

IOBC/wprs Bulletin, Vol. 66, 2011

Working Group “Insect Pathogens and Entomopathogenic Nematodes”.
Proceedings of the 13th European Meeting “Biological Control in IPM Systems” at
Innsbruck (Austria), 19 – 23 June, 2011. Editors: Ralf-Udo Ehlers, Neil Crickmore,
Juerg Enkerli, Itamar Glazer, Martin Kirchmair, Miguel Lopez-Ferber, Sigrid
Neuhauser, Hermann Strasser, Cezary Tkaczuk, Michael Traugott.
ISBN 978-92-9067-241-8 [XXV + 550 pp.].

Preface	I
List of Participants	III-XIV
Contents	XV-XXV

Plenary Session 1: Biological control in IPM systems

Position and perspectives of biological control in the European plant protection package

Johannes Jehle 3-6

Abstract: Driven by concerns on the human and animal health and on the environment of the use of pesticides in the European Union (EU), the European Commission has launched a new legislative framework, the so-called EU plant protection package, which will replace the hitherto existing registration regulations of active substances and plant protection products. It consists of four parts: (1) The Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and replacing the Directive 91/414/EEC; (2) the Directive 2009/128/EC aiming to achieve the sustainable use of pesticides; (3) the Directive 2009/127/EC regarding to machinery for pesticide application, and (4) the Regulation (EC) No 1185/2009 concerning statistics on pesticide marketing and use. Whereas the Directives need to be converted into national law in the EU member states, the Regulations are legally in force in all EU member states. This new framework aims to harmonize the plant protection legislation in Europe and to develop measures to reduce the risks posed from the use of plant protection products. As most biological control agents (BCAs) underlie the same legislative procedures as chemical plant protection products, these new regulations will also apply for most BCAs. Thus, they will have a strong impact on future registration and use of BCAs.

Biological control of arthropod pests in outdoor crops -present status and future challenges

Lene Sigsgaard 7-10

Abstract: There is little use of biological control in outdoor crops. While the use of biological control against insect and mite pests in greenhouses in Denmark had reached a high level already ten years ago and has managed to stay high, the use in outdoor crops remains low. The total amount of money spent on biological control in Denmark has reduced, but this is due to falling prices, since the amount of product used has not changed or increased slightly. However, still the use in outdoor crops is small and is to a high degree restricted to the use of *Bacillus thuringiensis* against lepidopterans in high value vegetable and fruits, and to the use of predatory mites in strawberries and predatory bugs in pear. The present paper discusses some main drivers and barriers decisive for the share of biological control in outdoor crops, using Denmark as an example.

Exploiting synergies to optimise the impact of entomopathogenic fungi

Tariq M. Butt 11-17

Abstract: The efficacy of entomopathogenic fungi (EPF) such as *Metarhizium anisopliae* and *Beauveria bassiana* is greatly improved when used with sub-lethal doses of insecticide or entomopathogenic nematodes (EPN) through additive or synergistic interactions. The latter

allows each agent to be used at reduced application rates with corresponding savings for growers. The exact mechanism for the synergism is unclear. It is postulated that sub-lethal rates of insecticide stress the pest making it more susceptible to infection. Some insecticides may increase pest mobility and increase acquisition of EPF conidia and, since mortality is dose-related, this increases pest mortality. Combined application of EPF and EPN is ergonomic and can result in synergism but some workers have noted stronger synergy if the EPN are applied 1-3 weeks after the EPF. There is a need to identify and optimise synergies as this strategy offers many benefits to growers including potential savings as application rates are significantly reduced. Furthermore, this strategy is in accord with the EU Directive 2009/128/EC.

Plenary Session 2: Trends in biological control

The evolutionary ecology of *Bacillus thuringiensis*; can social interactions maintain virulence and counteract strain attenuation?

Ben Raymond 21-25

Abstract: Social evolution theory makes a number of predictions about how bacterial virulence evolves in different environments and can have important implications for how we maintain or improve the virulence of biopesticidal bacteria such as *B. thuringiensis*. Investment in virulence should not be favoured in homogeneous artificial media, since social “cheats” that fail to synthesize virulence factors should be at growth advantage. There is some evidence that biopesticide derived strains are attenuated with respect to wild-type relatives. Selection of rifampicin resistant mutants of *B. t. kurstaki* in the diamondback moth *Plutella xylostella* led to increases in virulence and also reduced growth rates in artificial media, as predicted by theory.

Systematics and taxonomy of insect pathogenic fungi: current status, future directions

Stephen A. Rehner 27-28

Abstract only

The use of entomopathogenic nematodes in the US and issues related to genetic degradation

David Shapiro-Ilan 29-32

Abstract: Research and commercial application of entomopathogenic nematodes in North America has a long history. In the pursuit of commercial viability, there have been a number of success stories, but also quite a number of dead ends. In this presentation, we provide insight into new opportunities for entomopathogenic nematodes that are on the horizon. Some novel targets that are currently being researched include a variety of wood boring insects, weevil pests, as well as pests of pollinators. Novel application methods and formulations that are being developed will also facilitate expanded use of nematodes as microbial control agents. Advances in biocontrol using entomopathogenic nematodes will also be enhanced through fundamental studies on the microbial ecology, epizootiology, trait deterioration, population dynamics as well as basic studies on host-pathogen relationships.

Nucleopolyhedrovirus covert infection in *Spodoptera exigua*: sublethal disease and vertically transmitted infections likely provide unique opportunities for virus-based control on greenhouse crops

Oihana Cabodevilla, Elisabeth A. Herniou, Primitivo Caballero, Trevor Williams 33-36

Abstract: The multiple nucleopolyhedrovirus of *Spodoptera exigua* (SeMNPV) forms the basis for a number of highly effective biological insecticides. To assess the prevalence and diversity of SeMNPV genotypes in natural *S. exigua* populations, field populations were subjected to molecular analyses. The genotypes associated with covert infections, that are likely to be vertically transmitted, were compared to those associated with horizontally transmitted infections. Insights into the relationship between genetic diversity and transmission strategy were obtained by comparing the genetic composition and insecticidal properties, of genotypes associated with

vertically and horizontally transmitted infections. The results of these studies have clear applications in the development of custom designed biopesticidal products without the need for recombinant DNA technologies.

Bacteria

Bacteria Session 1

Bacillus thuringiensis sv *kurstaki* strain EG 2348: effect of formulation on efficacy against tomato leafminer (*Tuta absoluta*)

Edith Ladurner, Massimo Benuzzi, Sergio Franceschini 39-42

Abstract: The tomato leafminer, *Tuta absoluta* (Meyrick), is one of the most devastating pests on tomato. Severe losses due to this pest may occur on both fresh market and processing tomato. Current management strategies mainly rely on the use of chemical insecticides, but their integration with other control methods, such as microbial control agents, is of sound importance because of resistance management, residue, toxicological and ecotoxicological issues. *Bacillus thuringiensis* sv *kurstaki* (*Btk*) strains proved to be effective tools to be included into control strategies of the tomato leafminer. In the field studies, carried out in Southern Italy, the efficacy of two formulations of *Btk* strain EG2348, respectively a wettable powder (f.p. Lepinox Plus[®]) and a suspension concentrate (f.p. Rapax[®]), was investigated. The suspension concentrate proved to be more effective than the wettable powder.

Understanding the toxicity of *Bacillus pumilus* 15.1 toward the Mediterranean fruit fly (*Ceratitis capitata*)

C. Alfonso Molina, Juan F. Caña Roca, Diana García, Tania Dominguez,

Antonio Osuna, and Susana Vilchez 43-47

Abstract: *Bacillus pumilus* 15.1 has been recently reported as a strain active against larvae of the Mediterranean fruit fly, *Ceratitis capitata* (Molina et al. 2010). The 15.1 strain only reveals toxicity against this insect when cultures are incubated at low temperature for at least 96 h before the bioassay takes place, an interesting phenomenon worth to study and characterise it in detail. In this study we report as well that the virulence factor is of a proteinous nature. We characterised the protein expression profile of strain 15.1 along with its growth curve and found that the strain overproduces insoluble proteins that are secreted during sporulation.

Potential of a *Brevibacillus laterosporus* and azadirachtin combination in immature house fly integrated management.

Luca Ruii, Alberto Satta, Ignazio Floris 49-52

Abstract: Recent results of laboratory bioassays and comparative field treatments with *Brevibacillus laterosporus* (*Bl*) and azadirachtin [AZ] are discussed in order to evaluate their use in combination or integration, to suppress house fly immatures in natural breeding sites of animal farms. As determined by laboratory bioassays, the lethal effects of *Bl* and [AZ] are concentration dependent and the estimated median lethal concentrations (LC₅₀s) for second instar larvae are 1.7x10⁸ spore/g and 24.5µg/g of diet, respectively. Applications on the manure-covered cow pen in dairy farms, at a dosage of 3l/m², and concentrations corresponding to LC₅₀ levels caused a significant fly development depression in areas treated with [AZ] (63%) and *Bl* (46%), compared to the control. Preliminary laboratory observations show compatibility and interactive effects of *Bl* and [AZ]. Therefore, the integration of these generally regarded as safe natural insecticides is promising.

Bacillus thuringiensis treatment alters larval growth, hemocytes and modulation of Hsp70 in *Rhynchophorus ferrugineus*

Barbara Manachini, Mirella Vazzana, Monica Celi, Vincenzo Arizza 53-57

Abstract: To study the pathogen-host relationship, we used the model of the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) and *Rhynchophorus ferrugineus*, a quarantine pest that attacks palm trees. In particular, we focused on the Bt stress-induced infections. We studied the effect of Bt on larval growth, on hemocytes and on the expression of the heat shock proteins. HSPs are rapidly synthesized in the cell after exposure to stress including pathogens. Hsp70 was evaluated in the supernatant of the hemocyte lysate (HLS) obtained from larvae fed with Bt. This is the first time that the presence of Hsp70 has been recorded in *R. ferrugineus*. Bt has negative effects on larval growth, on total hemocytes counts and on the hemocyte type. In addition Hsp70 expression was modulated in time (3h, 6h, 12h, 24h) in response to Bt ingestion, highlighting that Bt is a stress factor for the *R. ferrugineus*. Further investigation is needed to understand the possible correlation between the reduction of hemocytes and Hsp70 modulation.

Preliminary selection of non-target Lepidoptera species for ecological risk assessment of *Bt canola* in Sicily

Barbara Manachini, Veronica Fileccia, Silvia Ruggieri, Filippo Castiglia 59-63

Abstract: It is essential to assess the environmental risk that *Bt canola* resistant to Lepidopteran pests may hold and to study its effect on species assemblages that fulfil a variety of ecosystem functions. Environmental risk assessment can be improved through the use of an ecological model which can be applied to a specific environment, so that local species can be classified functionally and prioritized to identify potential test species. Several other Lepidoptera species are also directly exposed to Bt toxin. In this paper an ecological approach was followed for selection of non-target Lepidoptera species for ecological risk assessment of *Bt canola* in Sicily, using data collected over a one-year period on Lepidoptera biodiversity on different habitats. Non-target Lepidoptera most likely to be affected were identified and prioritized for future testing and inclusion in risk assessments. Through use of the selection matrix, knowledge gaps were identified for future research and guidance for the design of ecologically realistic experiments.

Bacteria Session 2

Oral insecticidal activity of root colonizing *P. fluorescens* CHA0: a biocontrol agent with potential to control plant pests and diseases?

Monika Maurhofer, Beat Ruffner, Maria Péchy-Tarr, Alfred Rindlisbacher, Christian Obrist, Patrik Hoegger and Christoph Keel 67-70

Abstract: The application of microbial biocontrol agents for the control of fungal plant diseases and plant insect pests is a promising approach in the development of environmentally benign pest management strategies. The ideal biocontrol organism would be a bacterium or a fungus with activity against both, insect pests and fungal pathogens. Here we demonstrate the oral insecticidal activity of the root colonizing *Pseudomonas fluorescens* CHA0, which is so far known for its capacity to efficiently suppress fungal plant pathogens. Feeding assays with CHA0-sprayed leaves showed that this strain displays oral insecticidal activity and is able to efficiently kill larvae of three important insect pests. We further show data indicating that the Fit insect toxin produced by CHA0 and also metabolites controlled by the global regulator GacA contribute to oral insect toxicity.

Phylogenetic position of Fit insect toxin producing pseudomonads

Beat Ruffner, Maria Péchy-Tarr, Florian Ryffel, Christoph Keel, Monika Maurhofer 71-74

Abstract: Fit produced by *Pseudomonas fluorescens* CHA0 is a novel insect toxin in root colonizing pseudomonads, of which a homologue is described in *Photorhabdus* species.

However, occurrence and abundance of insect pathogenicity in plant-associated pseudomonads is still unclear. An extensive screening outside the *P. fluorescens* complex identified strains of *Pseudomonas chlororaphis* as further Fit toxin producing candidates. Sequences of five different *P. chlororaphis* strains generated in this study were used to reconstruct the evolutionary history of the Fit toxin gene and to analyse its mode of evolution. We found that *P. chlororaphis* is closely associated with a small subgroup of 2,4-diacetylphloroglucinol and pyoluteorin-producing pseudomonads, both when analyzing four housekeeping genes and the nucleotide sequences for the Fit toxin gene. Additionally, we identified purifying selection to be the predominant mode of Fit toxin evolution.

Identification and characterization of novel vip3-like genes in *Bacillus thuringiensis* strains from a Spanish collection
 Leopoldo Palma, Mireya Maeztu, Iñigo Ruiz de Escudero, Delia Muñoz & Primitivo Caballero 75-78

Abstract: A total of 400 strains of *Bacillus thuringiensis* (Bt) have been screened to identify the presence of vip3-like genes using a PCR-restriction fragment length polymorphism (PCR-RFLP) strategy to detect novel vip3 genes. This allowed identification of vip3A known genes in ~80% of the strains, whereas three new RFLP patterns revealed the presence of three novel vip3-like genes in three different Bt strains (B5.3, B8.1, B8.3). PCR amplicons from B8.1 and B8.3 strains produced fragments of 1646 and 1642 bp, respectively that were cloned and sequenced. These two fragments shared homologies of ~77% with known vip3 genes suggesting they may be novel vip3 genes. The strain B5.3 amplicon was 1620 bp and showed 91% homology with vip3Aa1 gene. The full-length sequence of this vip3A-like gene was obtained and found to be a new vip3Ag variant and was designated vip3Ag4 by the Bt Toxin Nomenclature Committee. This gene is 2364 bp in size, encodes a 788 amino acid (88 kDa) protein and shares between 81 and 91% homology with known vip3A genes. Furthermore, vip3Ag4 was successfully cloned in pET-28b(+) and expressed in *Escherichia coli* BL21(DE3). The protein obtained was toxic against *Chrysodeixis chalcites*, *Helicoverpa armigera*, *Spodoptera exigua*, *S. frugiperda* and *S. littoralis*.

