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Preface	v
List of Participants	vii
Contents	xvii

Plenary Presentations

Biological control of plant diseases: Future research goals to make it successful. <i>Claude Alabouvette, Christelle Cordier</i>	3-5
No abstract	
Fungal-based bio-pesticides, multiple solutions for Integrated Pest Management <i>Antoine Bonhomme, Samantha Besse</i>	6
Abstract only	
Insect pathogenic fungi in biological control: Status and future challenges <i>Jørgen Eilenberg, Nicolai Vitt Meyling, Annette Bruun Jensen</i>	7-10
Abstract: In Europe, insect pathogenic fungi have in decades played a significant role in biological control of insects. With respect to the different strategies of biological control and with respects to the different genera of insect pathogenic fungi, the success and potential vary, however. Classical biological control seem to have little indication of potential, whereas inundation and inoculation of fungi for biological control has several success stories, e.g. within the genera <i>Metarhizium</i> , <i>Beauveria</i> , <i>Isaria/Paecilomyces</i> and <i>Lecanicillium</i> (previously <i>Verticillium</i>). However, the genotypes employed seem to include a narrow spectrum of the many potentially useful genotypes. Conservation biological control with species of the genera <i>Pandora</i> and <i>Entomophthora</i> have a strong potential, but also <i>Beauveria</i> has a potential to be explored further. The main bottleneck for further exploitation of insect pathogenic fungi in biological control is the limited knowledge of host pathogen interaction at the fungal genotype level.	
Insecticidal proteins from <i>Bacillus thuringiensis</i> : their use in conventional and trans- genic plant biotechnology <i>Juan Ferré</i>	11-15
Abstract: The protection of plants against the attack of insects is an old problem that has been partly alleviated by the introduction of conventional resistance genes into crop plants and partly solved by the use of chemical insecticides for about half a decade. However, with the discovery of the insecticidal proteins from <i>Bacillus thuringiensis</i> and the introduction of their genes into plants (the so-called Bt-crops) we are witnessing a revolution in the field of plant protection. With the current trend of reduction of chemical insecticides and the growing demand of organic food, it is anticipated that use of <i>B. thuringiensis</i> -based insecticides will grow. Bt-crops are a different alternative to the use of chemical insecticides in plant protection; the global area planted to Bt-crops is steadily growing and this trend is expected to continue in future years. The main threat to the long term efficacy of this type of products (both sprays or transgenic crops) is the emergence of resistance in pest populations. The use of strategies for resistance management is therefore crucial to preserve this technology.	
The promise, practice and prospects of baculoviruses in biocontrol <i>Johannes A. Jehle</i>	16
Abstract only	

General perspective of the biocontrol industry

Sergio Franceschini, Edith Ladurner, Massimo Benuzzi 17-20

Abstract: Regulation and policies can strongly influence the opportunities for biological control agents to be used as plant protection products. There are a number of driving factors that can favour their use in alternation with or in addition to synthetic agrochemicals in IPM programmes.

Host factors that may affect susceptibility to *Bacillus thuringiensis* crystal toxins

Paul R. Johnston and Neil Crickmore 21-26

Abstract: Diverse physiological factors have been identified that have the potential to affect the susceptibility of lepidopteran larvae to the pesticidal crystal (Cry) toxins of *Bacillus thuringiensis* (Bt). Here, we review the results of comparative studies using both laboratory- and field-selected populations that have highlighted factors that may contribute to tolerance of, and resistance to, Bt Cry toxins.

Resistance to Cry toxins and epithelial healing

Anaïs Castagnola, Juan Luis Jurat-Fuentes 27-32

Abstract: Resistance to Cry toxins can develop by alterations in any of the steps in the Cry toxin mode of action. Most characterized mechanisms of resistance to Cry toxins involve alterations in enzymatic toxin processing or toxin interaction with receptors in the insect midgut epithelium. Previous reports have suggested an alternative mechanism of resistance to Cry1Ac toxin in *Heliothis virescens* larvae involving enhanced midgut regeneration after toxin-induced injury. Our current hypothesis is that as midgut mature cells interact with Cry1Ac and undergo injury, stem cells divide and differentiate to replace damaged mature cells. Considering this hypothesis, resistant *H. virescens* larvae may display a more effective regenerative mechanism that prevents compromising epithelial integrity. We report the detection of differences in the proteins secreted by mature cells upon Cry toxin treatment and an efficient regenerative response to intoxication in midgut cells from Cry1Ac-resistant *H. virescens* larvae.

Bacillus thuringiensis Cry34Ab1/Cry35Ab1: development in stacked *Bt* gene corn products for control of *Diabrotica* spp.

Ken Narva, Ed King, Nick Storer 33-34

Abstract only

Research needs for entomopathogenic nematodes – An industry’s perspective

Arne Peters 35-39

Abstract: Many disciplines from fundamental and applied sciences contributed to the development of entomopathogenic nematodes as a biocontrol industry. Studies on the nematode taxonomy, the biology of the pest insects and the symbiotic association with bacteria were as indispensable as the product development involving mass production techniques, formulation and application. The (incomplete) industry’s wishlist for future research includes fundamental research on the ageing of the infective juveniles, on how to expand their “shelf-life”, and on how to trigger infection behaviour. Solving these questions would decrease the cost of nematode production and the rates used per area and would open new markets. The exploration of new pest targets, also in the context of advances in application technology and reduced nematode prices is part of this wishlist as well as the exploration of whether pest associated rhabditid nematodes can be used as vectors for pathogenic micro-organisms. Recent advances show that two-party partnerships of public research institutes and industry were highly successful in the development of for instance the slug killing nematode or the liquid mass production of *Heterorhabditis* sp.. It is argued that within the EU framework programmes less stringent requirements for the formation of research consortia would enable the project initiators to choose the best suited partners and ultimately contribute to a more efficient use of EU-funding. Often, substantial discoveries are made by chance and outside specific public-private research projects (like the discovery of *Aplectana kraussei*). Fundamental and independent research from scientists with broad interest is most likely to delivery such innovations and should therefore be maintained. In industry the view tends to become short-termed and narrow since all activities are rated against profits made per time unit. While indispensable for a quick product development, this is not a good environment for the emergence of new ideas.

Concepts and visions to overcome problems with microbial biocontrol agent registration

Hermann Strasser41-46

Abstract: On January, 13th 2009 the European Parliament voted on a new EU pesticides regime. A compromise had been reached between the European Commission and the European Parliament which now helps to evaluate all plant protection products following a stringent evaluation system. Although all relevant stakeholder groups welcome the new legal registration regime, the proposals of the EU-funded project REBECA still have to be considered in order to improve primarily the registration of bio-control organisms. The purpose of this presentation is to discuss visionary approaches and concepts which could be implemented rapidly by EU member state rapporteurs and registration authorities to overcome the very diverse problems with microbial bio control agents.

Research needs and promising approaches for the biological control of *Diabrotica* and other emerging soil insect pests with pathogens or nematodes

Stefan Toepfer, Juerg Enkerli, Ulrich Kuhlmann47-58

Abstract: This paper suggests priority areas for Europe-wide research cooperation on biological control of newly emerging soil pests, such as the invasive maize pest *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae), with insect pathogens or insect parasitic nematodes. The conclusions presented in this paper were derived from discussions by international experts. Discussions revealed that insect pathogens and nematodes will be useful elements of a strategic approach to the control of soil pests. Although biological control may not always be competitive with soil insecticides or transgenic crops in conventional production, an integrated or biological pest management strategy is likely to incorporate application of biological control products, classical releases of biological control agents, attract-dissemination-kill methods, and modifications of cultural techniques to enhance natural enemy populations. Overall, there is good evidence that accelerated exploration of biological control options may provide the advances in soil pest management we urgently need.

Microbiological insecticides against lepidopteran pests in greenhouse horticulture in Almeria, Spain

Jan van der Blom59-62

Abstract: Biological control has recently become widely established in greenhouse horticulture in Almeria. During the crop season of 2008-2009, the application of beneficial arthropods is the basis for all pest control measures in approximately 50% of the total of 27.000 ha. This offers a good solution for most pest problems, but is insufficient against others, especially lepidopteran pests. Since the presence of beneficial insects and mites sets a severe limit to the use of chemical pesticides, the available selective microbiological insecticides are widely used to resolve remaining problems. In this paper, the current use of microbiological insecticides, as well as new developments relating this issue, will be discussed.

The mode of action of *Bacillus thuringiensis* Vip3A and its applications for transgenic insect control

Frederick S. Walters, Mi Kyong Lee, Narendra Palekar, Ryan Kurtz, David O'Reilly, Jeng S. Chen63-68

Abstract: The vegetative insecticidal protein, Vip3A, from *Bacillus thuringiensis* (*Bt*), has great value to agriculture due to a broad lepidopteran spectrum and a unique mode of action compared to that of *Bt* insecticidal crystal proteins. Further observations comparing studies used in determining Vip3A mode of action are shared, as well as recent bioassays used to examine potential interactions of Vip3A with Cry1Ab. Data indicate no interactions, in agreement with a novel mode of action for Vip3A. Individual component Vip3A and full-length (FL) Cry1Ab transgenic cotton events and the stacked, VipCot™ product, are also examined for high dose status and discussed in the context of insect resistance management (IRM) practice.

Baculoviruses as biopesticides: Need for further R&D

Heiri Wandeler, Franz Bollhalder, Daniel Zingg, Martin Andermatt69-74

Abstract: Baculoviruses (BV) are the most frequently used viruses as biopesticides. Many studies were carried out on this virus-family, making broad knowledge accessible to science and industry. So far, this resulted in at least 20 commercially available BV products worldwide. However, a lot has to be done to extend the product range and to allow these products to compete with chemical

pesticides. The most important fields for further investigation are: viruses for key pests, isolation and selection of new virus isolates, optimization of insect and virus production, development of new formulations, improvement of application, and insect resistance against virus. The following article focuses on the need for further research and development, from an industrial point of view.

Basic research to widen the use of nematode-based biocontrol agents

Michael J. Wilson 75-76

Abstract: The most important aspect of designing an applied research project is choosing the right questions. In terms of use of nematode-based biological control agents, improved performance could be obtained by a greater understanding of mode of action, production, resistance to stress and understanding the behaviour of nematodes in soil following application. Recently developed scientific techniques, particularly related to sequencing of genomes and transcriptomes have much to offer, but in some cases, “low-tech” research methods may provide greater insight. All research is limited by the availability of funding, and different funding agencies have different priorities. Entomopathogenic nematodes and slug parasitic nematodes are useful models to study evolution, physiology and ecology of soil organisms. Agencies that fund research on basic biology may fund hi-tech, high-cost projects on our nematodes if they believe the outcomes will be sufficiently broad to interest biologists working with a range of organisms. Conversely, many funding agencies are interested in funding short term “problem solving” grants e.g. to control newly emerging pests. In such cases, problems can often be solved using low-tech solutions. The ability to maintain long term research programmes on nematode-based biological control agents is likely to be secured by combining both approaches.

Virus

Investigations on the mechanism of CpGV resistance in *Cydia pomonella*

S. Asser-Kaiser, J. A. Jehle..... 79-81

Abstract: After the successful use of CpGV in control of codling moths in organic and integrated farming for decades, some populations of *Cydia pomonella* developed resistance against CpGV products. Resistant populations were detected in several orchards in Germany, France, the Netherlands, Italy and Switzerland. The resistance is dominant, monogenic and sex-linked inherited. Beside the knowledge of heredity, it is mandatory to know the mechanism of resistance for the development of successful resistance management strategies. Three different experimental approaches were followed to investigate the mechanism involved in CpGV resistance: First, virus replication in three different tissues of susceptible (CpS) and resistant (CpRR1) insects was estimated by quantitative PCR. Second, humoral and cellular immune responses as a reason for resistance were investigated by transfusion of haemolymph from resistant into susceptible insects. Third, by-passing the midgut budded virus was directly injected into the insect’s haemocoel in order to investigate whether resistance is present in others than midgut cells.

