a biological control agents. The susceptibility of *G. pyloalis* to infection by *H. bacteriophora* and *S. carpocapsae* infective juveniles (IJ) was tested under laboratory conditions. Individuals of IV instar larvae were collected from mulberry trees in Georgia, Tbilisi (village Digomi). Nematode suspensions at a concentration of 1500 IJs/ml were used for treatment of mulberry leaves. After 72 h, the mortality of *G. pyloalis* caused by *H. bacteriophora* was 54%, whereas *S. carpocapsae* caused 76% mortality. The results suggest that nematode suspensions of *H. bacteriophora* and *S. carpocapsae* can be used to control *G. pyloalis* in urban plots.

Attract and kill against western corn rootworm larvae

with entomopathogenic nematodes

Mario Schumann, Felicitas Kaemena, Anant Patel, Stefan Vidal 317-318

Abstract only

Bacteria

Poster

Cloning strategy for recovering phage-displayed Cry1Aa13 mutants from phages

with affinity towards proteins present in the gut of *Ceratitis capitata*

Tania Domínguez, Juan F. Caña Roca, Diana C. García,

Antonio Osuna, Susana Vílchez 321-325

Abstract: Using the phage display technique, a pool of phages from a library of bacteriophages expressing Cry1Aa13 toxins with modified loops 2 at the domain II was selected that showed affinity toward proteins present in the guts of the Mediterranean fruit fly, *Ceratitis capitata*. The sequences of the hypervariable regions of the *in vivo* selected phages were analysed and an almost identical sequence was obtained in all of the selected phages. Those phages bearing toxins different from the wild type toxin at the loop 2 were selected in order to recover the Cry1Aa13 mutant toxins. Here we describe the cloning strategy designed and used to clone the toxins from the phage genome in order to be expressed.

Efficacy evaluation of different *Bacillus thuringiensis* sv *kurstaki* strain EG2348 formulations against *Malacosoma neustria* (Lepidoptera: Lasiocampidae)
Luca Ruii, Achille Loi, Giovanni Falchi, Edith Ladurner, Andrea Braggio, Pietro Luciano 327-330

Abstract: Cork oak forest protection and management require continuous monitoring of defoliator moth species. Among these, the European tent caterpillar, *Malacosoma neustria* L., can cause widespread and extensive defoliation of host plants, and the implementation of appropriate management programs becomes necessary. Sustainable control methods may include the use of entomopathogenic microorganisms, such as *Bacillus thuringiensis* serovar *kurstaki* (*Btk*). However, the formulation of the microbial control agents can be a key factor for the success of application programs. The results of an efficacy trial with different formulations of *Btk* strain EG 2348 against larvae of *M. neustria* conducted in a cork oak forest in Sardinia (Italy) are reported. In the trial, a commercial and an experimental formulation of *Btk* strain EG 2348 were tested (henceforth Rapax[®] and Rapax Experimental) in comparison to two *Btk*-based reference products (Foray 48B[®] and Delfin[®]). Both formulations of *Btk* strain EG 2348 proved to be effective in controlling the pest.

Development of a new bio-insecticide for controlling lepidopteran pests
Kazım Sezen, Remziye Nağcıoğlu, İsmail Demir, Hüseyin Tepe, İslam Yıldız, Zihni Demirbağ 331
Abstract only

Bioluminescence determination of antibacterial activity of *Bombyx mori* and *Galleria mellonella* haemolymph
Libor Vojtek, Pavel Dobes, Ender Buyukguzel, Pavel Hyrs 333-338

Abstract: We describe an antibacterial assay based on bioluminescence of two Gram negative bacteria *Photobacterium luminescens* and transformed *Escherichia coli*, which can be used for a real-time measurement of antibacterial activity. We observed a significant dose-dependent decrease of bioluminescence using both bacterial species during one hour after exposure to *Bombyx mori* or *Galleria mellonella* haemolymph. The humoral origin of the antibacterial activity observed in whole haemolymph was confirmed in haemolymph plasma without haemocytes. Antibacterial activity operating against Gram negative bacteria was measured in unaffected insect larvae as well as after septic injury; increased antibacterial activity in haemolymph was detected in the latter case which confirms inducibility of antimicrobial agents. This method can be widely used for determination of antibacterial activity in insects and supposedly in other invertebrates.