Proteolytic processing of *Bacillus thuringiensis* Cry3Ca toxin by different protease digestion treatments
 María Martínez-Solís, Patricia Hernández-Martínez, Baltasar Escriche 79-82

Abstract: *Cylas puncticollis* (Coleoptera: Brentidae) is one of the most important biological threats to sweet potato productivity in East Africa. The control of this insect pest is difficult due to their cryptic feeding behaviour. Recently, the Cry3Ca toxin was found to be toxic to *Cylas* spp. However, little is known about the mode of action of this Cry toxin active against *C. puncticollis*. The present work studied the proteolytic activation pattern of the Cry3Ca 70kDa protoxin after treatment with commercial enzymes, midgut juice and brush border membrane vesicles (BBMVs). Our results showed that the activated fragment obtained after all treatments is a polypeptide of about 50 kDa.

Poster Bacteria

In vivo selection of Cry1Aa13 toxin mutants with high affinity for intestinal proteins of the Mediterranean fruit fly *Ceratitis capitata* from a phage display library
 Tania Domínguez, Juan F. Caña Roca, Diana C. García, Colin Berry, Antonio Osuna and Susana Vilchez 85-88

Abstract: In this work we aim to test the hypothesis that *in vitro* evolution of Cry toxins and their selection using phage display technology can be a useful strategy for developing novel insecticides actives against non-target insects. It was demonstrated that it is possible to select Cry toxin mutants that bind with high affinity to the intestinal proteins of *Ceratitis capitata*, from a library of bacteriophages expressing Cry1Aa13 toxin mutants on their surface. Selected mutants showed a completely different amino acid sequence in the loop2 of the domain II compared with

the lepidopteran active wild type protein. These toxins could potentially be active against this non naturally target insect, as binding to epithelial cells proteins is the first step required for Cry toxin activity.

Fungi

Fungi Session 1: Endophytes

Entomopathogenic fungi as endophytes: a new plant protection strategy?

Stefan Vidal 91-97

Abstract: Since several years entomopathogenic fungi, specifically *Beauveria bassiana*, *Lecanicillium lecanii*, and *Metarhizium anisopliae*, have been reported as organisms able to colonise different plant species and plant parts, thereby maintaining their entomopathogenic potential against various insect pests. This review discusses different aspects of the use of these entomopathogenic fungi as a plant protection strategy. Although the endophytic growth and establishment of these fungi can be initiated either by foliar spore application or by incorporation into the soil, the growth and maintenance within the plant tissues need to be guaranteed during the whole cropping season. New or improved formulation strategies need to be developed to enhance the colonization process of these organisms in the different plant tissues. So far the infection process of herbivores feeding on colonized plant tissues has not been studied in detail and mycosis has not been reported in all cases. Whether production of mycotoxins by endophytic entomopathogenic fungi may create a problem for humans consuming colonized plant parts needs to be addressed in forthcoming studies, as well potential non-target effects on natural enemies. The potential of endophytic entomopathogenic fungi as a new strategy for plant protection is discussed in the light of these open questions.

In planta detection and quantification of an endophytic *Beauveria bassiana* strain

Cristina López-Díaz, Blanca B. Landa del Castillo, F^o. Javier Muñoz-Ledesma, Enrique Quesada-Moraga 99

Abstract only

Systemic effects of fungal endophyte seed treatment on the development of the spotted stalk borer, *Chilo partellus* (Swinhoe)

Juliet Akello, Jean N. K. Maniania, Richard Sikora 101-103

Abstract: Greenhouse experiments were undertaken to investigate the systemic effects of cereal associated fungal endophytes on the development of *Chilo partellus* neonates in maize. Maize seeds were treated with seven endophytic fungal isolates and infested with *C. partellus* neonates, 17 days after emergence. The results indicate that seed treatment with endophytic strains E3RF20, S4ST7, S4SU1 and G1LU3 adversely affected larval feeding and development, resulting in less dead hearts and damage as compared to the untreated controls, four weeks after infestation. Thus, seed treatment with the above isolates show promise for early protection of maize seedlings. However, field studies under subsistence farming conditions in the subtropical and tropical environments are warranted if these isolates are to be considered as an IPM tool for lessening yield reductions attributed to stem borers.

Effects of *Metarhizium anisopliae*, *Bacillus thuringiensis* and new chemistry insecticide on the mortality and pupation of field populations of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)

Waqas Wakil, M. Usman Ghazanfar 105-108

Abstract: Two dose rates of *Bacillus thuringiensis* (0.5, 1µg g⁻¹), one dose rate of *Metarhizium anisopliae* (1.3×10⁶ conidia ml⁻¹) and chlorantraniliprole (0.01ppm) each were applied alone and in combination against second larval instar of the tomato fruitworm *Helicoverpa armigera*

Hübner. The overall results showed that all the treatments gave significant control of *H. armigera*, however, the population from Gujranwala proved most tolerant and of Rawalpindi was highly susceptible.

- Exploring and exploiting indigenous entomopathogenic fungi for managing the wood boring beetle, emerald ash borer, *Agrilus planipennis*, in North America
George Kyei-Poku; Shajahan Johny 109
Abstract only

Fungi Session 2: Molecular detection of fungal BCAs

- Molecular diversity of the *Metarhizium anisopliae* lineage in an agricultural field
Bernhardt Michael Steinwender, Jürg Enkerli, Franco Widmer,
Jørgen Eilenberg, Nicolai Vitt Meyling 113-115

Abstract: Entomopathogenic fungal isolates identified by morphology as *Metarhizium anisopliae* may belong to different species when identified by molecular characters. We isolated *Metarhizium* spp. from a Danish agricultural field using *Tenebrio molitor* as bait insect to assess the molecular diversity within the soil of a single field. Isolates were analyzed using DNA sequencing and applying SSR markers. Within the former *M. anisopliae* lineage, we found *M. brunneum* (86.3%), *M. robertsii* (11.3%) and *M. majus* (3.4%) in the soil samples. Several genotypes of each species were identified based on SSR markers. Differences in abundance of the species and their genotypes suggest different adaptations to the soil environment of the agricultural field.

- Microsatellite markers for cultivation-independent monitoring of persistence of the entomopathogenic fungus *Beauveria bassiana* in soil samples
Sandhya Galidevara, Monika Bischoff-Schaefer, Jacqueline Hirsch,
K. Uma Devi and Annette Reineke 117-120

Abstract: If bulk DNA extracted from soil samples should be monitored for the occurrence and persistence of a particular strain of an entomopathogenic fungus, strain specific molecular methods are needed. Here, we report on the applicability of five *Beauveria bassiana* specific microsatellite (SSR) markers for amplification of strain specific bands in the *B. bassiana* isolate ATCC 74040, which is present in the commercial product Naturalis. Discriminatory power of these markers was initially determined on *B. bassiana* DNAs from a world-wide collection of this entomopathogen. Single clearly separated peaks were evident in chromatograms after capillary electrophoresis, which had a characteristic size for most of the respective *B. bassiana* isolates. These microsatellite markers were accordingly applied for monitoring the establishment and persistence of *B. bassiana* isolate ATCC 74040 in the field. Experiments were conducted in a commercial nursery on a 100m by 25m field planted with *Paeonia* spp. Two defined plots within this field each with a size of 2m x 2m were treated with a 3% Naturalis-water suspension. Soil samples were taken before and after inoculation with *B. bassiana*. From the treated plots soil was taken and analysed 4, 10 and 19 weeks, respectively, after Naturalis application. *Beauveria bassiana* was successfully established and isolated from soil samples up to a period of 19 weeks after inoculation as indicated by the amplification of *B. bassiana* strain specific SSR profiles from soil DNA preparations.

- Using 454 pyrosequencing for assessing the effect of artificially applied *Beauveria bassiana* on fungal diversity in agricultural soils in India
Jacqueline Hirsch, Sandhya Galidevara, Stephan Strohmeier,
Martin Pfannkuchen, K. Uma Devi and Annette Reineke 121-124

Abstract: Due to optimal climatic conditions, entomopathogenic fungi (EPF) like *Beauveria bassiana* are frequently used as biocontrol agents against insects in tropical countries. EPF are

usually sprayed onto the plant's foliage or directly onto the soil. So far little is known on the effect of applications of EPF towards the naturally occurring soil microflora. In 2010, two agricultural fields in India were examined to define the structure and richness of the indigenous soil fungal community and to determine the influence of artificially applied *Beauveria bassiana* (strain ITCC 4688) on these populations. We performed this study by using tag-encoded 454 pyrosequencing of the fungal nuclear ribosomal internal transcribed spacer-1 (ITS-1) region. Our study validates the effectiveness of high-throughput 454 sequencing technology for assessing soil fungal diversity in agricultural soils and gives first insights into the effects of the application of a microbial biocontrol agent on soil fungal community compositions.

Investigation of *Metarhizium* spp. abundance in different habitat types based on cultivation-independent detection and quantification

Salome Schneider, Katja Jacot, Franco Widmer, Jürg Enkerli 125-128

Abstract: The genus *Metarhizium* includes insect pathogenic fungal species, which are currently used as biological control agents. The development of effective biological control strategies requires detailed knowledge of their biology and the environmental factors affecting their distribution and abundance. The goal of this study was to monitor *Metarhizium* spp. including *M. anisopliae*, *M. pinghaense*, *M. majus*, *M. brunneum*, *M. robertsii*, and *M. guizhouense* in soil using a cultivation-independent PCR-based detection and quantification tool. Soil was collected from different habitat types including semi-natural habitats, which may provide a refuge for *Metarhizium* spp. Abundance of *Metarhizium* spp. was significantly higher in semi-natural habitats, i.e., improved field margins and permanent grassland compared to cereal fields. Furthermore, uneven distribution of *Metarhizium* spp. was observed. Assessment of correlations between PCR and cultivation-dependent based data demonstrated the reliability and robustness of the PCR-based tool. The findings illustrated the value of fast and reliable detection and quantification tools for analyzing large numbers of soil samples and for further investigations of factors causing the uneven distribution of *Metarhizium* spp.

The development of GFP-expressing *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) on susceptible and resistant ticks (Acari: Ixodidae)

Dana Ment, Galina Gindin, Alice C. L. Churchill, Asael Rot, Eduard Belausov, Itamar Glazer, Michael Samish 129-132

Abstract: The fungus *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) is an efficient biological control agent of various arthropods, actively penetrating host cuticles via penetration pegs, colonizing the host body, and causing host death. Laboratory assays against various hosts (ticks and insect) have revealed that while some hosts are highly susceptible to fungus, other hosts are resistant to its infection. Results of previous studies of the factors involved in host susceptibility have suggested that cuticular compounds from susceptible and resistant hosts may differentially influence fungus development. This work describes the microscopic development of a GFP-expressing *M. anisopliae* strain on susceptible tick (Acari: Ixodidae), *Rhipicephalus annulatus*, and the resistant tick, *Hyalomma excavatum*. Conidia were observed germinating on all hosts examined. However, the fungus was observed penetrating the cuticles of the susceptible hosts only and never into the resistant hosts. Moreover, growth of germinating conidia and the hyphae that developed from them was inhibited on resistant hosts and died within a few days. Analyzing these observations provides new information on the specificity and importance of each event of the host-pathogen interaction for a successful mycosis.

Fungi Session 3: Formulating BCAs & taxonomy

Encapsulation materials and methods applied to biological insect control

Desirée Jakobs-Schönwandt, Rieke Lohse, Anant V. Patel 135-138

Abstract: There is still little systematic research on formulation materials and methods. Here we will present materials, methods and applications for encapsulation of bioinsecticides. The first

results of a recently granted project on mass-production and encapsulation of an endophytic *Beauveria bassiana* isolate for rape plant protection will be shown.

Furthermore encapsulation of artificial CO₂ sources for attract and kill capsules resulted in beads which released CO₂ under semi field conditions for 15 days and attracted western corn rootworm larvae.

Development of formulations based on *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) for the biological control of *Bemisia tabaci* (Hemiptera: Aleyrodidae)

Erika Paola Grijalba, Mauricio Cruz, María V. Zuluaga, Alba Marina Cotes,

Martha Isabel Gómez 139-143

Abstract: The whitefly *Bemisia tabaci* causes very important losses in more than 500 species of plants such as tomato, cucumber, beans, potato, cotton, melon, peanut, soybean and others (Rodríguez & Cardona, 2001). Two formulations (P1 and P2) designed as wettable powders based on *I. fumosorosea* (Pc013) were developed for the control of *Bemisia tabaci*. For both formulations, P1 and P2, two different conidia harvesting processes were evaluated. After six months of storage at 8°C formulation P1 germination was reduced by 34.35%, while it remained above 85% in formulation P2. With regards to bio-controlling activity P1 showed a lower efficacy (less than 35%), while P2 showed an efficacy above 80%. The higher efficacy and stability of formulation P2 led to the selection of this product.

Studies on the storage of formulated *Metarhizium anisopliae* conidia: effect of temperature on conidia germination and virulence against *Tenebrio* larvae

Sarah Sonderegger, Verena Niedermayr, Hermann Strasser 145-149

Abstract: The entomopathogenic fungus *Metarhizium anisopliae*, applied under field conditions, needs to be formulated to reduce the damage that can be caused, because of (i) UV-radiation, (ii) high temperatures and (iii) contact with water. The aim of our work was to study the longevity and virulence of conidia of *M. anisopliae*, mixed with six different anorganic fillers (Seramis[®], CN- Blähton[®], Liadrain[®], Clever Cat Litter[®], Green Clay[®], TixosilTM). The formulated and unformulated conidia were stored over several time intervals (1 week, 1 month, 3 and 6 months) at different temperatures (25°C, 4°C, -20°C).