A *Phthorimaea operculella* granulovirus (PhopGV) containing several genotypes is highly efficient on *Tecia solanivora*

Carlos Espinel-Correal, Xavier Léry, Laura Villamizar, Jean-Louis Zeddari,

Miguel López-Ferber, Alba Marina Cotes..... 83-86

Abstract: The granulovirus of *Phthorimaea operculella* (PhopGV) is becoming an alternative for biological control of potato moth pests. A PhopGV isolate from *P. operculella* in Peru, multiplied for several years on the alternative host *Tecia solanivora* in Colombia (named #Col6) appears to successfully control this pest, in spite of previous studies showing the inefficacy of PhopGVs on other hosts including *T. solanivora*. In this work, we studied the possible reasons for the high performance of this PhopGV. Restriction profiles and PCR analyses were performed on #Col6 and a reference PhopGV isolate (#1346). Submolar bands were found in #Col6 particularly with *Sma* I and *Nru* I endonucleases. PCR amplification of the 90-91 gene of #Col6 gave two bands, one of 767 bp and another of 619 bp, corresponding to a deleted gene found previously in viral isolates from *T. solanivora*. This indicates that #Col6 is a mixture of several genotypes, one of them probably coming from a local latent GV infection in the *T. solanivora* laboratory colony used for virus multiplication. The LC₅₀ was assessed on *T. solanivora* with #Col6, #1346, the

original Peruvian isolate and a PhopGV isolated from *T. solanivora* in Colombia (#VG003). #Col6 exhibited the highest virulence, probably because of the presence of several genotypes coming from *P. operculella* and *T. solanivora*.

Isolation and characterization of a Colombian *Spodoptera frugiperda* nucleopolyhedrovirus

Juliana Gómez, Laura Villamizar, Gloria Barrera, Magda García, Alba Marina

Cotes87-90

Abstract: A native *Spodoptera frugiperda* nucleopolyhedrovirus was isolated after analyzing 652 larvae collected from the department of Córdoba (Colombia). It was codified as NPV003. This isolate presented a mean particle size of $2.0 \pm 0.5 \mu\text{m}$ and a LC_{50} of 2.3×10^5 occlusion bodies (OBs)/mL determined over neonatal larvae. The sensitivity of the isolate to different temperatures (30°C, 40°C and 50°C) and to UV radiation was also determined, obtaining a viral inactivation of 32.1% when OBs were exposed to 50°C during four hours and UVC light caused a significantly viral inactivation after six hours of irradiation.

Using African armyworm NPV as a strategic biological control agent: Will studying pathogen ecology provide the answers?

Robert I. Graham, David Grzywacz, Wilfred L. Mushobozi, Jennifer S. Cory, Alan

D. Shirras, Yamini Tummala, Kenneth Wilson91-94

Abstract: The African armyworm *Spodoptera exempta* (Lepidoptera: Noctuidae) is one of the most devastating agricultural pests in sub-Saharan Africa, destroying staple crops such as maize, wheat, sorghum, millet, rice and pasture grasses. We are interested in the ecology of a natural disease, *S. exempta* nucleopolyhedrovirus (SpexNPV), and examining how this virus may be utilised in a strategic control program. In this report, we describe preliminary findings from a single field-season, exploring the structure of natural overt SpexNPV populations; and the transmission strategies of the virus, specifically covert infections.

Dual reprogramming of the AcMNPV chitinase (*chiA*) and cathepsin (*v-cath*) expression profiles

Jeffrey J. Hodgson, Noha Gerges, Basil M. Arif, Peter J. Krell95-98

Abstract: The co-dependent function of the baculoviral CHIA and V-CATH enzymes is to aid viral progeny dissemination by promoting host tissue liquefaction. Mis-expression (earlier or increased) of these endogenous viral enzymes may increase the virulence of the baculovirus infection, and might accelerate host debilitation, and thereby might enable enhancement of a baculovirus-based biopesticide. Recombinant AcMNPV with *chiA* transcription reprogrammed by AcMNPV *polh* or *p6.9* promoters adjacent to the native intergenic *v-cath* promoter had *chiA* expression reprogrammed as expected, but lacked detectable *v-cath* transcription or cathepsin enzyme activity. Dual reprogramming of the adjacent *chiA/v-cath* genes with AcMNPV-derived *p6.9/polh* promoters, respectively, simultaneously modulated the transcription profiles of both genes. The reprogrammed transcription profiles of the two genes were characteristic of the alternate *p6.9/polh* promoter sequences and resulted in a 1.5 and 1.15 fold increase in the relative chitinase and cathepsin activities at 40 hours post infection.

Spodoptera exigua nucleopolyhedrovirus is not infectious for *Agrotis segetum* larvae per os, but only after intrahemocoelic injection

Agata K. Jakubowska, Ikbal Agah Ince, Salvador Herrero, Just M. Vlæk, Monique

M. van Oers99-102

Abstract: *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) and *Agrotis segetum* NPV are close relatives, but distinct baculovirus species. Their genomic organization is remarkably similar. The cross infectivity of these two viruses for *S. exigua* and *A. segetum* larvae has been analyzed. AgseNPV was able to infect *S. exigua* orally, although the dose needed was high (LD_{50} of 8.3×10^5 OBs/larvae) and the yield of progeny polyhedra very low. In contrast, SeMNPV appeared to be non-infectious for *A. segetum* larvae when administered *per os*. The course of SeMNPV infection in *A. segetum* was monitored by real-time PCR in a time course experiment and showed that SeMNPV was able to infect and produce polyhedra in *A. segetum* larvae by intrahemocoelic injection. Infection of *A. segetum* with a mixture of AgseNPV and SeMNPV, only resulted in AgseNPV polyhedra. So, in the case of SeMNPV the midgut is the major barrier for infection. The possible mechanisms of *A. segetum* resistance for SeMNPV *per os* are discussed.

- Covert infections in *Spodoptera exigua* laboratory culture
R. Murillo, M. Hussey, R. Hails, R.D. Possee103-106
Abstract: A laboratory colony of *Spodoptera exigua* was investigated to assess the role of covert (latent or persistent) baculovirus infections on spontaneous disease outbreaks. Two nucleopolyhedrovirus (NPV) species were found to be reactivated into fully lethal forms and were identified as SeMNPV and *Mamestra brassicae* by REN and PCR-sequencing. By use of q-PCR, both NPVs species have been quantified in asymptomatic individuals over several generations to elucidate the fluctuating trends of latent or persistent infections.
- Stability of *Spodoptera frugiperda* multicapsid nucleopolyhedrovirus and Sf29null bacmid genomes after sequential rounds of *per os* infection: implications for virus transmission
Oihane Simón, Trevor Williams, Robert D. Possee Primitivo Caballero107-109
Abstract: The Sf29 gene of the *Spodoptera frugiperda* nucleopolyhedrovirus is a viral factor that determines the number of virions within the occlusion bodies. We investigated the stability of the SfMNPV bacmid (Sfbac) and Sf29null bacmid after serial rounds of *per os* infections. The bac replicon was lost in both viruses. In addition, the Sf29null virus was able to recover the Sf29 gene. We hypothesize that deletion of the Sf29 gene supposes a selection pressure, favouring recombination between the Sf29null virus and a covert nucleopolyhedrovirus infection already present in the host insect. Sf29null virus recovers this gene to improve the likelihood of transmission.
- Influence of diet composition on mortality of *Cydia pomonella* larvae infected with CpGV
Tereza Zichová, Jitka Stará, Vladan Falta, Jiban Kumar, Johannes A. Jehle.....110-113
Abstract: Various diets and methods of virus application can be used in bioassays to test the efficacy of *Cydia pomonella* granulovirus (CpGV) biocontrol agents against codling moth (*Cydia pomonella* L.). Usually, diet incorporation of virus occlusion bodies (OBs) or application of the OBs on the diets surface is used in bioassays. Here, we compared the effect of three diet compositions, the Ivaldi-Sender diet, Guennelon diet and Manduca Premix-Heliothis Premix, on the mortality of codling moth larvae when CpGV OBs were incorporated into the diets. Statistical differences were found between the mortality of codling moth on the Premix diet and Ivaldi-Sender and Guennelon diets, with a significantly higher median lethal concentration (LC₅₀) value of CpGV-M on the Premix diet. The impact of the diet composition on bioassay results is discussed.
- Virulence management as a long term strategy to overcome virus resistance of codling moth
Daniel Zingg, Markus Züger, Philip Kessler, Franz Bollhalder, Heiri Wandeler, Martin Andermatt.....114-116
Abstract: Many virus isolates which can overcome virus resistance have been found in the field or through a selection process on resistant codling moth populations. Differences of efficacy of resistance breaking virus isolates on highly resistant codling moth populations lead to the strategy of virulence management by switching to new isolates every three to five years.
- Curriculum Vitae and Scientific Accomplishments of Dr. Juerg Huber
Johannes A. Jehle117-118
 No abstract
- More than 20 years of CpGV-Commercialization
Martin Andermatt.....119-120
Abstract: Products against the codling moth based on CpGV were first mainly used by organic growers. But as resistance of the codling moth against most of the chemical insecticides became more and more obvious, it was soon regarded as an interesting tool for IPM, especially as it has a long lasting effect on the codling moth population and as it allows producing residue free fruits. CpGV is now well accepted in all important apple producing countries where the codling moth is a key-pest.
- Genomic and proteomics of hymenopteran baculoviruses
Shannon Escasa, Peter J. Krell, Basil M. Arif121-124
Abstracts: To date, a total of 48 baculovirus genomes have been sequenced including three of hymenopteran baculoviruses that are, so far, the smallest and appear to be more ancient than

lepidopteran nucleopolyhedroviruses. The genomes of hymenopteran baculoviruses lack a number of genes that were thought of as essential for the replication of lepidopteran baculoviruses. Most notably are those encoding for IE-1 protein and membrane fusion proteins needed for budded virus formation. Hence, the latter phenotype may not play a role in the biology of hymenopteran baculoviruses. Comparative genomic analyses of three hymenopteran baculoviruses revealed co-linearity in most of the genome but an obvious non-syntenic region that contains many genes and ORFs with closer identity to insect genes than those of viral origin. Proteomics analysis by LC-MS/MS has so far demonstrated the presence of 18 proteins associated with the virion, three of which are totally novel and do not exist in any other sequenced baculovirus genome.

Resistance to baculoviruses - new answers to an old question

Johannes A. Jehle, Annegret Schmitt 125-127

Abstract: The *Cydia pomonella* granulovirus (CpGV) is one of the most important commercialized baculovirus biocontrol agents. It has been successfully used for codling moth (CM) control in Europe since more than 20 years. This success was threatened when first reports on CM populations became available in 2005, demonstrating an up to 1000-times reduced susceptibility to CpGV products. Intensive research supported by the European Union and national funding agencies in Germany and France were initiated to understand the prevalence, the molecular mechanism and the genetics behind this phenomenon. New CpGV strains overcoming the resistance were identified or selected from existent isolates. This contribution will provide an overview on recent developments in resistance research of baculoviruses.

An overview of the molecular biology of *Cydia pomonella* granulovirus

Doreen Winstanley 128

Abstract only

Comparative analysis of a granulin fragment of Colombian granulovirus isolated from *Tecia solanivora*

Gloria Barrera, Paola Cuartas, Laura Villamizar 129-132

Abstract: A 410bp fragment of the granulin gene was sequenced from a granulovirus (GV) obtained from *Tecia solanivora* larvae sampled in Cundinamarca, Colombia. This isolate has been obtained as part of a project to control the Guatemalan potato moth (*T. solanivora*) in Colombia. Degenerate primers were designed to amplify the granulin gene. The PCR product was cloned in a plasmid vector and sequenced. The deduced amino acid sequence was aligned with 16 granulin/polyhedrin sequences obtained from Genbank. A dendrogram displaying genetic similarities was constructed using UPGMA method. The granulin of Colombian isolate showed 99% of identity with *Phthorimeae operculella* (PhopGV) gene. The topology of the dendrogram was congruent with the previously described division of lepidoteran granulovirus and nucleopolyhedrovirus (NPV).