Intramolecular cleavage at the loop between α 3-helix and α 4-helix is critical for cytotoxic activity of Cry8Da
Takuya Yamaguchi, Hisanori Bando, Shin-ichiro Asano 339-342

Abstract: Cry8Da from *Bacillus thuringiensis galleriae* SDS-502 has the toxicity against both larvae and adult *P. japonica*. Cry8Da is processed into three fragments (64 kDa, 54 kDa and 8 kDa) by gut juice of *P. japonica*. Fragments of 54 kDa and 8 kDa are derived from the cleavage of 64 kDa fragment at the loop between α 3-helix and α 4-helix in Domain I. Binding assays showed that the 54 kDa fragment bound to both larvae and adult *P. japonica* brush-border membrane vesicles while the 64 kDa and 8 kDa fragments did not. We constructed a protease-resistant mutant, 8Da-R163A, in which R¹⁶³ on the loop was changed to A¹⁶³. To directly investigate whether intramolecular cleavage is critical for insecticidal activity of Cry8Da, we performed cytotoxic assays against midgut epithelial cells (MECs) prepared from adult *P. japonica* using purified uncleaved (64 kDa) and intramolecularly cleaved (mixture of 54 kDa and 8 kDa) Cry8Da toxin. Cytotoxic assay showed MECs were destroyed by only intramolecularly cleaved Cry8Da toxin. Intramolecularly cleaved Cry8Da toxin also formed oligomeric structure

after incubation with MGCs. These results strongly support our idea that the cleavage at the loop between α 3-helix and α 4-helix is critical for toxicity of Cry8Da.

Electron microscope and genetic analysis of an intracellular bacterium associated with the common rough woodlouse, *Porcellio scaber* (Isopoda, Porcellionidae)
Regina G. Kleespies, Andreas Leclerque 343-347

Abstract: The common rough woodlouse, *Porcellio scaber*, is a common and widespread isopod species of Western and Northern Europe. A previously unknown intracellular bacterium has been identified in a diseased *Porcellio* larva. Microscopic studies revealed the subcellular structures characteristic of infection by *Rickettsiella*-like bacteria. Molecular phylogenetic analysis based on the 16S ribosomal RNA encoding *rrs* gene demonstrated that the woodlouse pathogen belongs to the taxonomic genus *Rickettsiella* (Gammaproteobacteria; Legionellales). Moreover, genetic analysis makes it likely that this new pathotype should be considered a member of the “*Rickettsiella armadillidii* complex”, i.e. a group of *Rickettsiella* bacteria found mainly in terrestrial isopods. *R. armadillidii* is currently placed in synonymy with the nomenclatural type species, *Rickettsiella popilliae*. The present study does not lend support to this synonymization.

Miscellaneous

Poster

Impact of various oilseed rape productions on biological potential of endogaeic active ground beetles (Coleoptera: Carabidae)
Ivan Juran, Tanja Gotlin Čuljak, Wolfgang Büchs, Dinka Grubišić, Ivan Sivčev 351
Abstract only

Ground beetles (Coleoptera: Carabidae) in sugar beet fields as the base for conservation biological control
Tomislav Kos, Renata Bažok, Zrinka Drmić, Željka Graša 353-357

Abstract: The fauna and abundance of ground beetles (Coleoptera, Carabidae) in arable crops can be an indicator of influence of different agricultural measures on biodiversity. The aim of our study was to determine ground beetle fauna abundance and frequency in two fields with different herbicide and insecticide application practice, and to determine differences in total number of species and individuals, collected with two capturing methods. The study was conducted in 2012 in the eastern part of Croatia (County of Vukovar-Srijem). Beetles were collected in a period of twenty-one weeks (April- September) by setting four modified pitfall traps aimed to collect above ground fauna and four probes (WB PROBE II[®] Trap, Trece inc.) aimed to collect endogaeic fauna in each field. Nine different species and eight genera were identified in the study. Most abundant were *Pseudoophonus rufipes* (De Geer 1774) and *Bembidion* sp. (Latreille 1802). Both are classified as eudominant. The most frequent species was *P. rufipes* classified as constant (71.42%) and the most frequent genus was *Bembidion* sp. (38.04%) classified as accessory. There was no significant difference between fields among total number of established species and/or genus no matter if they were captured by pitfall trap or probe. Significantly more individuals were captured in pitfall traps on the field No. 1 (33.3) than on the field No. 2 (8.8), respectively. Opposite, significantly fewer individuals were captured with probe on the Field No. 1 (0.5) than on the field No. 2 (6.6), respectively.

Impact of *Entomophaga maimaiga* on gypsy moth populations in Bulgaria
Plamen Mirchev, Andreas Linde, Daniela Pilarska, Plamen Pilarski, Margarita Georgieva, Georgi Georgiev 359-363

Abstract: The entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales) (Entomophthoraceae) was introduced into three populations of gypsy moth (*Lymantria dispar* L., Lepidoptera: Erebidiae) in Bulgaria in 1999. After the first strong epizootics

in 2005, the species was introduced in six outbreak populations of gypsy moth in different regions of the country from 2008 to 2011. Due to the resulting fungal epizootics, the calamities of the pest in Bulgaria were totally suppressed. The pathogen increased its impact by a natural range extension and it is now present in nearly all regions of the country in which *L. dispar* occurs.