No remarkable differences were seen between formulated and unformulated conidia stored at 4°C and -20°C. Formulated conidia stored at 25°C for six months lost hundred percent of their vitality in comparison to unformulated conidia and those, which were stored at colder temperatures. All four expanded clay materials and Green Clay[®] were well suited for the storage of a virulent *M. anisopliae* at 4°C and -20°C. Neither the production methods nor the different expanded clay materials had a negative effect on the virulence of BIPESCO 5 conidia.

Taxonomical and epizootiological studies on the arthropod-pathogenic fungi in Poland and Austria

Cezary Tkaczuk, Stanisław Bałazy, Rudolf Wegensteiner..... 150

Abstract only

Bacterial-type endochitinases as phylogenetic markers for fungal entomopathogens and mycoparasites

Kakhramon Ergashev, Anaida G. Guzalova, Zafar F. Ismailov,

Hans U. Schairer, Andreas Leclerque 151-155

Abstract: Two types of endochitinases referred to as bacterial or B-type and fungal or F-type are known to operate in fungi. While F-type chitinases are generally believed to have morphogenetic functions, B-type chitinases are often associated with pathogenesis in fungal entomopathogens and mycoparasites. Comparative genomics has previously identified at least four fungal B-type chitinase subfamilies termed *chiB1* through *chiB4*. Here we explore the suitability of the *chiB1* gene as a phylogenetic marker for the classification of a particular subgroup of filamentous fungi,

i.e. entomopathogenic and mycoparasitic hyphomycetes, and apply this marker to the classification to a set of fungal isolates from Uzbekistan.

Fungi Session 4: *Beauveria*

In vitro thermal regimes of the entomopathogenic fungi

Beauveria bassiana (Vuill.) Bals. and *Metarhizium anisopliae* (Metch.) Sorok.

Monica Oreste, Eustachio Tarasco, Michele Poliseno, Giovanni Bubici &

Oreste Triggiani 159-162

Abstract: The effect of temperature on the mycelial growth of 28 isolates of the entomopathogenic fungus *Beauveria bassiana* (Vuill.) Balsam (Ascomycota, Hypocreales, Cordycipitaceae) and five of *Metarhizium anisopliae* (Metch.) Sorok. (Ascomycota, Hypocreales, Clavicipitaceae) was evaluated *in vitro* on Sabouraud Dextrose Agar with yeast extract (SDAY). The five temperatures 15, 20, 25, 30 and 35°C were tested, and the fungal radial growth was measured over 15 days at two-day intervals. Radius data were used to calculate the growth rate (mm d⁻¹) per each temperature, isolate and replicate by performing linear regressions, and growth rate data were used for an analysis of covariance. Optimal and maximum temperatures and maximum growth rate were estimated by fitting a modified β function to the growth rate data. The thermal characterization of entomopathogenic fungal candidates for microbiological pest control is essential to assess their applicability in the field. According to the *Bio - Rational* approach, virulence against the target insect should be considered together with the adaptability to the thermal condition in the habitat involved. In particular, tolerance to high temperatures is a prerequisite for the application of entomopathogenic fungi in microbiological pest control in the Mediterranean areas.

Isolation of *Beauveria bassiana* from soil and its horizontal infection

among overwintering Colorado potato beetle – *Leptinotarsa decemlineata*

(Coleoptera, Chrysomelidae)

Medea Burjanadze 163

Abstract only

The action of *Beauveria bassiana* introduced strains on the Colorado potato beetle

Tsisia Chkhubianishvili, Manana Kakhadze, Iatamze Malania, Levan Ninua 165-167

Abstract: The Colorado potato beetle, *Leptinotarsa decemlineata*, is a major pest insect of vegetable crops in Georgia. Key elements of our present investigations are the evaluation of introduced *Beauveria bassiana* strains for Colorado potato beetle management. Results of our laboratory and field experiments demonstrated activity and a perspective for biological control.

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 169-173

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 *ef1 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.

Artificial hideouts with entomopathogenic fungi: A strategy for biological control of the plum fruit moth *Cydia funebrana*?

Dietrich Stephan, Melanie Herker 175-178

Abstract: Especially in organic agriculture, *Cydia funebrana* (Plum fruit moth) is a serious pest of plum fruits. Therefore, we investigated whether the insect can be controlled by artificial hideouts treated with conidia of entomopathogenic fungi. Because we were not able to establish a mass rearing of *C. funebrana* we did additional experiments with the related species *Cydia pomonella*, *Cydia molesta* and *Eupoecilia ambiguella*. In previous experiments we proofed that corrugated cardboard and bark mulch substrates were accepted for pupation. Additionally, we investigated the effect of water and oil based formulation of conidia of *Beauveria bassiana*. The presented data demonstrate that vegetable oils have a dramatic effect on larvae and oil-based formulations can improve the efficacy of entomopathogenic fungi. In case of *C. pomonella* a mortality of 46% and 92% was determined for Tween80 and sunflower oil, respectively. The addition of *Beauveria bassiana* conidia did not enhance the mortality but even when low concentrations of conidia (10^4 conidia/ml) were applied in oil, 90% mycosis was achieved. In contrast high concentrations of conidia were needed to achieve at least 70% mycosis when formulated in water containing Tween 80. In further experiments we compared the efficacy of *B. bassiana* conidia formulated in sunflower and rape oil. The results demonstrate that both oils caused a high larval mortality and that the mycosis of the larvae was significantly higher when conidia were formulated in rape oil. As we did not achieve high mortality with *B. bassiana*, we tested other entomopathogenic fungi like *Lecanicillium lecanii*, *Isaria fumosorosea* and *Metarhizium anisopliae*. *M. anisopliae* and *I. fumosorosea* caused the highest mortality to all moth species. Furthermore, *C. pomonella* seems to be more sensitive to entomopathogenic fungi than *C. funebrana*. In additional experiments we investigated the persistence of *M. anisopliae* formulated in oil or water and two *I. fumosorosea* isolates formulated only in water on chipped wood under natural conditions. The results demonstrate that the oil itself maintained its efficacy over the observed time of two months. Furthermore, the efficacy of the three tested isolated in the water based formulation was influenced by the weather condition. Better results were obtained with *I. fumosorosea* compared to *M. anisopliae*.

Efficacy of *Beauveria brongniartii* as Melocont in the control of the European cockchafer (*Melolontha melolontha*)

Barbara H. Łabanowska, Halina Bednarek 179-182

Abstract: The European cockchafer is a dangerous soil pest of strawberry, sour cherry and apple trees as well as other fruit orchards in some regions of Poland. The first experiments concerning the control of white grubs of *Melolontha melolontha* using the fungus *Beauveria brongniartii* (product Melocont) were conducted in 2009-2010 at the Research Institute of Pomology and Floriculture in Skierniewice. The fungus *B. brongniartii* applied at the end of May 2009 to bare soil (without growing plants), in young plum orchards and in a nursery on a field with *Spiraea japonica* considerably reduced the number of pest insects. The best results were obtained with Melocont on a field with white mustard (2009) when *B. brongniartii* was applied at the rate of 50kg/ha at the end of May and in the following year (spring 2010) at the lower rate of 30kg/ha.

Poster Fungi

Isolation and characterisation of entomopathogenic fungi from phylloplanes and soil of different Spanish ecosystems, and insecticidal activity of their fungal extracts

Inmaculada Garrido-Jurado, María Fernández-Bravo, Enrique Quesada-Moraga..... 185

Abstract only

Entomopathogenic fungal infections of hibernating pupae of horse chestnut moth
Cameraria ohridella Deschka & Dimic
Alicja Sierpinska , Katarzyna Kubiak 186
Abstract only

Determination of mortality factors of *Bemisia tabaci* in cotton with
Lecanicillium lecanii applications for its control
María Victoria Zuluaga, Edgar Herney Varón, Camilo Piedrahita,
Martha Isabel Gómez 187-190

Abstract: The effect of a *Lecanicillium lecanii* based biological control product on *Bemisia tabaci* was assessed at a field managed according to Integrated Crop Management rules (ICM) field and farmer managed fields in the Tolima department of Colombia. An instar dependent survival was seen for N4 nymphs in the farmer managed field, in which chemical insecticides were used, while the ICM field showed an N4 survival of 16%. Mortality was mostly related with the activity of *L. lecanii*, parasitoids and predators.

Potential of *Aphidius colemani* as a vector of entomopathogenic fungi
Lecanicillium spp.
Daigo Aiuchi, Yuuna Saitou, Junya Tone, Masanori Koike 191-194

Abstract: The ability of *Aphidius colemani* to vector *Lecanicillium* spp. conidia during host searching and/or oviposition to a colony of uninfected *Aphis gossypii*, and the ability of *A. colemani* to discriminate *Lecanicillium* spp. infected aphid from uninfected individual was demonstrated. In bioassays for discrimination ability of *A. colemani* against fungal infected aphid, there were no significant difference between control plot and *Lecanicillium* spp. treated plots on the number of ovipositional behaviours. Only fungus-infected aphids covered with mycelia were avoided as host insect for oviposition. Results indicate that *A. colemani* does not have the ability for discriminate between healthy and *Lecanicillium* spp. infected aphids for a period of up to 48h after infection. In bioassays to assess the fungal vectoring ability of *A. colemani* to aphid population, the aphid population increased ca. 26 times in control plot during 10 days. In contrast, aphid population did not increase in plots with *A. colemani* release up to 8 days after parasitoid release and then exponentially increase in 10 days. In *Lecanicillium* treated parasitoid release plots, aphid density was maintained at low level up to 10 days after parasitoid release. Considering that the number of mummies of *A. gossypii* was at the same level in parasitoid and parasitoid + fungi plots, the decrease of the aphid population density might have been an effect of *Lecanicillium* vectored by *A. colemani*. In this study, it was revealed that *Lecanicillium* spp. can act additively to control the aphid by *A. colemani* vectoring.

Biological control of greenhouse whitefly, *Trialeurodes vaporariorum* by
entomopathogenic fungus *Lecanicillium muscarium* hybrid strain 2aF43
in greenhouse
Hiroto Shinomiya, Daigo Aiuchi, Willem J. Ravensberg, Masayuki Tani,
Masanori Koike 195-198

Abstract: The *Lecanicillium muscarium* hybrid strain 2aF43-formulation (average of conidial concentration 1.7×10^4 spore ml^{-1}), which was obtained by protoplast fusion and shown to have a high control potential *in vitro*, and the Mycotal[®]-formulation (3.0×10^6 spore ml^{-1}), which is a commercial biological agent with a high virulence against greenhouse whiteflies on tomato plants in greenhouses, was investigated. On untreated tomato plants, the number of adult whiteflies significantly increased to 354 adults per leaf during 8 weeks. In 2aF43-formulation and Mycotal[®]-formulation plots, adult density remained at low levels (0.6 to 15.9 adults per leaf and 0.5 to 11.3 adults per leaf, respectively) for 7 weeks. Evidence suggests that the hybrid strain 2aF43-formulation, which has a substantially lower conidial concentration than the Mycotal[®]-formulation, has the potential for controlling early emergence of greenhouse whiteflies and the possibility for long term effects in greenhouse use.

The influence of white light exposition on the growth, sporulation and pathogenicity of entomopathogenic fungi *Isaria fumosorosea* and *Beauveria bassiana*
Tomasz Kuźniar 199-203

Abstract: The aim of research was to evaluate the influence of white light on the growth, sporulation and pathogenicity of entomopathogenic fungi *Isaria fumosorosea* and *Beauveria bassiana*. The study indicated that white light was an important factor, affecting the sporulation of entomopathogenic fungi. Fungi grown in total darkness sporulated very faintly. However the fungi which were irradiated during the period of growth, showed considerably better ability to produce spores. The highest production of spores was achieved when fungi were grown in total darkness for the first three days, and next under exposure to white light. The exposure of mycelium to white light did not influence the germination ability and pathogenicity of harvested spores against test insects.

Beauveria bassiana on overwintering adults of *Stereonychus fraxini* in Croatia
Milan Pernek, Nikola Lackovic 205-207

Abstract: The ash weevil, *Stereonychus fraxini*, occurred in high infestation levels in 2009 causing serious damage on different ash species in lowland forests in Lipovljani, Croatia. Overwintering adults in moos on the bottom of trees showed high mortality rates (over 80%). Most of these beetles were infected with the fungus *Beauveria bassiana*, which reduced the population density of adults by 53.7%. The naturally occurring strain was isolated and tested on overwintering individuals under laboratory conditions. Laboratory experiments demonstrated that this strain was very virulent on adult weevils. Due to restrictions in use of insecticides in Croatian forests, biological control using *B. bassiana*, is a promising option.

Infecting *Ips typographus* (Coleoptera, Curculionidae) with *Beauveria bassiana*,
Metarhizium anisopliae or *Isaria fumosorosea* (Ascomycota)
Florian Herrmann, Rudolf Wegensteiner 209-212

Abstract: Adult *Ips typographus* were inoculated with *Beauveria bassiana*, *Metarhizium anisopliae* or *Isaria fumosorosea* alternatively by dipping the beetles directly into conidiospore suspensions or by forcing them to walk on spore suspension treated spruce bark. Effect of temperature was tested by incubating experiments at 15°C, 20°C or 25°C.

Direct inoculation was successful with all three fungal species tested. Infection rates varied between the three fungal species (75.7%-8.1%) depending on incubation temperature. Infection rate was significantly higher only with *B. bassiana* at 20°C compared to the other two fungus species. Mean life span was prolonged at 15° and shortest at 25°C with all three fungus species.

Direct inoculation caused always higher infection rates and shorter mean life span compared to indirect inoculation with all three fungal species. Limited contact with suspension treated bark resulted in successful infection, but duration of beetles' contact with the conidia on bark influenced the infection success. Infection was highest when beetles had contact with freshly suspension treated bark for three minutes with *B. bassiana* and *I. fumosorosea* at 20°C and for five minutes with *M. anisopliae* at 25°C. Infection rates were significantly lower in most cases after shorter contact with treated bark compare to shorter contact or testing beetles after one week incubation of log sections compared to freshly treated bark.