The *Sf32* gene of *Spodoptera frugiperda* multicapsid nucleopolyhedrovirus influences occlusion body yield

Inés Beperet, Oihane Simón, Trevor Williams, Delia Muñoz, Primitivo Caballero .133-135

Abstract: The recently sequenced *Spodoptera frugiperda* nucleopolyhedrovirus genome from the Nicaraguan isolate (SfNIC) contains ten unique ORFs, among which is *Sf32*, of unknown function. A PCR and a bacmid-based recombination system was used to delete *Sf32* from the SfMNPV genome that had been previously inserted into a bacmid (Sfbac). Deletion of *Sf32* had not apparent effect on SfMNPV pathogenicity in terms of OB lethal concentration or speed of kill, but a significant decrease was observed in OB production per larva in the *Sf32* mutant compared to the wild-type. This indicates that the *Sf32* gene product influences OB yield.

Do vertically and horizontally transmitted variants of *Spodoptera exigua* multiple nucleopolyhedrovirus differ in their insecticidal characteristics?

Oihana Cabodevilla, Itxaso Ibáñez, Oihane Simón, Rosa Murillo, Delia Muñoz, Trevor Williams, Primitivo Caballero 136-139

Abstract: The coexistence of a wide range of genotypic variants within different *Spodoptera exigua* nucleopolyhedrovirus populations has led us to examine the hypothesis that genotypes may be specialized for different transmission routes: vertical and horizontal. To test this, we analyzed NPV-killed larvae originating from the progeny of field-collected adults, as well as from laboratory reared adults for the presence of vertically-transmitted genotypes. Seven different genotypes were identified among the progeny of laboratory adults (Se-OX1, Se-OX2, Se-OX3,

Se-OX4, Se-OX5, Se-OX6 and Se-OX7), two of which were identical to those found among the progeny of field-collected adults (Se-AL1, Se-AL2). Three of these isolates, Se-AL1, Se-AL2 and Se-OX4, and another three that had been previously reported from soil isolates (Se-G24, Se-G25 and Se-G26), were selected to determine their insecticidal characteristics. We conclude that transmission route and insecticidal properties do not appear to be correlated in this virus.

The selection of defective and complete co-occluded genotype mixtures in a nucleopolyhedrovirus occurs during entry into midgut epithelial cells

Gabriel Clavijo, Trevor Williams, Delia Muñoz, Miguel López-Ferber, Primitivo Caballero

140-142

Abstract: An isolate of the *Spodoptera frugiperda* multiple nucleopolyhedrovirus comprises a stable proportion of deletion genotypes (such as SfNIC-C) that lack *pif1* and *pif2* rendering them non-infectious *per os*. Deletion genotypes survive by complementation with a complete genotype (SfNIC-B) in co-infected cells. To determine whether selection for particular ratios of complete and deletion genotypes occurs mainly during the establishment of the primary infection in insect midgut cells or during subsequent systemic infection, we examined genotype frequencies in insects inoculated with OBs comprising different co-occluded mixtures of genotypes. Dramatic changes in genotype frequencies were observed between the OB inoculum and budded virus samples taken from larvae inoculated with OBs comprising 10% SfNIC-B + 90% SfNIC-C, indicating that a marked reduction of SfNIC-C genotype had occurred in the insect midgut due to the immediate elimination of all non-infectious OBs that originated from cells that had been infected only by SfNIC-C. In contrast, immediate changes were not observed in OBs comprising mixtures of 50% SfNIC-B + 50% SfNIC-C or those comprising 10% SfNIC-B + 90% SfNIC-C. Subsequent changes in genotypic frequencies during the systemic infection were fairly small in magnitude for all genotypic mixtures. We conclude that the prevalence of defective genotypes in the SfNIC population likely depends on a balance between the frequency of OBs produced in cells infected by SfNIC-C alone and selection for fast-replicating deletion genotypes. Selection is probably modulated by changes in the average number of genomes that infect cells during the infection period.

Effects of the *Spodoptera littoralis* granulovirus on the development and reproduction of cotton leafworm (Lepidoptera: Noctuidae)

Adel El-Sayed Hatem, Sergio Pérez-Guerrero, Hani K. Aldebis, Enrique Vargas-Osuna

143

Abstract only

Biological activity and compatibility with chemical pesticides of a Colombian granulovirus isolated from *Tecia solanivora*

Carlos Espinel, Juliana Gómez, Laura Villamizar, Alba Marina Cotes, Xavier Léry, Miguel López-Ferber

145-148

Abstract: The Guatemalan potato moth *Tecia solanivora* is the most limiting potato pests in Venezuela, Colombia and Ecuador. In a previous work, a native granulovirus isolated from *T. solanivora*, appeared to be one promising tool for managing this insect and the other potato moth *Phthorimaea operculella*. The compatibility with chemical pesticides used for potato crops protection was determined. Biological activity was not affected after 24 hour of contact, suggesting that native granulovirus could be used combined with these agrochemicals in an Integrated Potato Crop Management Programme.

Presence of nuclear polyhedrosis viruses in populations of pine looper *Bupalus piniarius* L (Lepidoptera: Geometridae)

Liga Jankevica, Rīta Seskena, Julija Halimona, Zane Metla, Ivars Zarins, Agnī Smits

149-152

Abstract: Baculoviruses are potential agents for the control of different forest pests. Research on baculoviruses of forest pests were carried out in the University of Latvia, Institute of Biology. The aim of our studies was to obtain new isolates of nuclear polyhedrosis viruses (NPVs) and to investigate occurrence of persistent infections in *Bupalus piniarius* populations. Monitoring of *B. piniarius* populations was done in different regions of Latvia. The insects collected from natural habitats were checked for presence of NPV. In 2008 we observed very low NPV infection level (mortality <5%). NPVs were isolated from *B. piniarius* by subjecting asymptomatic larvae to stress-factors: extreme temperatures, NsNPV, 0.5% ZnSO₄ or 1.0% H₃BO₃. An optimised

method of virus DNA determination by PCR was used for *in-situ* determination NPVs in different populations of *B. piniarius* (Lepidoptera: Geometridae). PCR confirmed that endemic insect populations had NPV infection.

Application of juvenile hormone analogue and optical brightener technologies to the production of *Spodoptera frugiperda* multiple nucleopolyhedrovirus

Rodrigo Lasa, Iñigo Moreno, Primitivo Caballero, Trevor Williams 153-156

Abstract: Final instar larvae of *Spodoptera frugiperda* grew to a maximum weight of 622 ± 13 mg on diet treated with 1% piriproxyfen, a juvenile hormone analogue (JHA), or 695 ± 17 mg on diet treated with 1% fenoxycarb, both of which were significantly greater weights than observed in larvae that developed on untreated diet (512 ± 9 mg). Virus mortality in insects inoculated with SfMNPV occlusion bodies (OBs) was approximately 50% in all treatments, reflecting the resistance of late instar *S. frugiperda* larvae to infection. However, JHA treatment did not result in a significant increases in the total OB yield, or OBs per mg larval weight, compared to untreated infected insects. We determined whether resistance to infection could be overcome by inoculation with mixtures of OBs and optical brighteners. Potentiation of OB activity was >2500-fold in mixtures with 1% Leukophor AP or Blankophor BA, or >15-fold in mixtures with 0.1% of either brightener, compared to SfMNPV OBs alone. We conclude that SfMNPV production was not increased in JHA treated larvae because the JHA did not result in a supernumerary instar in *S. frugiperda*. However, optical brighteners were highly effective in overcoming developmental resistance to infection in this species.

Detecting positively selected genes in geographically distinct *Spodoptera frugiperda* nucleopolyhedrovirus genotypes: Potential applications in the development of biological insecticides

Leopoldo Palma, Delia Muñoz, Oihane Simón, Trevor Williams, Miguel López-Ferber, Primitivo Caballero 157-160

Abstract: The complete genomic sequences of three *Spodoptera frugiperda* nucleopolyhedrovirus (SfMNPV) genotypes purified from North, Central and South American wild-type isolates were compared in an effort to identify genes potentially involved in virulence or in determining host species barriers. As a first step, the sequencing of the complete dominant genotype within the SfMNPV Nicaraguan isolate, named SfNIC-B, was performed, analyzed, and compared to the complete genome sequences of two other genotypes from the US (SfMNPV-3AP2) and Brazil (SfMNPV-19). The nucleotide sequences of these three genomes were highly conserved and collinear. The genome size of SfNIC-B was the largest and comprised 143 ORFs. Selection pressure analysis over the SfNIC-B ORFs resulted in pairwise ω value combinations greater than one (positive selection) for ten ORFs. These genes are currently under analysis to identify potential positively-selected sites (amino acids) in each single ORF. Positively selected genes will be functionally examined in future studies.

Bacteria

Efficacy of *Bacillus thuringiensis* ssp. *tenebrionis* against different European populations of *Tribolium confusum* in combination with spinosad

Christos G. Athanassiou, Nickolas G. Kavallieratos 163-166

Abstract: The potential of using *Bacillus thuringiensis* Berliner ssp. *tenebrionis* (Bacteria: Bacillaceae) and spinosad against several European populations of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) larvae was examined. The insecticide formulations used were Novodor that contains 3% of *B. thuringiensis* ssp. *tenebrionis* (AI) and NAF-313 that contains 0.12% of spinosad (AI). At the lowest dose rate of *B. thuringiensis* ssp. *tenebrionis*, there were significant differences among the mortality of the populations tested. With the increase of the *B. thuringiensis* ssp. *tenebrionis* dose, these differences were reduced and all populations had similar mortality levels. The addition of spinosad also increased mortality of the exposed *T. confusum* larvae. All larvae died on wheat treated with spinosad and the highest dose rate of *B. thuringiensis* ssp. *tenebrionis*.

Translocation of *Bacillus thuringiensis* in plants and insecticidal activity

Colin Berry, Erica S. Martins, Guy de Capdeville, Rose Monnerat 167-169

Abstract: *Bacillus thuringiensis* was isolated from within the tissues of cotton plants from fields that had never been treated with commercial formulations of this bacterium. Bt *kurstaki* marked by transformation with a plasmid encoding GFP could be introduced to cotton plants by application of spores to the roots. Such bacteria appear to migrate through the xylem and could be recovered from all parts of the plant. Leaves from inoculated plants were able to cause toxicity when fed to *Spodoptera frugiperda*. These results give further insight into the natural ecology of Bt and may provide a novel method for insect control.

Does *Diabrotica*-resistant Bt-maize promote pests like fruit flies and aphids? –

Indications from biosafety research on effects of Cry3Bb1-Bt-maize on Diptera

Wolfgang Büchs, Sabine Prescher, Oliver Schlein 170

Abstract only

Bacillus thuringiensis susceptibility variation among *Ostrinia nubilalis* populations

Maria Cristina Crava, Yolanda Bel, Baltasar Escriche 171-174

Abstract: *Ostrinia nubilalis*, Hübner, (Lepidoptera: Crambidae) has been considered one of the most important corn pests and nowadays is widely controlled with transgenic plants expressing one activated *Bacillus thuringiensis* (Bt) toxin (Cry1Ab). However, this insect species is a secondary pest of other organic farming crops where it is kept under control with Bt formulations. We have tested the susceptibility of an *O. nubilalis* European laboratory strain to a model formulation (Standard Bt HD-1-S-2005) and to their protoxin components; Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa. The principal components of the formulation, Cry1Ab and Cry1Ac, were as toxic as the whole model formulation. Lower toxicity was found for Cry1Aa and Cry2Aa (about 10 and 100 times, respectively). Reported results from North American pointed to a different susceptibility pattern of *O. nubilalis* strains showing a similar toxicity of the tested protoxins and a higher activity of the formulated product. Though the bioassay methodology makes difficult the comparison of results obtained in different laboratories, intrapopulation susceptibility pattern variation has been reported in other lepidopteran species. The present study shows this also happens in *O. nubilalis* and suggests that a susceptibility screening could help to increase the effectiveness of the Bt-based bioinsecticide by choosing the most efficient product for the target population treatment.