Strain-specific PCR-based diagnosis for *Beauveria brongniartii* biocontrol strains
Ana-Cristina Fatu, Viorel Fatu, Ana-Maria Andrei, Constantin Ciornei,
Daniela Lupastean, Andreas Leclerque 213-216

Abstract: A Romanian *Beauveria brongniartii* biocontrol isolate, strain ICDPP #1a, was genetically compared to the producer strain of the commercially available mycoinsecticide "Melocont®". Analysis of 18S rRNA gene and elongation factor 1 alpha (EF1α) peptide sequences revealed a very close phylogenetic relationship of both strains even in comparison to further *B. brongniartii* strains. However, a difference in the respective 18S rRNA gene exon-intron structures was detected. Based upon this genetic difference, a PCR-based diagnosis tool was developed that renders the two-sided positive discrimination of the strains possible.

Morphological and genetic diversity among Polish isolates of *Beauveria bassiana*
Julia Budziszewska, Marta Wrzosek, Cezary Tkaczuk, Joanna Matuska-Łyżwa 217
Abstract only

Use of mycelium overgrown corn in biological control after producing the
entomopathogenic fungus *Lecanicillium muscarium* on wheat
Sandra Lerche, Helga Sermann, Kathleen Schmidt, Martin Tschirner,
Carmen Büttner 218
Abstract only

Colonization of glacier foreland soils with entomopathogenic fungi
Mirjam Zoderer, Kerstin Hashold, Sigrid Neuhauser, Martin Kirchmair 219-223

Abstract: Since mid of the 19th century the Alpine glaciers are retreating continuously. The "Rotmoos" valley glacier foreland (Tyrolean Central Alps) is 2km long with a chronosequence of developmental stages from recently deglaciated terrain to sites which have been ice-free for 145 years at the terminal moraine dated 1858. Due to the gradual retreat of the glaciers we can now observe all age stages of community assembly within a limited spatial scale. Such chronosequences represent natural experiments showing the sensitivity and the resilience of alpine ecosystems exposed to changing environmental conditions. In this study the colonization of entomopathogenic fungi in young soils was investigated. Soil samples were taken in spring summer and autumn at eleven sites at the glacier foreland. The abundance and density of entomopathogenic fungi in soil were determined. Fungal isolates were identified morphologically. Entomopathogenic fungi (*Beauveria bassiana*, *Isaria farinosa*, *Hirsutella sp.*, *Syngliocladium sp.*, the anamorph of *Cordyceps militaris*) as well as fungi recognized as weak entomopathogens (*Paecilomyces carneus* and *P. marquandii*) could be detected along the chronosequence. In the first years after deglaciation, soils are sporadically "contaminated" with anemochorically distributed entomopathogenic fungi. In fifty year old soils entomopathogens are frequent and diverse, similar to their possible hosts (collembolans, larvae of midges, mites etc.), that can be found in higher abundances at older soils.

Beauveria brongniartii in the protection of forest plantations and nurseries
against white grubs of *Melolontha* spp. in Poland
Alicja Sierpinska, Halina Bednarek 224
Abstract only

Integrated control of *Melolontha melolontha* L. in Romanian forest nurseries
Constantin Ciornei, Ana-Maria Andrei, Daniela Lupăștean 225-228

Abstract: The research was conducted in 2008-2010 in forest nurseries in eastern Romania, infested with larvae of *Melolontha melolontha* L. Biological product Melocont Pilzgerste based on *Beauveria brongniartii* (Sacc) Petch has been used. It was applied in different doses (40, 50, 60kg/ha), single or in combination with sublethal doses of chemical insecticides. The treatment effect was positive, the mortality recorded by direct action of the entomogenous fungus reached values up to 75% and the density of larvae in the soil in the third year implementation was considerably reduced.

Impact of Harpellales (Kickxellomycotina, Zygomycota) on Chironomidae larvae
(Diptera, Insecta) in alpine streams
Gerald Andre, Leopold Füreder, Reinhold Pöder 229-232

Abstract: Harpellales are placed within the Kickxellomycotina, Zygomycota. Like some other orders within the Zygomycota (*e. g.*, Entomophthorales, Zoopagales, Asellariales), Harpellales strongly interact with animal hosts, but it is not yet fully understood whether or not an infestation with these fungi is beneficial or disadvantageous for their host. Literature holds evidence that the nature of symbiosis with Harpellales depends on variables like specific host-fungus affiliations, the physiological status of the host, and its developmental stage

This study is focused on the impact of Harpellales on Chironomidae (Diptera, Insecta) in alpine streams, and particularly aims at evaluating potential benefits or disadvantages of this symbiosis in alpine streams, which represent harsh and hostile habitats.

Genetic diversity among strains of the entomopathogenic fungus, *Beauveria* ssp. as revealed by RAPD markers

A. Imoulan and A. El Meziane 233-236

Abstract: The genetic relatedness of *Beauveria* isolates collected from Morocco soil at different geographic origin was determined by comparison of the products of polymerase chain reaction amplification of genomic DNA with single arbitrary sequence oligonucleotide primers. *Beauveria* isolates have a high genetic variability and they were clearly distinguished from references isolates including *B. bassiana* and *B. brongniartii*. RAPD banding patterns did not, however, revealed a potential relatedness with geographical origin.

Assessment of oxalate and oosporein accumulation by endophytic

Beauveria bassiana isolates

Hermann Strasser, Pamela Vrabl, Lukas Tartarotti 237-240

Abstract: *Beauveria bassiana* secretes a broad range of low molecular weight metabolites, some which are referred as relevant toxins. In submerged culture five selected endophytic *B. bassiana* isolates excreted more than 20mmol l⁻¹ oxalate but not the secondary metabolite oosporein. Compared to the quantities of oxalate normally detected in plant tissues or in the environment it can be concluded, that there are no risk to humans, plants and the environment.

Potential of the strain of entomopathogenic fungus *Isaria fumosorosea* CCM 8367 as a biological control agent against Colorado potato beetle

Hany M. Hussein, Rostislav Zemek, Eva Prenerova 241-244

Abstract: *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) (WIZE) Brown & Smith (Deuteromycota) is potentially useful for the biological control of economically important agricultural and forest insect pests. Selection of effective, highly virulent strains is a prerequisite for development of successful biological control agents. Our strain CCM 8367 of *I. fumosorosea* was isolated from a lepidopteran pest. The aim of our study was to evaluate the effects of this strain on larvae and pupae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). In addition, we compared it with the effects of *I. fumosorosea* strain isolated from the commercial product PreFeRal[®] WG (Biobest, Belgium; *I. fumosorosea* strain Apopka 97 as an active ingredient). Obtained results revealed high virulence of CCM 8367 blastospores to the late last instar larvae, prepupae and pupae of *L. decemlineata*. Obvious differences in virulence between our strain and the strain isolated from PreFeRal[®] WG were found using fresh blastospores, where corrected mortality of CCM 8367-treated larvae eight days after the treatment was 96.5% while mortality in Apopka 97-treated larvae reached only 54.4%. We can conclude that the strain *I. fumosorosea* CCM 8367 has strong insecticidal effects on *L. decemlineata* and has a potential to be implemented as a novel biocontrol agent.

Effects of *Beauveria bassiana* on *Frankliniella occidentalis*

(Thysanoptera: Thripidae) through different routes of exposure

Andrea Boaria, Letizia Rossignolo, Alberto Pozzebon, Carlo Duso245-248

Abstract: *Beauveria bassiana* is an entomopathogenic fungus widely used in biocontrol tactics over the world. Its potential has been tested on several pest species, included the Western Flower Thrips *Frankliniella occidentalis* Pergande. However, knowledge on *B. bassiana*-thrips interactions is limited. In laboratory bioassays, we exposed different developmental stages of *F. occidentalis* (first and second instar larvae, adults) to residual or topical applications of a *B. bassiana* commercial strain (strain ATCC 74040, Naturalis[®]). A moderate to high mortality was noticed for first instar larvae, late second instar larvae and adults when the two routes of exposure were combined. The results stress on the importance to favor the contact of thrips with *B. bassiana* to obtain satisfactory control of this pests. The significant reduction in thrips survival after *B. bassiana* applications suggests that the latter can be included in IPM tactics.

The influence of the larval food plants on microsporidia (*Nosema mesnili* P.) infection in diapausing *Pieris brassicae* L. pupae
Katrin Jõgar, Luule Metspalu, Külli Hiisaar, Angela Poomi, Marika Mänd, Irja Kivimägi, Tea Tasa and Anne Luik 249-253

Abstract: The Large White Butterfly, *Pieris brassicae* L., is a cosmopolitan insect, and is found wherever cruciferous plants are grown. The *P. brassicae* is one of the most important pest insects in cruciferous plants of Estonia. The experiments were carried out in the experimental laboratory of the Estonian University of Life Sciences. In our experiment six foodplants were used: white cabbage (*Brassicae oleracea* L. var. *capitata* f. *alba*), german turnip (*B. oleracea* var. *gongylodes* f. *gongylodes*), ornamental cabbage (*B. oleracea* var. *acephala*), broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*) and Garden Nasturtium (*Tropaeolum majus* L.). The aim of the present work was to investigate the effect of larval food plants on natural infection by microsporidia (*Nosema mesnili* Paillet) in diapausing *Pieris brassicae* L. pupae and to assess the influence on hibernation of *P. brassicae* pupae. Physiological state of *P. brassicae* pupae was estimated by measuring standard metabolic rate (SMR). The infection by *N. mesnili* was determined by microscopic analysis after SMR.

A statistical analysis of the results indicated that infection by *N. mesnili* of *P. brassicae* pupae was the lowest on white cabbage. Infection was highest on german turnip and garden nasturtium. The experiments showed that less pupae were infected by *N. mesnili* in ornamental cabbage, broccoli and cauliflower variants. Healthy pupae on nasturtium plants had a higher SMR rate than on white cabbage. Comparing infected and healthy pupae in white cabbage and garden nasturtium variants revealed that the value of SMR was about three times higher in both infected variants. The higher metabolic rate of infected *P. brassicae* pupae indicated that those pupae would not have been able to survive winter conditions.

Biological control of greenhouse whitefly, *Trialeurodes vaporariorum* by entomopathogenic fungus *Lecanicillium* spp. hybrid strain in greenhouse
Daigo Aiuchi, Sayaka Horie, Toshihiro Watanabe, Satoshi Yamanaka and Masanori Koike 255-258

Abstract: The utility of *Lecanicillium* spp. hybrid strain 2aF43 which shown to have high control potential at *in vitro*, to control greenhouse whitefly on tomato and cucumber foliage in greenhouse were investigated. The fungal epiphytic ability on the leaf surface was simultaneously evaluated. On tomato foliage, the number of adult whitefly in a control plot significantly increased to 130 adults/leaf during 12 weeks. Whereas in 2aF43 and Mycotol-formulation plots, adult density was remained extremely low level (0 to 2 adults/leaf) for 10 weeks. In addition, on cucumber foliage, both fungal strains application resulted in lower density of adults (under 2 adults/leaf) compared to control plot for 5 weeks. The density of 2aF43 propagules on both plant foliages was significantly higher than formulated Mycotol. Especially, 2aF43 was detected in high density (6.6×10^4 cfu/cm²) on tomato foliage, indicating possibility that not only persisting, this strain also growing on foliage under given conditions. Evidence suggested that hybrid strain 2aF43 has the potential for controlling early occurrence of greenhouse whitefly and the possibility for long term effect in greenhouse use.

Nematodes

Nematodes Session 1: Use against insects

Are entomopathogenic nematodes effective biological control agents against the cereal leaf beetle (*Oulema melanopus* [L.]?)
Žiga Laznik, Tímea Tóth, Tamás Lakatos, Matej Vidrih, Filip Vučajnk, Stanislav Trdan 261-265

Abstract: The efficacy of entomopathogenic nematodes (EPN) in controlling *Oulema melanopus* on winter wheat was tested in a field experiment. A Slovenian strain of *Steinernema carpocapsae*

(C101) was compared with the commercial product NemasysC and the insecticide thiametoxam. The highest yield was obtained when thiametoxam was used ($7.3 \pm 0.13t \text{ ha}^{-1}$), while the lowest yield was in the control treatment ($5.6 \pm 0.16t \text{ ha}^{-1}$). In treatments with EPN we determined statistically significant differences between both strains. Weaker response was recorded for the commercial strain NemasysC (low nematode concentration: $5.96 \pm 0.05t \text{ ha}^{-1}$; high conc.: $6.1 \pm 0.15t \text{ ha}^{-1}$), while the Slovenian strain provided higher results (low conc.: $6.6 \pm 0.14t \text{ ha}^{-1}$; high conc.: $6.8 \pm 0.2t \text{ ha}^{-1}$). In the field experiment different EPN concentration did not result in statistically significant differences in wheat yield. Observing the population dynamics of the pest, it has become apparent that EPN are effective biological agent in controlling cereal leaf beetle.

Efficacy of entomopathogenic nematodes against the larvae and adults of the tomato leafminer *Tuta absoluta* in soil treatments and their compatibility with the insecticides used against this insect

Fernando Garcia-del-Pino, Xavier Alabern and Ana Morton 267-270

Abstract: The efficacy of soil treatments of three species of entomopathogenic nematodes (EPN) (*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*) against *Tuta absoluta* larvae and adults was examined under laboratory conditions. The effect of some insecticides used against *T. absoluta* in the survival, infectivity and reproduction of these native species of EPNs was also evaluated.

Soil application of EPNs gave a high control of larvae when falling into the soil for pupation: 52.3%, 100%, and 96.7% efficacy was recorded for *S. feltiae*, *S. carpocapsae* and *H. bacteriophora*, respectively. The mortality of the adults emerging from soil was 0.5% for *S. feltiae* and 79.1% for *S. carpocapsae*. The insecticides tested have a negligible or moderate effect on EPN survival, infectivity and reproduction. For all insecticide treatments and exposure times, *H. bacteriophora* was more sensitive than *S. feltiae* and *S. carpocapsae*. Nematodes that survived were able to infect *G. mellonella* larvae with no significant difference from the control. The larvae affected by the insecticides served as suitable hosts for the infection and reproduction of the nematodes. These results suggest that the larvae of *T. absoluta* falling from leaves following insecticide application could be a suitable host for nematodes, thereby increasing their concentration and persistence in the soil. This study demonstrates the suitability of soil application of EPN for controlling *T. absoluta*.