Consumption of *Bacillus thuringiensis kurstaki* causes an immune response in

Bt-resistant and *Bt*- susceptible *Trichoplusia ni* colonies

Jerry D. Ericsson, Alida F. Janmaat, Judith H. Myers, Carl Lowenberger..... 175-177

Abstract: Increasing evidence implicates a role for the innate immune system of *Trichoplusia ni* in mitigating the effects of spore-crystal formulations of *Bacillus thuringiensis kurstaki* (*Btk*). We studied the immune response to *B. thuringiensis kurstaki* (*Btk*) in susceptible and resistant *T. ni* after consumption of low doses of *Btk*, and after an injection challenged with a cocktail of bacteria. We measured the expression of genes encoding antimicrobial peptides (AMPs) in the fat body, and the differential number of circulating hemocytes in resistant and susceptible individuals.

Cloning and expression of modified *Bacillus thuringiensis cry1A.105*, *cry2Ab2* and

cry3Bb1 genes from Bt-maize event MON89034 x MON88017

Hang Thu Nguyen, Johannes A. Jehle..... 178

Abstract only

Occupational exposure to airborne *B. thuringiensis* in environments treated with

Dipel

Vinni Mona Hansen, Jørgen Eilenberg, Anne Mette Madsen..... 179-180

Abstract: Knowledge of the natural occurring bioaerosols in horticulture environments can aid in the risk assessment of introducing new organisms into the working environment through bio-control agents. Few studies have quantified vegetable growers' exposure to *Bacillus thuringiensis* (*Bt*) during working hours. In this study we have collected air samples from greenhouses and open fields obtained from workers breathing zone and studied the presence of *Bt* and mesophilic bacteria. The vegetable growers worked in 3 greenhouses or open fields where Dipel had been applied and in 5 greenhouses or fields where Dipel was not applied. Preliminary results showed that vegetable growers to some extent were exposed to *Bt*, although the level did not seem to resemble any hazard.

- Insecticidal activity in root-associated, plant-beneficial pseudomonads
Christoph Keel, Monika Maurhofer..... 181
 Abstract only
- Development of a new microbial insecticide based on *Chromobacterium subtsugae*
Marja Koivunen, Lisa Chanbusarakum, Lorena Fernández, Ratnakar Asolkar,
Eunice Tan, Dan Wallner, Pam Marrone..... 183-186
Abstract: Marrone Organic Innovations has licensed a technology based on a novel species of *Chromobacterium* from USDA and is developing it into a microbial bioinsecticide. The development work includes media optimization to maximize the yield of secondary metabolites responsible for insecticidal activity as well as formulation development for increased efficacy and storage stability. Bioactive compounds are extracted from fermentation broths and the resulting crude extracts are fractionated for compound isolation and identification. Our studies confirm the previous data from USDA; the insecticidal activity of fermentation broths develops over time and coincides with the cell death during the stationary growth phase. Cell-free extracts have good activity against insect pests. The active compounds in the whole-cell broth seem to be heat-stable but some activity is lost during freeze drying. Work is in progress for media optimization, formulation development and active compound identification. Spectrum testing against various insect pests is continuing through bioassays as well as greenhouse and field studies.
- Preliminary studies for the attract-and-kill strategy against the mosquito *Culex pipiens* employing oviposition pheromone and *Bacillus thuringiensis* subsp. *israelensis*
Antonios Michaelakis, Dimitrios C. Kontodimas, M. Anagnostou-Veroniki, Elias Kioulos, George Koliopoulos, Elias A. Couladouros..... 187-190
Abstract: The attract-and-kill strategy requires an intelligent and an efficient combination of an attractant and a killing agent such as a pheromone and an insecticide respectively. The production of the synthetic oviposition pheromone of the mosquito species *Culex quinquefasciatus* (Diptera: Culicidae) was already achieved and its combination with a microbial insecticide was tested. Furthermore two microbial larvicides based on *Bacillus thuringiensis* subsp. *israelensis*, commonly used in Greece, were tested in the laboratory against *Culex pipiens* biotype *molestus* (Diptera: Culicidae) as agents that can keep water free from mosquito larvae. Larvicidal activity, over a 50-day period, revealed no good results. The results from oviposition bioassays revealed that all the tested larvicidals repel gravid females of laying eggs for the first two days. However, when synthetic pheromone is combined with one of the microbial agent, oviposition bioassays revealed the same attractant pattern as synthetic pheromone independently.
- High activity of a *Bacillus pumilus* strain against *Ceratitis capitata*
C. Alfonso Molina, Juan F. Caña Roca, Tania Domínguez, Antonio Osuna, Susana Vilchez..... 191-194
Abstract: *Ceratitis capitata* is one of the world's most destructive and damaging fruit pests. Chemical insecticides are the most used control method, but they are unspecific, environmentally harmful, and in most cases inefficient. Given all these disadvantages and the fact that no efficient field-applicable product is commercially available at the moment, searching for new alternative methods is essential. The entomopathogenic bacteria from *Bacillus* genus are natural agents for biological control of invertebrate pests. Here we report the isolation of a novel strain of *B. pumilus* 15.1 highly toxic against *C. capitata* larvae. We demonstrate that toxicity is revealed only when sporulated cultures are exposed to low temperatures. Isolation of this pathogenic bacterium to *C. capitata* could signify an important finding for the future development of new control strategies.
- Molecular detection of the entomopathogenic bacterium *Pseudomonas entomophila* using Polymerase Chain Reaction (PCR)
Antonios Papagiannoulis, Dimitris Mossialos, Konstantinos Mathiopoulos, Panagiotis Markoulatos..... 195-198
 No abstract

Cloning and expression of modified *Bacillus thuringiensis cry3Bb1* gene from *Bt* maize event MON88017

Hang Thu Nguyen, Johannes A. Jehle.....199-202

Abstract: The gene *cry3Bb1* of transgenic *Bt* maize MON88017 is a modified variant of the wild-type gene of *B. thuringiensis* subsp. *kumatosiensis* encoding δ -endotoxin against coleopteran larvae. Its open reading frame was amplified from transgenic maize leaves by PCR, cloned into pET-28b(+) vector and expressed in *E. coli* strain BL21-DE3 under the control of T7-promoter. When expressed in *E. coli* the protein accumulated as inclusion bodies. As expected, the Cry3Bb1 was expressed as a 77 kDa protein. Cry3Bb1 produced in *E. coli* and in transgenic plants were compared in SDS gel electrophoresis and Western blot. Bioassays using first instar larvae of *Leptinotarsa decemlineata* will be conducted to examine the toxicity of Cry3Bb1 protein.

Phylogeny and pathogenicity of *Xenorhabdus* strains for *Spodoptera littoralis*

Sylvie Pagès, Nadège Ginibre, Christine Laroui, Sophie Gaudriault, Patrick Tailliez, Alain Givaudan.....203-206

Abstract: We used an approach based on a multilocus sequence analysis (MLSA) to address the genealogy within the genus *Xenorhabdus*. The concatenation of four gene sequences – *recA*, *gyrB*, *dnaN* and *gltX* – yields a phylogenetic tree with remarkable robustness that was used to analyse the distribution of the pathogenicity for *Spodoptera littoralis* within the *Xenorhabdus* genus. The results show that the majority of the strains distributed along the phylogenetic tree are not highly pathogenic for *S. littoralis* except *X. nematophila* and *X. indica*.

Field persistence of *Bacillus thuringiensis* subsp. *kurstaki*

Christian Petrucci, Guido Marchi, Tiziana Panzavolta, Rizio Tiberi, Giuseppe Surico, M. Filindassi, L. Ruii.....207-210

Abstract: After an aerial application, the possible persistence of *Bacillus thuringiensis* subsp. *kurstaki* strain HD1 (Btk-HD1) in the soil of an oak forest near Florence (Italy), was assessed by means of morphotyping followed by a high throughput staining methodology and PCR analysis. The results show that although the numbers were relatively high on the phylloplane 24h after the insecticidal treatment, individuals belonging to the subsp. *kurstaki* were never detected among the members of the resident edaphic community of *Bacillus thuringiensis*.

How do toxin producing *Bacillus thuringiensis* strains persist in the field? An evolutionary-ecology perspective

Ben Raymond, Mike Bonsall.....211-214

Abstract: The ecology of *Bacillus thuringiensis* (*Bt*) and selective processes maintaining the highly costly cry toxins are still unresolved. Here, we examined whether *Bt* toxins represent cooperative virulence traits and explored the evolutionary ecology of competition between toxin-producing (cooperators) and toxin-null (cheat) strains in the laboratory and the field. We found that *Bt* toxin have the essential properties of cooperative public goods: within-hosts toxin null cheats can out-compete toxin-producing cooperators but cheats cannot infect hosts in the absence of cooperators. In the field, the toxin-producing phenotype shows strong negative frequency dependence at the level of the host plant: patches dominated by toxin-producers are rapidly invaded by cheats and vice versa. Evidence of competition between cheats and cooperators can also be seen in the strong bimodal distribution of cooperator frequency. We argue that the cooperative nature of toxin production can explain several aspects of *Bt* ecology.

Identification, biosynthesis and synthesis of selected natural products from *Xenorhabdus* and *Photorhabdus*

Daniela Reimer, Alexander O. Brachmann, Edna Bode, Katharina Neitzel, Helge B. Bode.....215-218

Abstract: Together with their symbiotic nematodes of the genera *Steinernema* and *Heterorhabditis*, *Xenorhabdus* and *Photorhabdus* are useful biocontrol agents against various insect pests. Moreover, these bacteria are potent producers of new and interesting natural products that might play a role in the complex life cycle of bacteria, nematodes and insects but that might also be useful as antibiotics or other pharmaceuticals. Therefore, we have investigated the biosynthesis and synthesis of several new natural products including new amides, the blue pigment indigoidine, the depsipeptide xenematide, and the cytotoxic xenortides.

Brevibacillus laterosporus preliminary testing in diary farms for the house fly management

Luca Ruiu, Alberto Satta, Ignazio Floris219-222

Abstract: A biopesticidal formulation containing spores of a *Brevibacillus laterosporus* strain was employed to test its potential for the management of immature house flies. In outdoor cage experiments, simulating a natural house fly environment, a 58% immature flies inhibition was achieved. In diary farms trials, a reduction in immature fly development was obtained with repeated treatments of the cow pens. In 2003, with a formulation at a concentration of 1×10^8 spores/ml applied at a dosage of 2 l/m^2 , reduction was around 30%. In 2008, with a concentration of 2×10^8 spores/ml and a dosage of 3 l/m^2 , the formulation was able to cause an average depression of immature fly development of 46 %. Therefore, the introduction of this bacterial preparation in Integrated Pest Management programmes in diary farms is promising.

Concentration of mycotoxins in maize grains and its relation to the European corn borer damage

Adriana Simanska, Peter Bokor, Ludovit Cagan223-224

Abstract: Maize grains were collected at three localities at the end of two growing seasons. In each variant there were selected the cobs without visible damage and the cobs damaged by the *O. nubilalis* larvae and with the symptoms of Fusarium ear rot. From both variants there were created representative samples and these were analysed for mycotoxin content. Mycotoxin analysis was carried out using appropriate ELISA methods for each toxin. Mycotoxins concentration varied among localities, maize hybrids, years and it was affected by the damage caused by *O. nubilalis* larvae. Mycotoxins level in the grains of Bt maize hybrids was similar to that which was found in the samples from non-damaged cobs of non-Bt maize hybrids.

RVA (Rapid Virulence Annotation) as a functional genomics tool to compare two *Photorhabdus* species with different host ranges

Nicholas R. Waterfield, Andrea Dowling, Paul Wilkinson, Richard H. French-Constant, Maria Sanchez-Contreras225-228

Abstract: We describe an assumption-free approach, Rapid Virulence Annotation (RVA), for the high-throughput parallel screening of genomic libraries against four different taxa: insects, nematodes, amoeba and mammalian macrophages. These hosts represent different aspects of both the vertebrate and invertebrate immune systems. We have applied RVA to the emerging human pathogen *Photorhabdus asymbiotica* using “gain of toxicity” assays of recombinant *Escherichia coli* clones. We describe a wealth of potential novel virulence loci and attribute biological function to several putative genomic islands, which may then be further characterized using conventional molecular techniques.

Preliminary investigations on bacterioses in cambiophagous beetles with special reference to *Scolytus ratzeburgi*

Stanisław Bałazy, Cezary Tkaczuk229

Abstract only

Characterization of Cry1Ac binding in Cry1Ac-resistant *Helicoverpa zea* (Boddie)

Silvia Caccia, Jayadevi Chandrashekhar, William J. Moar, Juan Ferré230

Abstract only

Susceptibility of *Tortrix viridiana* L. to *Bacillus thuringiensis* var. *kurstaki* at different level of larval development

Filippo Castiglia, Barbara Manachini231-234

Abstract: Recently out-breaks of the green oak leaf roller *Tortrix viridiana* L. (Lepidoptera, Tortricidae) were recorded in Natural Reserve in Sicily (Italy) where treatments are generally forbidden. The commercial, social and environmental value of the wood in the forest needs to be preserved and biological control could be applied in particular case. To optimise the permitted biological treatment, not only in terms of product amount but also for the time of application baseline susceptibility of *T. viridiana* from the Natural Reserve of Ficuzza (Palermo, Italy) to a commercial formulation of *Bacillus thuringiensis* was determined for neonates and 2, 5, 10 and 15 day old larvae. The differences in susceptibility of the different larval development were recorded. For neonates the calculated DL50 was $0.63 \mu\text{g/ml}$, while after five days it was $10.01 \mu\text{g/ml}$. The same doses had little effect on the older larvae, showing a clear decrease in susceptibility with age

and larval growth. A relationship was also found between susceptibility and body length. The implications of these data in controlling this pest in the natural reserve are discussed.

Studies on the proteolytic processing and binding of *Bt* toxins Cry3Bb1 and Cry34Ab1/Cry35Ab1 in the midgut of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

Renate Kaiser-Alexnat, Wolfgang Büchs, Jürg Huber235-238

Abstract: The Western corn rootworm (WCR) is one of the most economically important corn pests worldwide. One possibility for controlling this pest is the cultivation of *Bt*-corn. However, widespread cultivation of *Bt*-corn may increase the probability of the development of pest populations resistant to the respective *Bt* toxins. To establish test systems for identifying resistance mechanisms in the case of resistance development, different parameters involved in the processing of *Bt* toxins in the midgut of third instars larvae of WCR (European strain) were studied. The proteolytic processing of *Bt* toxins Cry3Bb1 and Cry34Ab1/Cry35Ab1 by WCR midgut juice was examined, but no degradation of any of these toxins was observed. Ligand-blot binding analyses with Cry3Bb1 as well as Cry34Ab1 and Cry35Ab1 revealed specific receptors in the WCR midgut epithelium. The molecular weights for Cry3Bb1, Cry34Ab1, and Cry35Ab1 receptors were characterized as having molecular weights of approximately 30 kDa, 110 kDa, and 50 kDa, respectively.

Potential effects of *Bacillus thuringiensis* against adults and older larvae of *Rhynchophorus ferrugineus*

Barbara Manachini, Paolo Lo Bue, Ezio Peri, Stefano Colazza239-242

Abstract The Red Palm Weevil, *Rhynchophorus ferrugineus* (Oliv.) (Coleoptera, Curculionidae) is the most important pest of the date trees in many countries. It reached Italy in 2004. One of the alternatives for its control could be the utilization of *Bacillus thuringiensis* (Bt), an entomopathogenic bacterium characterized by its production of insecticidal crystal proteins. Larvae and adults of *R. ferrugineus* were assayed on a meridic diet containing a commercial product of Bt active against Coleoptera. The LC50 for adults was very high superior to 2.0 mg/ml. Larval mortality, body weight, and number of surviving larvae that did not gain significant weight (<0.5 mg per larva) were also recorded after 21 days. Actual and practical mortality were calculated for RPW larvae. The calculated LC50 value of Bt based on practical mortality was 0.45 mg/ml. Negative effect of Bt treated diet on the weight of RPW larvae was recorded.

Regulation of the expression of *Fit* insect toxin locus genes in the root-associated biocontrol pseudomonad CHA0

Maria Péchy-Tarr, Olivier Binggeli, Monika Maurhofer, Christoph Keel243-247

Abstract: The root-colonizing *Pseudomonas fluorescens* strain CHA0 is a biocontrol agent of soil-borne plant diseases caused by fungal and oomycete pathogens. Remarkably, this plant-beneficial pseudomonad is also endowed with potent insecticidal activity that depends on the production of a large protein toxin termed Fit (for *P. fluorescens* insecticidal toxin). In our present work, the genomic locus encoding the *P. fluorescens* insect toxin is subjected to a detailed molecular analysis. The Fit toxin gene *fitD* is flanked upstream by the *fitABC* genes and downstream by the *fitE* gene that encode the ABC transporter, membrane fusion, and outer membrane efflux components of a type I protein secretion system predicted to function in toxin export. The *fitF*, *fitG*, and *fitH* genes located downstream of *fitE* code for regulatory proteins having domain structures typical of signal transduction histidine kinases, LysR-type transcriptional regulators, and response regulators, respectively. The role of these insect toxin locus-associated control elements is being investigated with mutants defective for the regulatory genes and with GFP-based reporter fusions to putative promoter regions upstream of the transporter genes *fitA* and *fitE*, the toxin gene *fitD*, and the regulatory genes *fitF* and *fitH*. Our preliminary findings suggest that the three regulators interact with known global regulators of biocontrol factor expression to control Fit toxin expression and secretion.

Toxicity of *Bacillus thuringiensis* Cry proteins against the olive moth *Prays oleae*

Sergio Pérez-Guerrero; Hani K. Aldebis, Enrique Vargas-Osuna248-250

Abstract: *Prays oleae* (Bernard) is one of the most important pests of olive orchards in countries from the Mediterranean basin. *Bacillus thuringiensis* is being included in Integrate Pest Management programs as an effective biological control agent. For the first time, in this work, activity against third-instar *P. oleae* larvae of nine Cry proteins have been analysed. All Cry

proteins tested were toxic against *P. oleae* showing Cry2Aa, Cry1Ca and Cry1Aa the highest larval mortality percentages. Toxicity of the most active toxin as mean lethal concentration (LC₅₀) value was also calculated.

Occurrence and molecular diversity of the Fit insect toxin locus in plant-beneficial pseudomonads

Beat Ruffner, Maria Péchy-Tarr, Christoph Keel, Monika Maurhofer251-254

Abstract: The application of plant-beneficial pseudomonads provides a promising alternative to chemical pest management in agriculture. The fact that *Pseudomonas fluorescens* CHA0 and Pf-5, both well-known biocontrol agents of fungal root diseases, exhibit also potent insecticidal activity is of particular interest, as these plant-beneficial bacteria naturally colonize the rhizosphere of important crop plants. Insecticidal activity in these strains depends on a novel locus encoding the production of a protein toxin termed Fit (for *P. fluorescens* insecticidal toxin). To gain a better understanding of the ecological relevance of the *Pseudomonas* anti-insect activity, we have begun to investigate the occurrence and molecular diversity of the Fit toxin genes among root-associated pseudomonads. To this end, we have screened a large world-wide collection of fluorescent *Pseudomonas* sp. isolated from the roots of different plant species using molecular fingerprinting techniques. The strains are already well characterized for exoproduct patterns and disease-suppressive ability and are currently being tested for insecticidal activity in a greater wax moth larvae assay system.

Interaction of loop 1 of *Bacillus thuringiensis* Cry3Aa domain II with Colorado

Potato Beetle midgut epithelium cells generates cytotoxicity

Jorge Sánchez, Camila Ochoa-Campuzano, M. Dolores Real and Carolina

Rausell 255

Abstract only

Fungi

Potential of *Lecanicillium* spp. for use against the melon aphid and cucumber powdery mildew

Tsisia Chkhubianishvili, Manana Kakhadze, Iatamze Malania, Tina Kuprashvili,

Mark Goettel259-262

Abstract: The melon aphid *Aphis gossypii* and the cucumber powdery mildew fungus *Erysiphe cichoracearum* D.C. f. *cucurbitacearum* Poteb. are the major pest organisms of cucumbers in both open and closed environments in Georgia. Key elements of our present investigations are the establishment of pathogenicity of introduced *Lecanicillium* spp. to the above mentioned pest organisms. Results of our laboratory experiments demonstrated activity against both aphid and powdery fungus justification for further investigations under field conditions.

Biocontrol of whiteflies based on *Lecanicillium lecanii*

Alba Marina Cotes, Carlos Espinel, Laura Villamizar, Ilán Garzón, María Denis

Lozano, Aristóbulo López-Ávila263-266

Abstract: A native isolate of the fungus *Lecanicillium lecanii* VI026 originally isolated from an adult of *Trialeurodes vaporariorum* demonstrated biocontrol activity against *T.vaporariorum* and *Bemisia tabaci*. In order to develop a formulated biopesticide, initially, a massive production medium was standardized by evaluating two solid substrates supplemented with two nutrient solutions. No significant differences were observed among treatments as compared with substrates supplemented only with water obtaining a yield of 2.1 x 10⁹ conidia per cm² in S1 substrate after 8 days of incubation at 25°C, which was selected to produce this fungus. Produced conidia were separated and used as active ingredient for biopesticide. The product was designed as a wettable powder for foliar application, in which, a sunscreen and a drying protectant was included. This formulation presented concentration of 10¹⁰ conidia.g⁻¹, germination of 96%, moisture content of 4.2% and a protection against ultraviolet radiation of 100% and demonstrated efficacy.

Side-effect of the entomopathogenic fungus *Lecanicillium muscarium* on the predatory mite *Phytoseiulus persimilis*

András Donka, Helga Sermann, Carmen Büttner267-269

Abstract: In biological control, different beneficial organisms have to be combined for an effective management. If entomopathogenic fungi will be integrated, than the effect on non-target organisms like beneficial arthropods has to be considered as well. Because of the high importance of the predatory mite *Phytoseiulus persimilis* in biological control, side effects of *Lecanicillium muscarium* on this species were assessed. In two standardised bioassay in Petri dishes and on plants (*Phaseolus vulgaris*) individual mites were dipped in suspension or put on leaf after spraying with *L. muscarium* at different spore density. Results indicate pathogenicity of the fungus towards the predatory mite, however, the risk of infection decrease, the closer the conditions are to application density used under practical conditions. At concentration of 10^6 and 10^7 spores ml^{-1} no risk for predatory mite is expected on the plant.