Attraction of *Heterorhabditis* sp. toward synthetic (E)- β -caryophyllene, a plant SOS signal emitted by maize on feeding by larvae of *Diabrotica virgifera virgifera*

Samuel Anbesse, Ralf-Udo Ehlers 271-274

Abstract: Most plants when damaged by herbivore insects, synthesize and release indirect defense mechanism by release of various chemicals that attract parasitic or predatory insects, which are natural enemies of the herbivores. When attacked by Western corn rootworm, roots of many maize plant varieties emit (E)- β -caryophyllene that attracts the neighboring entomopathogenic nematodes to kill the feeding pest. Through plant genetics and biotechnology it was possible to manipulate this volatile compound in order to increase the effectiveness of entomopathogenic nematodes in reducing the damage of the pest. In order to further use this strategy to improve the effectiveness of *Heterorhabditis bacteriophora*, we investigate the applicability of the strategy to be used for genetic selection of enhance host finding and tested different standard laboratory bioassays using three different sand and one agar plate assay. Synthetic (E)- β -caryophyllene and *H. megidis* (the strain which showed in previous investigation, significant attraction to caryophyllene) were used. In all the bioassays tested no significant difference were observed between the treatment and control.

Entomoparasitic nematodes for control of the Western corn rootworm,

Diabrotica virgifera virgifera

Christina Pilz, Udo Heimbach, Giselher Grabenweger 275-279

Abstract: Entomoparasitic nematodes of the species *Heterorhabditis bacteriophora*, highly virulent against larval stages of the Western corn rootworm in laboratory studies, were tested in field experiments in the south-eastern part of Austria. *Diabrotica virgifera virgifera* was first recorded at this field site in 2002, since then high population densities developed in fields with continuous corn. Nematodes were applied to small field plots in two different formulations, suspended in water and as granules without water, and tested against an untreated control and Clothianidin coated seeds in five replicates. Maize plants were artificially infested with *D. v. virgifera* eggs to guarantee a homogenous pest population. Treatments were evaluated by (a) recording the emergence of adults in cages and (b) rating the damage of maize roots. Entomoparasitic nematodes significantly reduced the emergence of adult corn rootworms from treated plots, regardless of formulation. They were more efficient than the treatment of maize seeds with Clothianidin. However, nematode treatments could not prevent root damage, whereas treatment of seeds with Clothianidin was able to keep root damage below the economic threshold. Reasons for these contradictory results are discussed.

Steinernema feltiae to control Western flower thrips (*Frankliniella occidentalis*) in potted chrysanthemum

Andrew P. Brown, Julie Graesch, Mark Howieson 281-284

Abstract: Entomopathogenic nematodes such as a *Steinernema feltiae* are used in a variety of commercial growing systems to control a number of pests as part of an integrated pest management program. One such use is *S. feltiae* against Western flower thrips (*Frankliniella occidentalis*, WFT). Against this pest growers are using both foliar and soil applications to target the different susceptible lifestages. Two field trials were carried out to investigate the efficacy of regular foliar applications of a commercial formulation of *S. feltiae* (Nemasys[®] F, Becker Underwood) against WFT in potted chrysanthemum compared to a standard chemical and alternative bicontrol *Beauveria bassiana* strain GHA (BotaniGard, Bioworks).

Survey of nematodes associated with terrestrial slugs in the Western Cape Province of South Africa

Jenna L. Ross, Elena S. Ivanova, Willem F. Sirgel, Antoinette P. Malan,

Michael J. Wilsona 285-288

Abstract: A survey of slug-parasitic nematodes was conducted in the Western Cape Province of South Africa, in order to gather new data regarding diversity and distribution. 521 slugs were collected from 35 sample sites throughout the Western Cape. Slugs were dissected and examined for the presence of slug-parasitic nematodes. Extracted nematodes were identified using molecular (18S rRNA gene sequencing) and morphological techniques. Nematodes were found parasitizing slugs at 40% of sample sites and 6% of slugs were infected with nematodes. A total of seven species of nematode were identified in the province including; *Agfa flexilis*, *Angiostoma* sp., *Phasmarhabditis* sp. SA1, *Phasmarhabditis* sp. SA2, *Caenorhabditis elegans*, panagrolaimid sp. and *Rhabditis* sp. Of these species, four were slug-parasitic nematodes (*A. flexilis*, *Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2), as opposed to nematodes that form necromenic or phoretic associations. Three new species of slug-parasitic nematode were identified during this study (*Angiostoma* sp. (named as *Angiostoma margaretae*), *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2).

Nematodes Session 2: Genetics and ecology

Bioinsecticidal ability of virulence factors released by *Steinernema carpocapsae*

Nelson Simões, Duarte Toubarro, Natesan Balasubramanian,
Gisela Nascimento, Mónica Martínez, Yingjun Jing, You-Jin Hao,
Rafael Montiel

291-294

Abstract: *Steinernema carpocapsae* is a biological control agent largely used to control insects. Laboratorial and field assays evidenced the diversity of beneficial traits of this organism namely virulence. The analysis of transcripts and proteins in the excreted secreted products of the parasitic nematode allowed to the identification of genes and proteins participating in invasion, evasion and host death. Some of these genes present specific features that support its use in the genetic improvement of the nematode, in the improvement of *Bacillus thuringiensis* in combination with other insecticidal genes, and in plant transformation.

Transcriptome analysis of desiccation and heat tolerance of entomopathogenic nematodes

Yaari Mor, Koltai, Hinanit, Salame, Liora, Glazer, Itamar

295-300

Abstract: Under natural conditions entomopathogenic nematodes (EPNs) are exposed to extreme environmental stresses such as desiccation and heat. Only little is known on the molecular mechanism of stress tolerance in EPNs. Since an adaptation period is needed for acquisition of the anhydrobiotic state and survival at high temperatures, we have focused in our research on the adaptation period of heat and desiccation stresses, affecting nematode survival. We have characterized the heat and desiccation tolerance of selected *Steinernema* species and performed a whole transcriptome analysis. Our research is expected to provide a better understanding of the biological pathways and molecular mechanisms of EPNs heat and desiccation stress response. This is the first report of the next generation sequencing of EPNs.

Development of heat and desiccation tolerance of genetically selected hybrid strains of *Heterorhabditis bacteriophora* during in vivo and in vitro propagation with and without selection pressure

Samuel Anbesse, Nanette Hope Sumaya, Verena Dörfler, Ralf-Udo Ehlers

301-304

Abstract: Repeated culturing of entomopathogenic nematodes under laboratory or industrial scale can lead to deterioration of beneficial traits. Assessment of the development of heat and desiccation tolerance was performed on heat and desiccation tolerant hybrid strains of *Heterorhabditis bacteriophora*. The test was done in *in vivo* and *in vitro* production with or without selection pressure for heat and desiccation. Tolerance to heat and desiccation stress was usually lower when nematodes were propagated without selection pressure but were more pronounced when nematodes had been propagated *in vivo* compared to *in vitro* cultivation.

Desiccation tolerance among different isolates of the entomopathogenic nematode

Steinernema feltiae (Filipjev)

Prakijan Nimkingrat, Ralf-Udo Ehlers

305-308

Abstract: Poor storage capacity is a major constraint limiting further expansion of the use of entomopathogenic nematodes. In order to prolong shelf life, a quiescent state of the dauer juveniles (DJs) should be induced. This can be attained by means of desiccation of DJs. In this study, 24 natural isolations of *Steinernema feltiae* were exposed to desiccation stress in non-ionic polyethylene glycol 600. The dehydrating conditions were measured as water activity, a_w -value. Non-adapted and adapted DJs were tested separately under a series of dehydrating conditions. The mean tolerated a_w -value (MW_{50}) ranged from 0.85 for the isolate NEP1 to 0.95 for FIN1, ISR5 and PAL4 when not adapted to desiccation stress and from MW_{50} of 0.822 for CR1 to 0.98 for ISR6 when adapted to the stress conditions. CR1 tolerated the lowest desiccation stress at an a_w -value for the most tolerant 10% of the population (MW_{10}) at 0.65 when DJs had been adapted to stress. No significant differences were recorded between all isolates in non-adapted DJs populations MW_{10} was compared. No correlation between tolerance under non-adapted and

adapted conditions were found. Most tolerant isolates will now be used for cross-breeding and subsequent genetic selection to enhance desiccation tolerance.

Molecular phylogeny of *Heterorhabditis* and *Steinernema* and their symbiotic bacteria.

What is true and what is wrong: impact on the evolutionary history of these organisms?

Jean-Claude Ogier, Sylvie Pagès, Patrick Tailliez 309-310

Abstract: By associating sequences available in databases and those obtained from the entomopathogenic nematodes preserved in our own collection at INRA Montpellier, we obtained new insights into the phylogeny of *Heterorhabditis* and *Steinernema* and highlighted, by tree-topology reconciliation analyses, that *Heterorhabditis-Photorhabdus* and *Steinernema-Xenorhabdus* have evolved using different events including cospeciations; sortings and host switches.

Nematodes Session 3: Efficacy & biology

Beneficial trait stability in entomopathogenic nematodes

David Shapiro-Ilan 313-316

Abstract: A number of beneficial traits such as virulence, reproductive potential, and environmental tolerance are key factors in determining an organism's ability to produce high levels of efficacy in biological control. Deterioration or loss of beneficial traits during laboratory or industrial culture production is detrimental to biocontrol efficacy. During in vivo production, both partners in the nematode-bacterium complex can undergo change, which contributes to reduction in beneficial traits. The nematode's bacterial symbiont also deteriorates when repeatedly cultured in vitro. Changes in nematode beneficial traits may be due to genetic or non-genetic (e.g., nutritional) causes. Methods to deter trait deterioration include maintenance or improvement of media, creation of selected inbred lines, or improved cryopreservation techniques.

A review of the use of entomopathogenic nematodes for the control of *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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

Phil Northing 317-320

No abstract

Bacterial symbionts of entomopathogenic nematodes of the genus *Steinernema* from South Italy: phenotypic features and evolutionary implications

Giancarlo Rappazzo, Carla Avanzato, Giulio Petronio Petronio,

Mirella Clausi, Eustachio Tarasco, Maria Antonietta Buccheri,

Pio Maria Furneri, Maria Cristina Scuderi, Maria Teresa Vinciguerra 321-325

Abstract: Three strains of *Xenorhabdus kozodoii* and seven of *X. bovienii*, obtained from different species and isolates of the genus *Steinernema* (*S. feltiae*, *S. vulcanicum*, *S. kraussei*, *S. apuliae* and *S. ichnusae*), were analysed by genotypic and phenotypic assays in order to investigate in vitro biofilm production, antibiotics susceptibility and growth properties. For identification purposes we used a genotypic analysis, such as a 16S rDNA partial sequence and ERIC PCR profile. Phenotypic profiles were obtained by testing, among others: growth on selective media, haemolytic activity, β -galactosidase. Moreover, bacterial toxin was isolated and tested in vivo on insect larvae upon direct injection into the haemolymph. The results indicate that *X. bovienii* isolates present a broad phenotypic spectrum, particularly for biofilm production and cephalosporin susceptibility, suggesting that this species underwent adaptive changes, presumably following the evolutionary divergence of *Steinernema* species or isolates. *X. kozodoii* diverges from *X. bovienii* for lower levels of biofilm production and for lactose utilisation.

However, both species synthesized a highly active toxin, which killed larvae in a manner resembling direct infection by EPN.

Susceptibility of larvae and adults of *Leptinotarsa decemlineata* to entomopathogenic nematodes

Arne Peters, Tomke Appel 327-330

Abstract: Entomopathogenic nematodes of the species *Steinernema carpocapsae*, *S. feltiae* and *H. bacteriophora* were evaluated for their efficacy in killing larvae and adults of Colorado potato beetles. In laboratory assays on potato leaves, *S. carpocapsae* proved to be the most virulent species against the larvae. On whole plant assays in a climate chamber the efficacy was significantly enhanced by a surfactant-humectant formulation while the formulation alone resulted in a non-significant increase in larval mortality compared to the untreated control. Adults beetles could not be controlled by application of nematodes to the soil but were killed after being in contact with *S. carpocapsae* in a gel-formulation for as little as 30 minutes.

Nematode infections are affected by insect clotting system

Pavel Hyrs, Pavel Dobes, Zhi Wang, Ulrich Theopold 331

Abstract only

Biocontrol of leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae) using entomopathogenic nematodes in Iran

Mahbobeh Ashtari, Javad Karimi, Mohammad Reza Rezapanah and Mahnaz Hassani-kakhki 333-335

Abstract: Walnuts produced in the *Juglans* region is among Iran's strategic products and the Leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae) is the key pest of this tree. Difficulty in chemical application against this caterpillar and special habitats of its larvae encouraged us to address efficiency of some entomopathogenic nematodes (EPNs) against different larval stages. Native isolates of EPNs were isolated from soil habitats of this pest in Arak, Markazi province, Iran. Using ITS and D2/D3 expansions of 28S sequences they were identified as *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *S. carpocapsae*. In laboratory assays, the susceptibility of second, third and fourth larval stages to commercial products of *S. carpocapsae* and *H. bacteriophora*, and native strain of *H. bacteriophora* were studied. For field application, both strains were used via injection of nematode suspensions into the galleries bored in tree stems or branches. This study showed that both tested nematodes at 2,000 IJs/larva proved to be effective on *Z. pyrina*. The results indicated the higher efficiency of *S. carpocapsae* as compared to *H. bacteriophora* on larvae of this moth.

Poster nematodes

Effect of the timing of application on efficacy of entomopathogenic nematodes in control of *Hylobius abietis* (L.)

Iwona Skrzecz, Elżbieta Pezowicz, Dorota Tumialis 339-342

Abstract: The large pine weevil (*Hylobius abietis*) is a pest of great economic importance, damaging young coniferous plantations. In search for new methods to reduce *H. abietis* populations, the experiments were conducted aiming at the evaluation of preferred timing of application (June or September) of entomopathogenic nematodes to decrease the number of pest larvae developing in Scots pine (*Pinus sylvestris*) stumps. The treatment with *Steinernema carpocapsae* and *Heterorhabditis downesi* at a rate 3 million specimen/stump against *H. abietis* larvae in *P. sylvestris* stumps applied in June indicated low efficacy. Nematode infection was observed in approximately 19% of the large pine weevil larvae collected from the stump. Higher efficacy of treatments at the same rate was recorded when nematodes had been applied in September. Spraying of litter around Scots pine stumps resulted in infection of 85% of the larvae

developing in the stumps. These results were influenced by the weather conditions – unfavourable for nematode vitality during summer treatment and favourable during autumn treatment.