Entomopathogenic fungi and the codling moth, *Cydia pomonella*: Comparison of different fungal isolates and the effect of CpGV resistance on the infectivity of fungi

Julia Flammersfeld, Regina G. Kleespies, Gisbert Zimmermann, Kerstin Jung270

Abstract only

Metarhizium anisopliae interactions with ticks' eggs

Galina Gindin, Dana Ment, Asael Rot, Itamar Glazer, Michael Samish271-274

Abstract: Efficacy of two entomopathogenic hyphomycetes fungi, *Metarhizium anisopliae* var. *acridum* (*M. an. ac.*) and *M. anisopliae* var. *anisopliae* (*M. an. an.*) was evaluated against eggs of three tick species – *Hyalomma excavatum*, *Rhipicephalus annulatus* and *Rhipicephalus sanguineus*. Eggs laid by surface-sterilized females were spread on conidia-impregnated filter paper. Although *M. an. an.* differed in their virulence to the tested ticks, they reduced the hatching percentages of eggs of all three tick species to 0–32% compared with 80–90% in the control eggs. The *M. an. ac.* strains were highly virulent to *H. excavatum* and *R. sanguineus* eggs, reducing the hatching percentages to 2–6 %, but had no influence on hatching of *R. annulatus* eggs. The effects of lipid fractions from tick eggs surface on the development of conidia were tested. Both germination of *M. an. an.* conidia and the formation of appressoria were stimulated by extracts from egg cuticles of all three tested tick species. However, the stimulating effect was lower when the conidia were exposed to lipids from relatively less susceptible *R. annulatus* eggs than when exposed to lipids from *H. excavatum* or *R. sanguineus* eggs. Conidia of *M. an. ac.* exposed to such lipid extracts did not germinate or form appressoria.

Further progress with *Metarhizium* microsclerotial production

Stefan T. Jaronski, Mark A. Jackson275-278

Abstract: Microsclerotium production by *Metarhizium anisopliae*, previously reported for flask scale, was successfully achieved at a 100-Liter fermenter scale. The resulting granular formulations readily conidiated on water agar or in moist soil to the same extent as reported for flask fermentations. Both Pharmamedia and Hycase M were suitable substitutes for the original casamino acids, with the former being slightly superior in terms of subsequent conidial production in soil. Both were slightly superior to casamino acids in terms of efficacy against the sugarbeet root maggot in non-sterile clay soil. Rates, as low as 20 mg microsclerotial formulation per 100 g non-sterile clay soil yielded 50% larval sugarbeet root maggot mortality within 1 week and > 90% after 2 weeks.

DNA polymorphisms in hybrid strains of entomopathogenic fungi *Lecanicillium* spp.

Fusako Kaibara, Masanori Koike, Daigo Aiuchi, Hisayuki Oda, Yoshinori

Hatakeyama, Iwano Hidetoshi279-282

Abstract: The entomopathogenic fungi *Lecanicillium* spp. are exploited commercially as useful biological control agents all over the world. Three strains, Vertalec and Mycotal with a high specific virulence against aphids and whiteflies, respectively, and B-2 with a high ability to colonize leaves were fused to obtain a new strain combining the beneficial characteristics into one strain. We investigated diagnostic fragments among these parental strains and some hybrid strains in order to detect molecular markers to distinguish hybrid strains. The internal transcribed spacer (ITS) and intergenic spacer (IGS) region of ribosomal DNA (rDNA) were analyzed by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and

subsequently investigated DNA haplotypes by combining primers, which amplified the ITS region and microsatellites. A uniform biased tendency of the DNA banding pattern was observed depending on the combination of parental strains. Finally, we detected unique DNA patterns generated from genome profiling (GP).

Fungal BCAs in viticulture: Compatibility with common fungicides

Martin Kirchmair, Sigrid Neuhauser, Hermann Strasser, Lars Huber.....283-286

Abstract: According to EUROSTAT, approximately 150,000 tons of fungicides were sold in the EU 15 in 2001. Seventy percent of them were applied in viticulture. Thus, the application of fungal BCAs has to be coordinated with the necessity of the conventional control of fungal pathogens. We tested the effect of fourteen common fungicides on the growth of a *Metarhizium anisopliae* isolate which is intended to be used for the control of grape phylloxera. The ASTM standard D 5590-00 was applied to determine the susceptibility of *Metarhizium* to fungicides (Standard Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay). Polyram (Dithiocarbamates) and CAA-fungicides (Melody Combi, Forum Star) inhibit the growth of *Metarhizium* when half of the recommended concentrations are used. No inhibition of growth could be observed when *M. anisopliae* was exposed to QoI-fungicides (Collis, Flint, Equation Pro), DMI-fungicide (Topas, Systhane), amines (Prosper), quinolines and pyrimidines (Vento), PA-fungicides (Ridomild Gold Combi), phosphonic acid (Phosfik) or wettable sulphur. Also Electis (dithiocarbamates) lead to no inhibition of growth. Most fungicides induced a visible increase of sporulation when used at low concentrations.

Mechanisms important for the epidemic development of *Neozygites floridana* in

Tetranychus urticae populations

Ingeborg Kligen, Silje Stenstad Nilsen287-289

Abstract: In this study we aimed at understanding the mechanisms that affect an epidemic development of *Neozygites floridana* in a *Tetranychus urticae* population. This was done by comparing how many spores a cadaver infected with a *N. floridana* isolate could produce and at what distance and in which directions they could be thrown on a coverslip at temperatures relevant to the northern hemisphere (13, 18 and 23°C). The highest number of spores were produced at 13°C at a number of 1,886 per cadaver. Numbers of spores thrown at 18°C and 23°C were 1,733 and 1,302, respectively. Temperature had a significant effect on sporulation. Most of the spores were thrown at a distance of 0–0.6 mm from the cadaver. Cadavers placed on the underside of a coverslip were able to throw spores back up on the coverslip surface. A whole plant bioassay was also conducted to reveal where on a plant *T. urticae* infected with *N. floridana* die and sporulate. Cadavers showed a different vertical distribution on the cucumber plant compared to healthy spider mites. Most of the cadavers were located at the lower to the middle part of the plant, while healthy spider mites were more evenly distributed on the whole plant.

Occurrence of entomopathogenic fungi in soils from different habitats in agricultural landscape

Cezary Tkaczuk.....290

Abstract only

Rhizosphere competence of insect pathogenic fungi in the control of *Othiorhynchus sulcatus* in strawberries under cold climatic conditions

Ingeborg Kligen, Karin Westrum291-294

Abstract: The vine weevil, *Othiorhynchus sulcatus*, is a serious pest in strawberries in Norway and biological control methods are needed to combat this pest. In this study, the rhizosphere competence of two cold active Norwegian fungal isolates (*Metarhizium anisopliae* isolate NCRI 250/02 and *Beauveria bassiana* NCRI 12/96 and the well known Ma43, originating from Austria) were tested by estimating fungal concentrations counting colony forming units (CFUs) in the bulk and rhizosphere soil surrounding the strawberry plant roots. The highest numbers of *B. bassiana* NCRI 12/96 CFUs were recorded in the rhizosphere at 1.87×10^9 per liter soil 3 months after application. The highest numbers of *M. anisopliae* NCRI 250/02 CFUs were recorded in the rhizosphere at 2.41×10^9 per liter soil 1 year after application. Numbers of CFUs of *M. anisopliae* Ma43 CFUs were generally lower than for the Norwegian isolates, but also for this isolate a higher fungal concentration was found in the rhizosphere soil than in the bulk soil.

Persistence of the entomopathogenic fungus *Lecanicillium muscarium* under outdoor conditions

Sandra Lerche, Helga Sermann, Carmen Büttner295-298

Abstract: Results of laboratory trials, which demonstrated the efficacy of the entomopathogenic fungus *Lecanicillium muscarium* against mining larvae of the horse chestnut leafminer moth *Cameraria ohridella* led to outdoor trials. One aspect of these trials was to determine the persistence of two fungi, the commercial product Mycotal® (Koppert, NL) and strain V24 from our collection. Different spore concentrations and the influence of an oil-containing adjuvant (Koppert, NL) on persistence were tested. The persistence of the fungus was determined through the number of colony forming units (cfu) after impressing the leaves on agar plates. The fungus could be detected until 14 days post application (dpa), with differences between the variants, despite most unfavourable weather conditions, like above-average of temperature and hours with sunshine as well as low humidity and heavy rainfall. In all variants dead and moulding larvae were found within the mines.

The potential of *Beauveria bassiana* and *Metarhizium anisopliae* strains for control of the cattle tick *Rhipicephalus annulatus*

Dana Ment, Rula Abu-Jreis, Naim Iraki, Galina Gindin, Asael Rot, Itamar Glazer, Michael Samish299-302

Abstract: The pathogenicity of 3 *Beauveria bassiana* and of 5 *Metarhizium anisopliae* strains from Israel, Ethiopia and the Palestinian Authority against engorged *Rhipicephalus (Boophilus) annulatus* females and eggs was examined. Two *B. bassiana* and 4 *M. anisopliae* strains were found to be highly virulent toward ticks (>50% mortality) regardless of their geographic or host origin. The infection was evidentially transmitted from the females to the eggs they laid, resulting in a reduction of hatching to 0-12.5% by the virulent strains and 25-50% by the lower virulent strains. Direct inoculation of eggs by all tested strains except one resulted in a complete inhibition of larvae hatching.

Metarhizium anisopliae chlamydospores in tick eggs

Dana Ment, Galina Gindin, Itamar Glazer, Shmuel Perl, Elad Dani, Michael Samish303-306

Abstract: *Metarhizium anisopliae* chlamydospores were rarely described previously and the conditions needed for their development were not identified. For the first time, the presence of *M. anisopliae* chlamydospores in the tick eggs and the ambient conditions influencing its formation are reported. The infection of tick eggs by *M. anisopliae* involves the common events such as adhesion, conidia germination, penetration of the egg chorion and development of hyphae in the egg cytoplasm. However, the final fungal developmental stage in egg differs according to the ambient conditions. At high humidity (100%) and moderate temperature (25°C) the fungus emerges out of the dead eggs and forms conidiophores with conidia. Stress conditions, in which the temperature was elevated to 30 °C or the humidity was reduced to 55 – 75 %, induced the production of chlamydospores inside the eggs without emergence of fungi on the eggs surfaces. When eggs with chlamydospores were transferred to favorable conditions (25°C / 100 % RH), conidiogenesis was observed. Observations were done by means of histology and TEM.

The criteria for selecting *Metarhizium anisopliae* thermo-tolerant strains for the control of arthropods on vertebrates

Dana Ment, Galina Gindin, Itamar Glazer, Rula Abu-Jreis, Naim Iraki, Asael Rot, Michael Samish307-310

Abstract: The tolerance of fungi to high temperature is an important criterion for choosing strains for controlling ticks while feeding on the warm skin of vertebrates. The efficacy of *Metarhizium anisopliae* var. *anisopliae* strains with different thermal characteristics was compared in two model experiments: 1) at temperature reflecting the common conduction on animals surface (35°C) and 2) at a relatively short increase of temperature up to the maximum that was detected on cattle skin for limited time (37°C and 40°C). The present study show that isolates suitable for application to cattle must be able not only to germinate and grow at higher temperature but also to fast recover the conidia germination and keep their virulence after temporal rise of temperature above the one in which the fungus develop and infect.

Phylogenetic characterization of entomopathogenic fungi from Uzbekistan

Kakhramon Ergashev, Anaida G. Guzalova, Andreas Leclerque311-314

Abstract: As part of a wider study of the biocontrol potential of entomopathogenic fungi from Central Asia, the pathogenicity of a number of insect-derived fungal isolates from Uzbekistan was assessed and its previous morphological assignment to the genera *Beauveria*, *Metarhizium*, *Fusarium*, *Trichoderma*, *Paecilomyces*, or *Aspergillus* re-evaluated and in most cases confirmed molecularly by a phylogenetic analysis based on internal transcribed spacer (ITS1) sequences.