- The effect of the number of bacteria *Photobacterium luminescens* (Thomas and Poinar, 1979) on the population dynamics of nematodes *Heterorhabditis megidis* (Poinar, Jackson and Klein, 1987) in in vitro culture
Dorota Tumialis, Elżbieta Pezowicz, Anna Mazurkiewicz, Iwona Skrzecz, Elżbieta Popowska, Kornelia Kucharska 343-346

Abstract: The study aimed at estimating the correlation between the nematode *Heterorhabditis megidis* (strain KV-136) development and the number of bacterial colonies of *Photobacterium luminescens*. Analyses concentrated on the dynamics of the population growth of *H. megidis* in liquid cultures at different aeration (160rpm, 200rpm), at constant biotic and abiotic parameters (initial dose of nematodes introduced to the culture 2,340 dauer larvae (DL)/ml, temperature 25°C, the number of bacterial colonies 0.3×10^7). Performed experiments showed that aeration at 200 rpm favourably affected the number of colonies of *P. luminescens* (24.14×10^7). High density of bacteria at this aeration variant resulted in an earlier (on the fifth day of the culture) maximum increase in the number of nematode individuals (1,239.6 H/ml) than in the culture aerated at 160rpm.

- The effect of gold nanoparticles on the mortality and pathogenicity of entomopathogenic nematodes from Owinema biopreparation
Kornelia Kucharska, Dorota Tumialis, Elżbieta Pezowicz, Iwona Skrzecz 347-349

Abstract: The effect of gold nanoparticles on the mortality of entomopathogenic nematodes *Steinernema feltiae* from Owinema biopreparation was studied. The effect of different concentrations of nano-Au on pathogenic properties of entomopathogenic nematodes was also studied. It was found that mortality depended on nano-Au concentrations and on the time of larval contact with them.

- Occurrence of pathogens and nematodes of bark beetles (Coleoptera, Scolytidae) from coniferous forest in different region of Georgia
M. Burjanadze, M. Lortkipanidze, A. Supatashvili, O. Gorgadze 351-354

Abstract: The aim of this research was to identify pathogens and nematodes and establish their distribution in a variety of bark beetles species from different locations and different altitude. Various pathogen species and nematodes were observed. *Gregarina typographi* was present in *I. typographus* (9.2-24.7%) and *I. sexdentatus* (35.3 %) in high prevalence; *Chytridiopsis typographi* was found in *I. typographus* (3-6.7%) and in *Tomicus piniperda* (4.7%). Low infestations with *Metschnikowia* sp. (1-3%) *Menzbieria* sp. (1.8%) were detected in *I. typographus* only from one locality. Two species of parasitic nematodes was detected: *Contortylenchus diplogaster* (16.9-41.9%) occurred in the population of *I. typographus*, *Contortylenchus pseudodiplogaster* (21.2 %) in *I. sexdentatus*. Multiple infections were observed with *G. typographi* and nematodes in combination.

- The influence of intraspecific competition and substrate on *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae)
Jiří Nermuť, Vladimír Půža, Zdeněk Mráček 355-358

Abstract: *Phasmarhabditis hermaphrodita* is a slug associated nematode that kills many species of slugs and snails and is also able to live in the soil on various organic materials. This simple laboratory study shows how intraspecific competition and substrate can influence these nematodes. Causal relationships of lipid reserves, length, fecundity and development of nematodes colonies to different substrates (kidney, slugs, faeces, organic matter and insect), dose and strain (commercial and wild) were observed. We recorded some interesting trends and behavioral patterns in this nematode. Big differences between wild and commercial strains, especially in the fecundity and development of nematodes colonies, were also observed.

A new entomopathogenic nematode (Steinernematidae) from Ethiopia
Tewodros Tamiru, Tesfaye Hailu, Lieven Waeyenberge, Ralf-Udo Ehlers,
Vladimír Půža, Zdeněk Mráček 359-362

Abstract: Three strains of *Steinernema* sp.n., (Dero-1, Dero-8 and Mosisa-1) collected from Ethiopia were characterized based on morphological, morphometric, and molecular methods. Results confirmed that all these strains represent one undescribed species. Infective juvenile of the *Steinernema* sp.n. have a body length ranging from 906-965 µm (mean 929), eight identical ridges in lateral fields, position of excretory pore at mid of pharynx, hyaline layer occupies approximately half of tail, ratio c' about 3.4. First generation males without mucron whereas second generation possesses short spine-like mucron. Slightly arcuate spicules in golden-brown colour, with ellipsoid or oblongate manubrium. First generation females without postanal swelling and with minute protuberance on the tail tip. Second generation with postanal swelling and protruding vulva. Based on the morphology, morphometry and DNA analysis, the new species belongs to the *glaseri*-group. The closest relative species seems to be the Afro-tropical *S.karii* found in Kenya. The BLAST analysis of the ITS region of the rDNA shows a similarity of 93% with *S. karii* (GenBank accession number AY230173), supporting the designation to a new species. In maximum parsimony tree the new species groups together with *S. karii* which is supported by a bootstrap value of 100%.

Possible interaction of the phoretic mite *Centrouropoda almerodai* on the control of
Rhynchophorus ferrugineus by entomopathogenic nematodes
Ana Morton, Fernando García-del-Pino 363-366

Abstract: The phoretic mite species *Centrouropoda almerodai* is associated with the red palm weevil, using the insect to colonize new host-palms to complete its life-cycle. Entomopathogenic nematodes are successfully used to control the red palm weevil, but the possible interaction between *C. almerodai* and entomopathogenic nematodes is unknown. In this study the predation by *C. almerodai* adult mites on the entomopathogenic nematode *Steinernema carpocapsae* was evaluated. A low number of infective juveniles was recorded when the nematodes were exposed to *C. almerodai*, confirming that the mite species feeds on *S. carpocapsae*.

The abundance, distribution and natural host range of entomopathogenic
nematodes (Nematoda: Steinernematidae) in the experimental
GM maize MON 88017 field
Vladimír Půža, Oxana Habuštová, Hany M. Hussein, Zdeňka Svobodová 367-370

Abstract: The present study is a part of the project focused on the environmental impact of GM maize MON 88017. Our aim was to assess the occurrence, abundance and distribution of entomopathogenic nematodes and their insect hosts in the experimental field with MON 88017 GM maize and non GM cultivars. *Steinernema affine* was present in all maize cultivars and the most important factor affecting its occurrence was probably the type of the neighbouring environment, as EPNs were only present in plots in the part of the field neighbouring with a forest, regardless of the maize cultivar. No effect of MON 88017 on EPN distribution was recorded. The overall EPN density was low, probably reflecting the low number of soil insects. Thus the biocontrol potential of such a population would be limited.

A highly pathogenic *Steinernema websteri* isolated first time in *Agrotis segetum*
and in Turkey
Gokce, C., Erbas, Z., Yilmaz, H., Demir, I. and Demirbag, Z. 371
Abstract only

Histopathological changes and chitinase activity induced by *Meloidogyne incognita*
in resistant and susceptible potato
Mahfouz M. M. Abd-Elgawad, Marie-Claire Kerlan, Sanaa S. A. Kabeil,
Farid Abd-El-Kareem, Moawad M. Mohamed, Wafaa A. El-Nagdi 372
Abstract only

Susceptibility of the noxious social insect *Tapinoma sessile* (Say) to infection by entomopathogenic nematodes

Nona Mikaia 373-375

Abstract: The susceptibility of the house ant, *Tapinoma sessile*, to entomopathogenic nematodes (EPN) and their reproduction in this host was investigated. Mortality of ants by EPN nematodes after 24 h exposure to *S. feltiae* was 21% and 28% to *S. carpocapsae*. After 48 h exposure the mortality caused by *S. feltiae* achieved 43%, that of *S. carpocapsae* 52%. Mortality of insects caused by *S. feltiae* after 72 h exposure was 61% and 72% for *S. carpocapsae*. The reproductive potential of *S. feltiae* on the house ant, *Tapinoma sessile*, on the first, second and third day was 1500, 2000 and 2500. For *S. carpocapsae* it was 2800, 3300 and 1800. The reproduction of *S. feltiae* on the fourth, fifth, and sixth day was much higher (3000, 3200 and 3500, accordingly) and for *S. carpocapsae* – 2200, 3700 and 4000. On the seventh day the reproductive potential for *S. carpocapsae* made 4200 more than *S. feltiae* (3800).

Virus

Virus Session 1: PTM granuloviruses

Colombian granuloviruses related to *Phthorimaea operculella* granulovirus isolated from the Guatemalan tuber moth, *Tecia solanivora*

Carlos Espinel-Correal, Xavier Léry, Laura Villamizar, Juliana Gómez, Jean Louis Zeddám, Alba Marina Cotes, and Miguel Lopez-Ferber 379-382

Abstract: The guatemalan potato tuber moth, *Tecia solanivora*, progressively invaded Colombia, partially displacing the original potato tuber moth, *Phthorimaea operculella*. No appropriate biological control methods exist for *T. solanivora*, while for *P. operculella*, a granulovirus, PhopGV, is used. An extensive sampling in Colombia allowed find *T. solanivora* infected larvae. Five different isolates have been characterised. All appear to be well adapted to *T. solanivora*, showing control efficacies (measured by their LC₅₀) lower than 10 OB/mm². In addition, isolates collected in regions where both host species coexist are also efficient on *P. operculella* larvae. They are thus promising candidates for the development of biological insecticides.

Stored potatoes in Costa Rica are efficiently protected from *Phthorimaea operculella* and *Tecia solanivora* with an indigenous granulovirus strain

Y. Gómez-Bonilla, M. López-Ferber, P. Caballero, D. Muñoz 383-386

Abstract: The control efficiency of a *Phthorimaea operculella* granulovirus isolate from Costa Rica (PhopGV-CR1) against the insect pests *P. operculella* and *Tecia solanivora* under storage and field conditions was evaluated. The virus reduced damage by over 70% compared with the untreated controls. These data favour the inclusion of PhopGV-CR1 formulations in IPM programs.

Potato crops in Costa Rica are efficiently protected from *Phthorimaea operculella* and *Tecia solanivora* by an indigenous granulovirus strain

Y. Gómez-Bonilla, M. López-Ferber, P. Caballero, D. Muñoz 387-390

Abstract: The control efficiency of a *Phthorimaea operculella* granulovirus isolate from Costa Rica (PhopGV-CR1) against the insect pests *P. operculella* and *Tecia solanivora* under field conditions was evaluated. The virus reduced damage between 50 and 80% in fields compared with the untreated controls. These data favor the inclusion of PhopGV-CR1 formulations in IPM programs.

Transcriptome analysis of the *Cydia pomonella* granulovirus

D. Schneider, K. E. Eberle, J. A. Jehle 391-394

Abstract: The *Cydia pomonella* Granulovirus (CpGV) is widely used for the control of codling moth (*C. pomonella*) many apple growing regions worldwide. In recent years development of resistance to CpGV was observed in several European countries. On the other hand, new CpGV isolates overcoming this resistance were identified and applied in orchards with resistance. To understand better the interaction between CpGV and its host and to determine the genetic factors involved in the virulence of the virus the transcriptome of the virus and the expression profile of its genome is analysed. By quantitative reverse transcription PCR (qRT-PCR) analyses, the specific time frame of the infection process in midgut and fatbody tissue shall be discovered. The aim of this project is to find new ways of virulence management by expanding the knowledge on the gene regulation of CpGV in susceptible and resistant codling moth.

Virus Session 2: CpGV granuloviruses

The new CpGV-R5 variant overcomes the codling moth resistance to the virus

Samantha Besse, Ludovic Crabos, Olivier Soubabère, Antoine Bonhomme, Miguel Lopez-Ferber, Marie Berling, Benoît Sauphanor, Sophie-Joy Ondet 397-400

Abstract: *Cydia pomonella* is the most important pest in apples, pears, quinces and walnuts orchards all over the world. Its control with chemical insecticides comes up against many resistances developed by this insect. The biological control with a specific Granulovirus, the CpGV, is one of the key tools for farmers, especially in organic production. After more than 20 years of generalized use of a single virus isolate, insect populations resistant to the virus have been detected in Germany first, then in France, opening the question on the sustainability of such control approach. Codling moth resistant populations weaken the whole production field.

By selection on a resistant insect colony, virus variants able to kill resistant insects were obtained. The most promising is being patented. Field trials conducted in France and Germany in 2010 with one of these variants, the CpGV-R5, have shown a very interesting control level in resistant populations, compatible with agricultural practices. The registration of CpGV-R5 variant is on-going in Europe and its equivalence with the CpGV-M isolate has been recognized by the French authorities for its inscription at the Annex I. Its registration in France would be obtained in 2011 under “Carpovirusine Evo2” trademark.

This example has contributed to the modification of the European regulations framework on the registration of biological control agents, allowing the introduction of the co-evolution and of biodiversity concepts for Baculovirus.

However, this approach requires modify the way in which the CpGV is used in orchards. A strategy was drawn by Arysta LifeScience/NPP for the use of CpGV-R5 variant in order to sustain the use of CpGV by farmers.

Use of resistance overcoming CpGV isolates and CpGV resistance situation of the codling moth in Europe seven years after the first discovery of resistance to CpGV-M

Daniel Zingg, Markus Züger, Franz Bollhalder, Martin Andermatt 401-404

Abstract: New *Cydia pomonella* granulovirus (CpGV) isolates Madex Plus/Madex 100 or Madex Max have been registered and commercialised in Austria, Germany, Holland, Italy and Switzerland. In most plots with resistance problems the codling moth (CM) populations were reduced to a satisfactory level by using these resistance overcoming CpGV isolates.

More highly virulent resistance overcoming CpGV isolates are available today and offer possibilities for virulence management by changing the virus isolates every few years in order to prevent further resistance development.

Resistance of codling moth to *Cydia pomonella* granulovirus –
are there two types of resistance?
Johannes A. Jehle, Stefanie Schulze-Bopp, Eva Fritsch, Karin Undorf-Spahn 405
Abstract only

Control of Oriental fruit moth and codling moth with a new Granulovirus isolate
*Iris Kraaz, Markus Züger, Heiri Wandeler, Daniel Zingg, Edith Ladurner,
Massimo Benuzzi, Marketa Broklova, Gyula Laszlo* 407-411
Abstract: A new Granulovirus isolate for the combined control of codling moth (*Cydia pomonella*) and oriental fruit moth (*Grapholita molesta*) has been developed and field-tested by Andermatt Biocontrol, Switzerland. Three selected trial results from the 2010 season in Italy, Slovakia and Switzerland are presented in this paper. The effect of the new isolate against oriental fruit moth on stone fruit was a reduction in fruit damage of 68% (on total damage) and 77% (on active damage) and 90% (on active damage) for codling moth in apple.

Virus Session 3: Nucleopolyhedroviruses

Evaluation of a microencapsulated baculovirus bioinsecticide for controlling
Spodoptera frugiperda in maize
Juliana Gómez, Judith Guevara, Laura Villamizar 415-419

Abstract: A formulation prepared by microencapsulation of nucleopolyhedrovirus SfMNPV 003 occlusion bodies (OBs) with a methacrylic acid polymer was assessed on maize plants infested with *Spodoptera frugiperda* under mesh house conditions. No obvious differences in the level of insect plant damage were observed between plants treated with formulated and unformulated virus. On days 7 and 11, values of damages ranged from 2.5 to 17.5% for plants treated with virus, while in control plants damages varied between 90 to 70%. Formulated and unformulated virus at a rate dose of 1×10^7 OBs/ml (750g/ha) efficiently reduced insect damage under semi controlled conditions. Biopesticide was also evaluated under field conditions in a maize crop, by using three doses. Biological and chemical products reduced similarly the insect plant damage. The results obtained allowed to recommend treatments at a biopesticide dose of 750g/ha equivalent to 7.5×10^{11} OBs/Ha for controlling *S. frugiperda* under field conditions in maize crops with applications when fresh damage reaches 15%.

Physical maps and biological activity of seven isolates of *Helicoverpa armigera*
nucleopolyhedrovirus from the Iberian Peninsula
*Maite Arrizubieta, Oihane Simón, Delia Muñoz, Trevor Williams and
Primitivo Caballero* 421-425

Abstract: In the present study, seven previously identified *Helicoverpa armigera* NPV (HaSNPV) isolates from the Iberian Peninsula were subjected to molecular and biological characterization and compared with a Chinese genotype (HaSNPV-G4). Physical maps with *EcoRI* indicated that the Spanish strain HaSNPV-SP1 bore the largest genome while the others presented deletions of varying sizes. Biological characterization revealed no differences in pathogenicity (effective doses) among the different isolates. However, they showed variations in virulence (speed of kill), with HaSNPV-SP1 being, by far, the fastest in killing larvae, including the reference HaSNPV-G4. In terms of productivity (number of occlusion bodies yielded per larva), HaSNPV-SP1 was one of the most productive isolates. In conclusion, HaSNPV-SP1 evidences highly desirable biological characteristics for its development as a bioinsecticide.

Spodoptera frugiperda multiple nucleopolyhedrovirus as a potential biological insecticide: genetic and phenotypic comparison of field isolates from Colombia
Gloria Barrera, Oihane Simón, Laura Villamizar, Trevor Williams and Primitivo Caballero 427-431

Abstract: Thirty eight isolates of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV), collected from infected larvae on pastures, maize and sorghum plants in three different geographical regions of Colombia, were subjected to molecular characterization and were compared with a previously characterized Nicaraguan isolate (SfNIC). Restriction endonuclease analysis (REN) showed two different patterns among Colombian isolates, one profile was particularly frequent (92%) and was named SfCOL. The physical map of SfCOL was constructed and the genome was estimated to be 133.9 Kb, with few differences in terms of number and position of restriction sites between the genomes of SfNIC and SfCOL. The *Pst*I-K and *Pst*I-M fragments were characteristic of SfCOL. These fragments were sequenced to reveal the presence of seven complete and two partial ORFs. This region was collinear with SfMNPV *sf20* to *sf27*. However, two ORFs (4 and 5) had no homologies with SfMNPV ORFs, but were homologous with *Spodoptera exigua* MNPV (*se21* and *se22/se23*) and *Spodoptera litura* NPV (*splt20* and *splt21*). Biological characterization was performed against two different colonies of *S. frugiperda*, one originating from Colombia and one from Mexico. SfCOL OBs were twelve times more potent for the Colombian colony than SfNIC OBs. SfCOL and SfNIC showed a slower speed of kill (by ~50 h) in insects from the Colombian colony compared to the Mexican colony, which was correlated with a higher production of OBs/larvae. SfCOL is a new strain of SfMNPV that presents pathogenic characteristics that favor its development as the basis for a biopesticide product in Colombia.

Evaluation of the efficacy of two nucleopolyhedroviruses to suppress whitemarked tussock moth populations
G. Brodersen, R. Lapointe, G. Thurston, C. Lucarotti and D. Quiring 433-437

Abstract: The nucleopolyhedroviruses of *Orgyia leucostigma* (OrleSNPV) and *Orgyia pseudotsugata* (OpMNPV) were compared to assess their potential as microbial biological control agents against the whitemarked tussock moth (WMTM). The influence of virus species, dose and larval instar at inoculation on larval mortality was determined in laboratory bioassays. Mortality rates of larvae infected with OrleSNPV and OpMNPV were comparable, although OpMNPV had slightly higher efficacy at lower doses when fed to early-instar larvae. LT₅₀ experiments demonstrated that OrleSNPV carried a slight but significant advantage on the time to mortality. Manipulative field bioassays were also performed to determine the influence of pre- and post-ingestion diet on the mortality caused by both viruses. Both pre- and post-virus-ingestion nutrition impacted the mortality rates of larvae infected by each virus equally, which may be associated with varying nutritional qualities, especially protein content, of the diet and foliage used.

Poster Virus

Formulation effect over insecticidal activity of *Phthorimaea operculella* granulovirus VG003 for controlling *Tecia solanivora*
Juliana Gómez, Carlos Moreno, Karen Vega, Alba Marina Cotes, Laura Villamizar 441-445

Abstract: Two formulations of a Colombian isolate of *Phthorimaea operculella* granulovirus designed as emulsifiable concentrate (EC) and dispersible granules (WG) were evaluated under laboratory, mesh house and field conditions, against the Guatemalan potato moth *Tecia solanivora*. In the laboratory test both formulations showed lower potency than unformulated virus, although there were no significant difference between their lethal concentrations. EC formulation significantly reduced larval population, incidence and severity of damage caused by the insect in mesh house, while WG showed no effect on any variable. Formulation type had a

different effect over insecticidal activity and EC, with a concentration of 1×10^7 OBs/ml, a volume rate of 400l/ha and a biweekly frequency, was selected as the most promising treatment in the field, where it produced the highest field crop protection (83%).

Characterization and storage stability of a formulation based on a Colombian

Spodoptera frugiperda nucleopolyhedrovirus, SfNPV

Lizeth Tabima, Juliana Gómez, Laura Villamizar 447-451

Abstract: A formulation based on a Colombian isolate of *Spodoptera frugiperda* nucleopolyhedrovirus, prepared by the microencapsulation of viral occlusion bodies (OBs) with a methacrylic acid polymer was characterized to establish limits for its future quality control in manufacture process and its stability in storage was studied. Biopesticide showed a concentration of 10^9 OBs/mL, a moisture content of 1.8%, a pH of 7.1, a particle size less than $10\mu\text{m}$, total contaminant content lower than 10^5 CFU/g and a laboratory efficacy of 94%. Insecticidal activity of formulated and unformulated virus was stable for six months of storage at 8°C and 18°C . However, a significant reduction in efficacy was observed after six months of storage at 28°C . Contaminants content in formulated virus remained under acceptance limit during storage at three evaluated temperatures. Obtained results allowed to conclude that the developed SfNPV biopesticide could be stored for six months at 8°C and 18°C without quality losses.

Spodoptera ornithogalli nucleopolyhedrovirus: Preliminary study of Colombian isolate

Gloria Barrera, Paola Cuartas, Juliana Gómez, Judith Guevara

and Laura Villamizar 453-456

Abstract: *Spodoptera ornithogalli* (Guenée) (Lepidoptera) is a phytophagous insect of family Noctuidae. In Colombia, this specie had been reported in several crops including cotton and ornamental flowers with economic importance. The Nucleopolyhedrovirus of *Spodoptera ornithogalli* (SoNPV) is a natural enemy for the larvae stage. In this work, *S. ornithogalli* larvae collected from citric crop were reared until dead by viral symptoms. The SoNPV obtained were subject to morphological and biological characterization. Additionally, restriction endonuclease analysis (REN) patterns were made using four restriction endonucleases. The size of the polyhedra showed high variation although the most frequent range size was inferior compared to *S. frugiperda* polyhedra. Mean lethal concentration (LC_{50}) determined in neonates larvae was 1×10^5 OBs/mL and the yield per unit weight of the larvae was found to be 2×10^9 OBs/g. The REN showed a differential pattern compared with others baculoviruses. This is a preliminary study of SoNPV native from Colombia and could be the base for future works in order to develop a biopesticide.

Investigation of nucleopolyhedroviruses and experience of regulation of forest pest populations in Latvia

Jankevica, Liga, Halimona, Julija, Metla, Zane, Seskena, Rita, Zarins, Ivars 457-460

Abstract: Baculoviruses are potential agents for the control of different forest pests. The aim of studies was to extend the knowledge on insect viruses and to clarify their role in regulation of pest populations. Nucleopolyhedroviruses (NPVs) were isolated from 15 pest species. Four NPV isolates were isolated from Hymenoptera species, 11 isolates – from Lepidoptera. Isolated viruses were used as a basis for virus preparations. NPVs isolated in Latvia were found to be active against their corresponding pests and may be successfully used as biological control agents. The possibilities to enhance viral infection by synergistic additives were investigated.

Wireworms

Wireworm Session 1: Management and feeding ecology

Wireworm management: mitigating environmental risk with the development of new 'push-pull' strategies

Robert S. Vernon and Wim Van Herk 463-466

Abstract: Many higher risk insecticides commonly used for wireworm management are now obsolete worldwide, or soon will be, and wireworm populations and damage are on the rise globally. The search for lower risk replacements, however, has proven to be challenging. Our studies, for example, have shown that neonicotinoids (i.e. thiamethoxam, clothianidin and imidacloprid) applied as seed treatments (i.e. wheat, corn, potatoes) will preserve crop stand and yield, but this is due to wireworms entering a long-term but reversible state of intoxication rather than mortality. We have also found that synthetic pyrethroids (i.e. bifenthrin and tefluthrin) applied as wheat seed treatments are repellent to wireworms long enough to enable crop establishment, but once again without wireworm mortality. In contrast, the phenyl pyrazol, fipronil, applied at higher dosages to wheat seed and potato crops resulted in excellent crop protection and near extermination of wireworm populations. Applications of fipronil at lower dosages did not affect wireworm health immediately, but significant latent mortality (up to 90%) began occurring after about 40 days. Since fipronil has a higher environmental and health risk profile than the neonicotinoids or pyrethroids, a number of novel strategies were developed to reduce the amount of fipronil applied per ha while maintaining crop health and significantly reducing wireworms. A 'blend' of thiamethoxam (10g a.i./100kg seed) + fipronil (1g a.i./100kg seed) on wheat seed was found to be sufficient to protect wheat stand under moderate to high wireworm populations, as well as reducing neonate and resident populations by >90% (fipronil rate/ha = 1.24g a.i.). Similar field efficacy was also demonstrated with 'push-pull' strategies involving 1:1 mixtures of 'repulsive' tefluthrin-treated wheat seeds and 'lethal' blend-treated wheat seeds (fipronil rate/ha = 0.62g a.i.). Push-pull strategies to merely remove wireworms from fields, or for use as companion plantings were developed using up to 3:1 mixtures of untreated (attractive) wheat seeds and blend-treated wheat seeds (with 3:1 seed ratios, fipronil rate/ha = 0.095g a.i.). Practical uses for these strategies are discussed.

Crop rotation as a management tool for wireworms in potatoes

Christine Noronha 467-471

Abstract: Wireworm damage to potato tubers can result in serious economic losses for producers. The lack of efficacious insecticides in Canada and increasing wireworm pressure has intensified the need to evaluate other management strategies. The effectiveness of a three-year crop rotation on tuber damage was evaluated, with buckwheat, alfalfa, brown mustard or the standard barley/clover grown in years one and two, and potato in year three. Results showed a significant decrease in tuber damage and an increase in marketable tuber yield following the brown mustard and buckwheat rotations when compared to the alfalfa and barley/clover rotations.

Trap crops as a means to manage *Agriotes* larvae in maize

Bettina Thaling, Karin Staudacher, Nikolaus Schallhart, Corinna Wallinger, Anita Juen, Michael Traugott 473

Abstract only

Seasonal patterns in the dietary choice of *Agriotes* wireworms revealed by molecular analyses

Corinna Wallinger, Karin Staudacher, Nikolaus Schallhart, Evi Mitterrutzner, Eva-Maria Steiner, Bettina Thaling, Anita Juen, Michael Traugott..... 474

Abstract only

Attraction of wireworms to root-emitted volatile organic compounds of barley

Fanny Barsics, Eric Haubruge, François J. Verheggen 475-478

Abstract: The ability of wireworms (*Agriotes sordidus* Illiger) to orientate towards a blend of volatiles emitted by chopped roots of barley was tested. During individual tests, the larvae chose between the two sides of a Y-shaped olfactometer. One side was connected to a chamber containing the chopped roots and the other was connected to an empty chamber. Wireworms chose significantly more often the side of the olfactometer providing the blend of root volatiles. This result underlines the importance of the identification of these compounds and their role assessment alone or combined, as for their effect on wireworms. Such compounds could be used in IPM strategies.

Wireworm Session 2: Monitoring & identification

Factors influencing monitoring of *Agriotes* spp. wireworms

Hilfred Huiting and Klaas van Rozen 481-484

Abstract: Wireworms tunneling into potato tubers cause quality decline to the product. Due to the short time between initial wireworm presence in the topsoil in spring and the moment of potato planting, decisions whether or not to control wireworms based on monitoring in spring are insecure. Increased knowledge on temperature and soil moisture may help enhance a successful monitoring strategy. Two trials performed in climate chambers showed wireworms to be increasingly active between 5°C and 25°C. The preferent soil moisture level was 20%, ranging from 10 to 25%.