Mycotal, a microbial insecticide recently registered in Spain to control whiteflies in IPM programs

Magda Galeano, Jose Eduardo Belda, Willem Ravensberg 315

Abstract only

Occurrence and distribution of entomopathogenic fungi in Moroccan endemic forests of *Argania spinosa*

A. Imoulan, A. Alaoui, A. El Meziane 316-319

Abstract: The fungi *Beauveria* and *Metarhizium* have a world-wide distribution. These species are frequently found in the soil and are known by their ability to control a wide range of insects. The use of local entomopathogenic fungi will be appropriate to control insects since these strains may be better adapted to local environmental conditions. Our study aimed to analyze the occurrence and the natural abundance of *Beauveria* and *Metarhizium* spp. in Moroccan forests of *Argania spinosa* (endemic Moroccan tree) and to isolate and select potential strains for control of insects. The presence of entomopathogenic fungi was examined by using selective media for *Beauveria* and *Metarhizium* spp. and by the *Galleria* baiting methods. In approximately 55% of soil samples the occurrence of *Beauveria* on selective medium was recorded, whereas *Metarhizium* spp. were found only at low density in only three regions. The baiting method revealed the presence of entomopathogenic fungi in all soil samples. *Beauveria bassiana* was dominant in all soil samples, whereas *Metarhizium* spp. was recovered mainly from soil collected in South-Western Morocco. More than 400 isolates were identified and constitute the first Moroccan collection of entomopathogenic fungi. In addition, isolates were characterized for their temperature tolerance and potential to produce cuticle-degrading enzymes.

Assessment of *Metarhizium anisopliae* for control of the Japanese Beetle on the Azores

Aida Medeiros, José Mota, Stefan Jaronski 320

Abstract only

Entomopathogenic fungi isolated from soil in the vicinity of *Cameraria ohridella* infested horse chestnut trees

Eva Preserve, Rostislav Zemek, František Weyda, Lubomír Volter 321-324

Abstract: Occurrence of entomopathogenic fungi in soil samples collected near horse chestnut trees infested by its pest, *Cameraria ohridella*, was surveyed using the modified *Galleria* bait method. The results revealed that entomopathogenic fungi frequently occur in the soil collected from *C. ohridella* habitats. Dominant species found were *Isaria fumosorosea* (Wize) Brown et Smith and *Beauveria bassiana* (Balsamo) Vuillemin. The isolated strains are deposited in the CCEFO (Culture Collection of Entomopathogenic Fungi Olešná) in the Czech Republic. The virulence of 100 native strains was tested using standardised biotests.

Microbiological control of the leaf-footed bug *Leptoglossus occidentalis*

Pietro Rumine, Gian Paolo Barzanti 325-326

Abstract: The leaf-footed bug *Leptoglossus occidentalis* Heidemann is one of the many phytoparasites infesting pine trees in several forest areas of Italy. This species has shown strong development in recent years, seriously compromising pine-seed production. To assess the effectiveness of microbiological control of the pest, we conducted laboratory trials in 2006-2008 using isolates of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. collected from Italian agro-forest environments. In preliminary trials, apical sprigs of *Pinus nigra* Arn. and adult specimens of *L. occidentalis* were placed in plastic boxes and sprayed with conidial suspensions of the fungi. Further treatments were carried out directly on 1-year-old, 50-cm-high *Pinus nigra* plantlets sprayed with suspensions of the entomopathogenic fungi. The results showed that the leaf-footed bug is sensitive to infection by the fungal isolates, some of which killed the treated individuals within 10 to 30 days.

Evaluation of some parameters influencing the activity of a fungal biocontrol agent used for *Bemisia tabaci* control

Laura Villamizar, Erika Grijalba, Victoria Zuluaga, Martha Gómez, Alba M. Cotes. 327-330

Abstract: Some problems with using biological agents under field conditions are their susceptibility for sun radiation and their incompatibility with commonly used chemical pesticides. This is often due to the use of no or poor formulations. When photostability of a previously developed biocontrol product based on a *Lecanicillium lecanii* isolate was evaluated by exposure to sun radiation, a reduction of 69.7% in the germination of non-formulated *L. lecanii* (VL026) conidia was obtained, compared with 37.4% reduction observed with the formulated conidia, six hours after exposure to radiation. Chemical pesticides, like Chlortalonyl, Diphenconazole, Carbofuran and Dimethoate were not compatible with the fungus, whereas Sulfur, Cooper Oxichloride, Propiconazole and Carboxyn-Captan showed some compatibility. Under laboratory conditions, formulated and non-formulated conidia produced 80.6% and 69.1% mortality of *B. tabaci* nymphs, respectively.

Possibility of microbial control using entomopathogenic fungi *Lecanicillium* spp. hybrid strains and *Beauveria bassiana* against the diamondback moth

Keika Yamada, Daigo Aiuchi¹, Masanori Koike, Toshio Masuda..... 331-334

Abstract: For control of the diamondback moth (*Plutella xylostella*), the pathogenicities of *Lecanicillium* sp. and *Beauveria bassiana* were investigated. Conidial suspensions of 1×10^6 /ml of 13 hybrid isolates of *Lecanicillium* spp. were used in bioassays against second-instar larvae. Of these isolates, 2aF27 was selected based on the low LT_{50} value and the mortality 6 days after inoculation. Then suspensions of 2×10^7 /ml of 2aF27 and *Beauveria bassiana* (strain MG-Bb-1) were sprayed on a cabbage field. MG-Bb-1 treatment provided high control of the *P. xylostella* population, whereas Bt. 2aF27 was less effective. Results suggest that entomopathogenic fungi qualify as candidates for microbial control of *P. xylostella*.

Nematodes

Identifying genes that are involved in the recovery process of the entomopathogenic nematode *Heterorhabditis bacteriophora* TTO1 strain

Anat Moshayov, Hinanit Koltai, Itamar Glazer..... 337-340

Abstract: Characterizing the process of recovery in *Heterorhabditis bacteriophora* was done by identifying genes that are putatively involved in this process. For this purpose, a large scale bioassay for recovery was established and two subtraction libraries of recovered IJs subtracted by arrested IJs were constructed. Six hundreds expressed sequence tags (ESTs) were sequenced and annotated resulting in 300 useful ESTs that were compared to the *C. elegans* Wormbase and categorized into functional categories according to gene ontology. Of these, twenty three genes were chosen for further analysis. These genes were examined for their expression in the recovery process by quantitative (q) RT-PCR. The results of the RT-qPCR supported the results obtained from the subtraction libraries. Further analysis to these genes is being done by RNAi-based functional analysis in *H. bacteriophora*.

Improving application to enhance pest control with entomopathogenic nematodes.

Brown Andrew P..... 341-344

Abstract: Entomopathogenic nematodes (EPN) (Nematoda: Rhabditidae) have been commercially manufactured for over 20 years. Their use, as with other biological control agents, has increased in recent years due to desire for more environmentally sensitive growing and legislative restrictions on chemical use. Beneficial nematodes offer high levels of pest control which enable them to compete in conventional pest control markets against a number of chemical insecticides. In order to obtain the highest levels of control with these biological control agents, targeted application is very important. This paper will discuss the advances in understanding in application of *Steinernema feltiae* (Nemasys™) to control sciarid larvae (*Bradysia* spp.) and Western flower thrips (*Frankliniella occidentalis*) to a number of growing systems which has helped to improve the commercial use of EPN.

Control of carpophagous Lepidoptera in chestnut by means of entomopathogenic nematodes

Giovanna Curto, Alberto Reggiani, Elisabetta Dallavalle, Massimo Bariselli345-348

Abstract: *Cydia splendana* and *C. fagiglandana* are the main insect pests of chestnut groves in the Emilia-Romagna (Northern Italy). They damage fruits and cause sizeable quantitative and economic losses. A biennial investigation was carried out in some chestnut groves in the provinces Bologna and Ravenna with the aim of evaluating sustainable strategies for the control of both moth species with entomopathogenic nematodes (EPN). The effectiveness of different EPN species on moth larvae was tested in experimental trials. The application of EPN suspension on the ground showed encouraging results in decreasing the fruit damage at harvest.

Conditioning of *Steinernema kraussei* and *S. carpocapsae* through storage:

Enhancing their performance over wide temperature ranges

Adam G. Guy, Denis J. Wright, Michael Gaffney, Christine T. Griffin349-352

Abstract: The influence of conditioning of entomopathogenic nematodes *Steinernema kraussei* and *S. carpocapsae* by storage at low temperature was assessed. *S. kraussei* virulence greatly decreases between 15 and 20°C suggesting that it is a poor biocontrol agent in warm conditions; *S. carpocapsae* shifted from showing poor to higher virulence between these temperatures. Virulence of both EPN species can be enhanced, at times to over 90% host death, at both sub-optimal and optimal temperatures following storage at low temperatures, this being greatest following 9-12°C storage. The greatest conditioning cases occurred for *S. kraussei* bioassayed at 27°C, and *S. carpocapsae* bioassayed at 9-15°C. Results indicate that low temperature storage causes major conditioning effects in terms of EPN virulence.

Population dynamics of *Steinernema carpocapsae* and *S. feltiae* in liquid culture

Ayako Hirao, Ralf-Udo Ehlers353-356

Abstract: *Steinernema carpocapsae* and *S. feltiae* were cultured with their symbiotic bacteria in liquid media. Although these two species are broadly used as biological control agents, the life cycle in liquid culture has not been described well. In this study, the population dynamics of both *Steinernema* species in liquid culture was observed in relation to the development of their symbiotic bacteria in the monoxenic cultures.

Optimal incubation temperature for *Steinernema carpocapsae* and *S. feltiae* in liquid culture

Ayako Hirao, Ralf-Udo Ehlers357-360

Abstract: Mass production of *Steinernema carpocapsae* and *S. feltiae* is done in liquid culture by pre-culturing the symbiotic bacteria of genus *Xenorhabdus* spp. for 1 to 2 days prior to dauer juveniles (DJ) inoculation. Major problems for commercial production can originate from variable progeny DJ density. This study aimed to determine optimal incubation temperature for DJ reproduction to reduce process duration. Temperatures tested were 20, 23, 25, 27 and 29°C. The population development was observed. The onset of development of DJs (termed 'recovery') and the fecundity in *S. carpocapsae* were significantly reduced at 29°C. Only the fecundity but not recovery of *S. feltiae* was reduced at high temperature (27°C). The DJ yield was lower at high temperature and the development was delayed at low temperature in both *Steinernema* spp. Optimal growth temperature for both nematodes is 25°C. Highest DJ densities were obtained after 10 days incubation at 25°C.

First field experiment with entomopathogenic nematodes in Slovenia

Žiga Laznik, Tímea Tóth, Tamás Lakatos, Stanislav Trdan361-364

Abstract: Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) was the target pest in our research. The experiment had 6 different treatments: *S. feltiae* B30 Iconc., *S. feltiae* B30 hconc., Entonem Iconc., Entonem hconc., Actara and control all repeated in 4 blocks. Observing the population dynamics of CPB we conclude, that entomopathogenic nematodes have a big influence on larval stages but on the other hand no effect on egg and adults in field experiment. Insecticide Actara showed the best results among the observations. Here the mortality of the insect was the highest. We also studied the effect of controlling CPB on the yield of potato. There were no differences between EPN treatments, however the results using EPN were better compared to control treatments and less evident as the results of insecticide Actara.

- Dosage screening with *Heterorhabditis indica* for grub control in peanut fields
Liu Qi-zhi, Wu Han-dong, Arne Peters, Du Xiao-Kang, Zhang Li-juan, Liang Lin-lin, Xie Na, Ralf-Udo Ehlers.....365-368

Abstract: A trial with the entomopathogenic nematode (*Heterorhabditis indica* in dosages of 2,000, 3,000, 4,000, 5,000 and 10,000 nematode/peanut plant was conducted for selecting an effective dosage to control grubs. Damaged plants (%) were significantly less in all treatments compared to the control treated with water only. The dosages of >4,000/plant resulted in 4.1%, 5.7% and 7.5% damage, respectively, which was significantly lower than 10.2% damage recorded in the chemical control treated with 40% isofenphos-methyl. A 95.7% decrease in number of grubs was achieved with 4,000 and 5,000 nematodes/plant, which was significantly higher than in the chemical control (82.7%). Peanut yields at 4,000 nematodes/plant were 327.6 kg/667m², significantly higher than in the chemical control (292.6 kg/667m²). The results demonstrate that *H. indica* can successfully control chafer grub in peanuts and subsequently increase peanut yields and is superior to the chemical control strategy. The dosage of 4,000 nematodes/plant can be recommended.