Occurrence of *Agriotes* wireworms in Austrian agricultural land

Karin Staudacher, Nikolaus Schallhart, Peter Pitterl, Corinna Wallinger, Nina Brunner, Bernhard Kromp, Marion Landl, Johann Glauningner and Michael Traugott 485

Abstract only

How does wireworm damage in potato fields correlate with elaterid species as well as environmental and cultivation parameters?

Nina Brunner, Patrick Hann, Claus Trska, Bernhard Kromp 487-490

Abstract: Wireworm damages are one of the major recent plant protection problems in organic as well as in conventional potato farming. This study aimed at identifying parameters of environment and cultivation that correlate with wireworm damages in potato as well as the occurrence of *Agriotes* species. From 2007 to 2009, 29 organic potato fields, located in Eastern Austria were investigated by wireworm-bait traps and damage ratings. Crop rotation and cultivation methods were interrogated from the farmers for each field. Site-specific soil, climate and landscape parameters were collected from databases. Relevant parameters were selected by Principal Component Analysis and then correlated with the damage height and the number of baited wireworms. The greatest differences between the research sites were caused by a regional factor. The severity of damage seemed to increase alongside a regional gradient from the lower warm-dry Marchfeld over central Weinviertel, both Lower Austria, towards the higher, cool-humid and densely-wooded Wald- and Mühlviertel, Lower and Upper Austria, respectively. Higher air temperatures in July and August seemed to diminish the damage. Single wireworm species of the main pest-genus *Agriotes* showed differences in regional distribution. Only *Agriotes obscurus* and *Agriotes sputator* correlated with the damage height.

The importance of the identification of *Agriotes* larvae to implement IPM in arable crops

Lorenzo Furlan 491-494

Abstract: The implementation of IPM strategies against wireworms has been extremely difficult because until few years ago, there was a shortage of reliable information on the key aspects of the

species concerned. In the last couple of decades information about the behavior of the larvae of the most important species for agriculture and reliable economic threshold has been discovered. This information can be really useful to implement IPM in arable crops if the determination of the larva can be easily and quickly done. The results of this research with regard to the species *Agriotes brevis*, *A. sordidus*, *A. ustulatus* in north-eastern Italy and the practical implications for IPM are described. The above *Agriotes* species showed a different response to bait traps so it is necessary to assess thresholds (expressed as number of larvae/bait trap) for each of the associations crop-wireworm species provided the bait traps have been placed out in proper conditions. Thresholds for maize crop are reported.

Wireworm Session 3: Pheromones & dispersal

Click beetles and pheromones – an overview

Miklós Tóth 497-501

Abstract: Based on experience gained from the widely used moth pheromones. It was hoped that if pheromones of click beetles became known, they could be applied in similar ways in IPM strategies for the control of wireworms. A brief overview is presented on results of identification efforts of click beetle pheromones, new questions arising in the categorization of these pheromones into the class sex or aggregation pheromones, options for the development of female-targeted lures, and application perspectives.

A two year click beetle monitoring with pheromone traps in Germany: species distribution, trap specificity and activity pattern

*Stefan Vidal, Torsten Block, Thilo Busch, Frank Burghause and
Hans-Helmut Petersen* 503

Abstract only

Male *Agriotes* click beetle walking behaviour and response to sex pheromones

Carly Benefer, Rod Blackshaw, Mairi Knight and Jon Ellis 504

Abstract only

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

Antonela Kozina, Maja Čačija, Renata Bažok 505-509

Abstract: The main aim of recent investigation was to determine the distribution and abundance of *Agriotes lineatus* L. in four different regions of Croatia and correlate the abundance with the prevailed climatic conditions in each region. Investigation was conducted by the mean of pheromone traps (Csalomon) on 17 fields grouped into 7 microregions and four regions according to the climatic data. The average capture of *A. lineatus* beetles/field and the average dominance index were the highest in County of Zagreb where the most humid conditions were present. Going to the east the amount of rainfall is decreasing and mean average capture of beetles and the average dominance indexes became lower. In County of Vukovar-Sirmium where the highest temperatures and the lowest amount of rainfall is present the dominance indexes were the lowest and the species was classified as dominant or subdominant.

Dispersal of click beetle and crane fly species across an agricultural landscape

Rod Blackshaw, Helen Hicks 510

Abstract only

Wireworm Session 4: Microbial control & sampling

Natural infection of wireworm, *Agriotes* sp. (Coleoptera; Elateridae),

with *Rickettsiella* bacteria

Christina Schuster, Claudia Ritter, Regina G. Kleespies, Simon Feiertag,

Andreas Leclerque 513-516

Abstract: Wireworms, the polyphagous larvae of click beetles belonging to the genus *Agriotes* (Coleoptera: Elateridae), are severe and widespread agricultural pests affecting numerous crops worldwide. A previously unknown intracellular bacterium has been identified in a diseased *Agriotes* larva. Microscopic studies revealed the subcellular structures characteristic of rickettsiosis. Molecular phylogenetic analysis based on 16S ribosomal RNA encoding *rrs* gene demonstrated that the wireworm pathogen belongs to the taxonomic genus *Rickettsiella* (Gammaproteobacteria; Legionellales). The pathotype designation '*Rickettsiella agriotidis*' has been proposed to refer to this organism. Moreover, genetic analysis makes it likely that this new pathotype should be considered a synonym of the nomenclatural type species, *Rickettsiella popilliae*.

Potential control of Swiss wireworms with entomopathogenic fungi

Ursula Kölliker, Lorenzo Biasio, Werner Jossi 517-520

Abstract: The main wireworm species reducing the quality of potato tubers in northern Switzerland are *Agriotes obscurus*, *A. lineatus* and *A. sputator*. In this study, the effect of the Swiss *Metarhizium anisopliae* strain ART-2825 and of Naturalis[®] (*Beauveria bassiana*) on these wireworm species was evaluated in the laboratory, in the greenhouse and in the field. In a laboratory bioassay, larvae of the three *Agriotes* species treated with Naturalis[®] product and Naturalis[®] spores did not exceed the infection rate of the control treatment. Similarly, no significant effect of Naturalis[®] was observed in a potato field, with 79% percent damaged tubers in the control and 82% in the Naturalis[®] treatment. In contrast, *M. anisopliae* strain ART-2825 demonstrated a high virulence towards *A. obscurus* in two laboratory bioassays. Nine weeks post-inoculation, 80% and 97% infected *A. obscurus* larvae were observed in bioassay 1 and bioassay 2, respectively. However, the virulence of strain ART-2825 against *A. lineatus* and *A. sputator* was considerably lower with maximum infection rates of 50%. Application of strain ART-2825 onto sterile and non-sterile soil in pots in the greenhouse resulted in a significant reduction of surviving *A. obscurus* larvae. The corrected efficacy according to Abbott of strain ART-2825 was 61% in sterile and 50% in non-sterile soil. The results suggest that Naturalis[®] is not suitable to control wireworms in potato fields in northern Switzerland. However, *M. anisopliae* strain ART-2825 may be investigated further as a means to control wireworms in Swiss IPM programmes.

Alternative methods to control wireworms (*Agriotes* spp., Coleoptera: Elateridae)

in vegetable production – potential of calcium cyanamide

and *Metarhizium anisopliae*

Claudia Ritter, Kai-Uwe Katroschan, Ellen Richter 521-524

Abstract: Wireworms of *Agriotes* spp. are severe and widespread agricultural pests affecting especially maize, potatoes and numerous vegetable crops. Within the framework of a national cooperation project on pest management strategies against soil insects, special emphasis was placed on evaluating alternative wireworm control methods for vegetable production. Therefore, the potential of calcium cyanamide and *Metarhizium anisopliae* was investigated. Calcium cyanamide showed a repellent but no lethal effect on late instars of *Agriotes ustulatus*. A Swiss *Metarhizium anisopliae* strain revealed promising crop protection effects requiring further investigations concerning treatment reliability and commercialisation.

Evaluation of four different bait traps to sample wireworms (Coleoptera: Elateridae) infesting wheat and barley crops in Montana

Anuar Morales-Rodriguez, Aracely Ospina-Lopez and Kevin W. Wanner 525-528

Abstract: Wireworms are the most important soil insect pest of wheat and barley in Montana. Baited pitfall traps have been used in several studies in North America for sampling wireworms in soil. Wheat, wheat/corn mixtures, oatmeal, and grain sorghum seeds as well as pieces of carrot and potato have been used as bait in underground pitfall traps. In general, wireworms were equally attracted by all baits. Baited pitfall traps used to assess absence/presence of wireworms in crops and grassland is a more labour efficient method compared to sampling soil cores. We compared underground classical pitfall, pot, stocking and canister traps in spring wheat and barley fields during the spring and summer of 2010. Captures made with the four pitfall traps baited with a wheat/barley mixture, along with seasonal distribution of the different species that were collected, will be summarized.

Sampling for wireworms (Coleoptera: Elateridae) at sugarcane planting

Ron Cherry, Paul Grose, Emilio Barbieri 529-531

Abstract: Wireworms in Florida are primarily a pest in newly planted sugarcane where the larvae attack the underground portions of the plant by feeding on the buds and root primordia during germination and on shoots and roots after germination. Florida sugarcane growers usually apply a soil insecticide at planting to protect germinating seedpieces from wireworm damage. However, studies have suggested that this application in many cases may not be necessary. The objective of our research was to determine if a quick and easy sampling method could be used by Florida sugarcane growers to determine the need for soil insecticide at planting. Testing was conducted on fields located on King Ranch near South Bay, Florida. Wireworm samples were taken in a transect across the length of each field. Sugarcane yield parameters for each whole field were obtained through standard commercial harvesting procedures. Using our sampling method to determine the necessity of soil insecticide application, yield data showed that there was no significant difference in gross tons of sugarcane per ha, % sucrose, or tons of sugar per ha in fields with versus without insecticide application.

Dispersal of *Agriotes* beetles and their larvae revealed by stable isotope analysis

Nikolaus Schallhart, Manuel J. Tusch, Bettina Thalinger, Karin Staudacher, Corinna Wallinger, Anita Juen, Michael Traugott 532

Abstract only

Poster wireworm

Agriotes species: Comparison of species composition in pheromone trap catches with larval bait trap catches at the same site

Michèle Mangan, Marion Landl, Johann Glauningner 535-537

Abstract: In 2007 and 2008 several reportedly *Agriotes* infested sites in Lower and Upper Austria were selected. At each site, bait traps were placed in the soil to check for the presence of the agricultural harmful larvae. The larvae were determined with molecular methods; only *Agriotes ustulatus* was determined with morphological methods. At the same sites pheromone traps were placed and after 2 weeks their content was determined. The focus was on the five, in literature described as most harmful, species in Austria (*Agriotes ustulatus*, *Agriotes sputator*, *Agriotes brevis*, *Agriotes obscurus* and *Agriotes lineatus*). Interestingly, *Agriotes lineatus* larvae were very rare to totally absent in all the sites, which is contradictory to the adult catches. Due to these findings, the assumption that pheromone trap catches reflect the larval population cannot be made. Further studies analysing the origin of the beetles caught in the pheromone traps may help to understand this discrepancy.

- Morphological traits of *Agriotes obscurus* and *Agriotes ustulatus*
(Coleoptera: Elateridae) larvae
Michèle Mangel, Rudolph Wegensteiner, Johann Glauningner 539-540
Abstract: From 2007 until 2009 *Agriotes* larvae were collected at several sites in Upper and Lower Austria. These larvae were determined with molecular methods. As it is still a very complicated task to determine the *Agriotes* larvae based on morphological traits, we decided to have a closer look at the already reliably determined larvae under the scanning electron microscope (SEM). Up until now, no pictures of the morphological traits have been published and especially no pictures of individuals determined with molecular methods.
- Development of amplified fragment length polymorphism (AFLP) markers
for assessing *Agriotes* click beetle dispersal in agricultural land
Carly Benefer, Rod Blackshaw, Mairi Knight, Jon Ellis 541
Abstract only
- Ecology and control of wireworms in Florida sugarcane
Ron Cherry, Alvin Wilson 542
Abstract only
- Biodiversity of click beetles (Elateridae) in the agriculture landscape
of Saxony-Anhalt – Results of pheromone trap-monitoring
*Tackenberg, Maria, Wolff, Christian, Volkmar, Christa,
Lübke-Al Hussein, Marita* 543-547
Abstract: Wireworms become more important as substantial pests in the agriculture landscape of Saxony-Anhalt, because of the reinforced intercropping, the land set-aside, the greening, the weed infestation, the ploughing up of grassland and the reduced cultivation (Vidal & Petersen 2010). In 2001 a similar Monitoring had taken place in France to proof if the occurrence of *Agriotes sordidus* was spread. This species has a shortened cycle as the other ones, why the injuries increased (Furlan 2004). That's why a throughout Germany monitoring took place. Their adult occurrence was evaluated by an Elateridae-Monitoring in Saxony-Anhalt in 2009 and 2010. Concerning this we had pheromone traps of Furlan from Syngenta for the species *Agriotes lineatus*, *Agriotes obscurus*, *Agriotes sputator*, *Agriotes sordidus* and *Agriotes ustulatus*. In 2009 the traps were positioned at 4 habitats of effort and in 2010 at 5 habitats of effort in the different geographic natural environment in Saxony-Anhalt. The habitats were Poppau (Altmark), Giesenslage (Altmarkkreis Stendal), Quedlinburg (Harz), Bornum (Anhalt-Zerbst) and Dederstedt (Mansfeld-Südharz). The occurrence of *Agriotes sordidus* can't be proved in both years. The other species of *Agriotes* were found in different intensities at all habitats.
- Monitoring of click beetles with the use of pheromone traps in hop yards
of the Hallertau
Florian Weihrauch, Johannes Schwarz 548
Abstract only
- How plant identity and diversity affect food choices of *Agriotes* larvae
*Nikolaus Schallhart, Manuel J. Tusch, Corinna Wallinger, Karin Staudacher,
Michael Traugott* 549
Abstract only
- Detection rates of ingested plant-DNA in *Agriotes* wireworms
*Corinna Wallinger, Karin Staudacher, Nikolaus Schallhart, Anita Juen,
Michael Traugott* 550
Abstract only