- Use of entomopathogenic nematodes and chitosan against *Rhynchophorus ferrugineus* and *Paysandisia archon* in *Phoenix canariensis*, *P. dactylifera* and *Chamaerops humilis* in Spain
Mar Martinez de Altube, Alejandro Martinez Peña.....369-370
Abstract only

- Genetic breeding for heat tolerance of *Heterorhabditis bacteriophora*
John Mukuka, Olaf Strauch, Ralf-Udo Ehlers.....371-374

Abstract: High temperatures > 30°C can occur during transportation of entomopathogenic nematodes to the user and result in loss of viability and quality of nematode products. This study investigated the possibilities to improve heat tolerance of the nematode *Heterorhabditis bacteriophora*. The increase of heat tolerance in *H. bacteriophora* through genetic selection has been reported previously. This contribution screened sixty *H. bacteriophora* strains for their survival at high temperatures and subsequently crossed the most heat tolerant strains to further improve the tolerance. There were significant differences in heat tolerance among the *H. bacteriophora* strains. Adaptation to higher temperature for 3 h could not increase heat tolerance and no correlation was recorded for tolerance with or without adaptation. The mean tolerated temperatures ranged from 33.3°C to 40.1°C for non-adapted populations and from 34.8°C to 39.2°C for adapted populations. Prior to crossing the most tolerance strains, only the 10% with the highest tolerance of one population were propagated. The mean heat tolerance of hybrid strains was 40.3°C for non-adapted and 39.9°C after adaptation. These results confirmed superior performance of hybrid strains over parental nematodes.

- Use of entomopathogenic nematodes for the control of *Paysandisia archon*
Burmeister
Sandro Nardi, Emanuela Ricci, Roberto Lozzi, Francesco Marozzi, Edith Ladurner, Federico Chiabrando, Nunzio Isidoro, Paola Riolo375-378

Abstract: A preventative and a curative field trial were carried out in 2008 on in-ground and potted *Trachycarpus fortunei* (Hooker) Wendland palms in order to evaluate the efficacy of two different formulations of the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) in respectively preventing new *Paysandisia archon* Burmeister infestations and controlling the palm pest on already infested plants. The tested *S. carpocapsae* formulations showed very high efficacy (close to 100%) in both preventing new infestations and controlling *P. archon*. The entomopathogenic nematode can thus be considered a highly valuable tool for the control of this target pest.

- Analysis of secreted/excreted products of *Steinernema carpocapsae* (Nemata)
Nelson Simões, Duarte Toubarro, Natesan Balasubramanian, Gisela Nascimento, Yingjun Jing, YouJin Hao, & Rafael Montiel.....379-382

Abstract: A genomic and proteomic approach was used to identify transcripts of the parasitic phase of *Steinernema carpocapsae*. Among the 1,592 unique sequences obtained, 119 were predicted to be secreted and a few were hypothesized to be interacting with insect host. Four secreted/excreted serine-proteases were proved to cause disruption in insect host likely facilitating invasion and evasion.

Contribution to the knowledge of insect-nematode associations

Eustachio Tarasco, Oreste Triggiani383-386

Abstract: Among nematodes the species associated with insects are present in more than 30 families. During our research activities we found the following insect-nematode associations: *Eudiplogaster aphodii* Bovien (Rhabditida, Diplogasteridae) with *Aphodius fimetarius* Linnaeus, 1758 (Coleoptera, Scarabaeidae); *Oryctonema pentodonis* Poinar & Triggiani, 1979 (Rhabditida, Rhabditidae) with *Pentodon punctatus* Villers, 1789 (Coleoptera, Scarabaeidae); *Hexamermis albicans* von Siebold, 1848 (Mermithida, Mermithidae) in larvae of *Aleimma loeflingiana* Linnaeus, 1758, *Tortrix viridana* Linnaeus, 1758, *Archips crataegana* Hübner, 1799 (Lepidoptera, Tortricidae) and unidentified species of Geometridae; *Hexamermis* sp. in *Traumatocampa pityocampa* Denis & Schiffermüller, 1775 (Lepidoptera, Thaumetopoeidae) eopupae; *Sphaerularia bombi* Dufour, 1837 (Thylenchida, Sphaerulariidae) inside queens of *Bombus terrestris* Linnaeus, 1758, *Megabombus pascuorum* Scopoli, 1763 and *Pyrobombus sicheli* Radoszkowski, 1859 (Hymenoptera, Apidae). From soil samples of different biotopes 22 strains of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida, Heterorhabditidae), 25 *Steinernema feltiae* Filipjev, 1934, 11 *S. affine* Bovien, 1937, 8 *S. apuliae* Triggiani, Mráček & Reid, 2004, 3 *S. ichnusae* Tarasco, Mráček, Nguyen & Triggiani, 2008, 1 *S. carpocapsae* Weiser, 1955 and 1 *Steinernema* sp. of *S. arenarium* Artyukhovsky, 1967 group (Rhabditida, Steinernematidae) were isolated. *O. pentodonis*, *S. apuliae* and *S. ichnusae* are new species so far only reported from Italy.

Could Italy be considered a favorite place in Europe for EPN biodiversity?

E. Tarasco, M. Clausi, G. Rappazzo, M. Vinciguerra, A. Longo, O. Triggiani387-389

Abstract: EPN surveys carried out in Italy point out the presence of a high number of species if compared with other European and Mediterranean countries. Adding up the total, 106 EPN isolates belonging to 8 species were collected from about 1,500 soil samples in 20 years: 38 strains of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida, Heterorhabditidae), 39 *Steinernema feltiae* (Filipjev, 1934), 11 *S. affine* (Bovien, 1937), 4 *S. kraussei* (Steiner, 1923), 8 *S. apuliae* (Triggiani, Mráček & Reid, 2004), 3 *S. ichnusae* (Tarasco, Mráček, Nguyen & Triggiani, 2008), 2 *S. carpocapsae* (Weiser, 1955) and 2 *Steinernema* sp. Of the *S. arenarium* (Artyukhovsky, 1967) group (Rhabditida, Steinernematidae). *S. kraussei* was isolated only in the chestnut groves around the Enta Vulcano on Sicily; *S. apuliae* and *S. ichnusae* are new species so far only found in Italy.

Biological control of western corn rootworm larvae using nematodes

Stefan Toepfer, Ralf-Udo Ehlers, Regina Burger, Arne Peters, Ulrich Kuhlmann390

Abstract only

Inoculative release of entomopathogenic nematodes for control of *Phytodecta*

quinquepunctata (Coleoptera: Chrysomelidae) and other tree pests in urban green space

Marek Tomalak391-394

Abstract: *Phytodecta quinquepunctata* is a common leaf-feeding beetle causing extensive damage to bird cherry, black cherry, and rowan in urban parks. Laboratory and field experiments revealed high control potential of entomopathogenic nematodes against this pest during its pupation in the soil. Mass reproduction in the host and good persistence in the soil ensured high efficacy of *S. feltiae* for at least two seasons after introduction. Due to extinction of some infra-populations, *H. megidis* was less effective in the second seasons. Inoculative release of EPNs could be a valuable solution to management of this pest.

Two different bacterial symbionts of *Heterorhabditis megidis* and *Heterorhabditis downesi* inside one population

Tímea Tóth, Tamás Lakatos395-397

Abstract: During a detailed survey of Hungarian EPN fauna several areas were found, from where *H. megidis* or *H. downesi* with two different bacterial symbionts could be collected. The aim of this present study was to estimate the frequency of different *Heterorhabditis/Photorhabdus* combinations in one geographical location. EPN strains were isolated from 20 soil samples collected from an oak forest and a clearing near to the forest. Altogether 44 nematode strains (24 *H. downesi* and 20 *H. megidis*) were isolated from infected *G. mellonella* and *T. molitor* larvae. Two *Photorhabdus* taxa were isolated from IJs of the nematodes: *P. temperata* subsp. *temperata* (24 isolates) and *P. temperata* subsp. *cinerea* (20 isolates). These two bacteria could be isolated

both from *H. megidis* and *H. downesi*. Reproductive success of both nematode species associated with *P. temperata* subsp. *cinerea* were higher, than in the case of *P. temperata* subsp. *temperata* symbiont.

Efficacy of entomopathogenic nematodes against caterpillars in greenhouses with climatic conditions of Southern Spain

Magda Galeano, Ovidio Díaz, José Eduardo Belda399-402

Abstract: Some trials were carried out to improve the efficacy of the entomopathogenic nematodes applications to control pests in an IPM program. A bioassay has been carried out to test the most effective dose and a second trial in a commercial greenhouse to evaluate the application in the climatic conditions of Almería (Southern Spain). Some caterpillar pests are difficult to control in these conditions, due to the lack of compatible chemicals with beneficials and the implementation of IPM strategies. The results show that the treatment using *Steinernema carpocapsae* with a co-adjuvant is the most effective control of *Spodoptera littoralis* and the mortality surpassed 80% three days after application.

Virulence of entomopathogenic nematodes isolated in hazelnut orchards to *Curculio nucum* (Coleoptera: Curculionidae) larvae in the laboratory

Fernando García-del-Pino, Ana Morton, Alejandro Palomo403-405

Abstract: The hazelnut weevil *Curculio nucum*, (Coleoptera: Curculionidae) is a major pest of hazelnuts. Entomopathogenic nematodes have been shown to be pathogenic towards the larval stage of this weevil. In this study, the virulence of nine strains of nematodes isolated in hazelnut orchards with presence of this pest was tested in the laboratory against the last instar larvae. Larvae mortality ranged between 15 and 87.5% related to the nematode strain tested. One steinernematid and one heterorhabditid strain with 87.5% mortality could be promising candidates to use in future studies for the control of the hazelnut weevil pest.

Pathogenicity of *Steinernema feltiae* in oil-polluted soil

Janina Gospodarek, Magdalena Jaworska406-408

Abstract: Abiotic factors, such as heavy metal ions and oil fractions can reduce pathogenicity of entomopathogenic nematodes. Experiments conducted on wasteland contaminated with 2 dcm³/m² of oil derivatives revealed significant decrease in pathogenicity and reproduction of *S. feltiae* lasting throughout 2 month observation. Petrol was more toxic than motor oil and diesel fuel.

Insect pathogenic nematode, *Steinernema feltiae*, from Iran

Javad Karimi, Aziz Kharazi-pakdel, Toyoshi Yoshiga409-412

Abstract: A survey of entomopathogenic nematodes was conducted in the Iran. Out of a total of 194 soil samples, 9 were positive for an isolate of entomopathogenic nematodes of the genus *Steinernema*. Morphological and molecular studies as well cross hybridization tests were undertaken for characterization. The isolates were identified as *Steinernema feltiae*. ITS sequences confirmed this. 16S rRNA sequences and phenotypic characters of its symbiotic bacterium were determined and used for identification. In the phylogenetic analysis the relationship among different isolates of native *Steinernema* were indicated. Laboratory test on *Spodoptera exigua* showed high pathogenicity of these entomopathogens.

***Pristionchus* sp. (Rhabditida: Diplogastridae) from Italian populations of *Anoplophora chinensis* Forster (Coleoptera: Cerambycidae)**

Simona Landi, Barbara Manachini, Marta Valentini, Costanza Jucker, Mario Colombo413-416

Abstract: *Pristionchus* sp. n. (Rhabditida: Diplogastridae) is described from a population of Citrus Longhorn Beetles. Alive adults of this Cerambycid were collected from *Acer saccharinum* trees at Assago and Parabiago (Villastanza), two localities near Milan (Italy). The natural occurrence of the entomogenous nematode in the beetle was not frequent and widespread in the study area. Preliminary laboratory infectivity tests highlight the potential parasitic nature of the nematode. However, it was not successfully maintained in storage and culture. The diplogasterid *Pristionchus* sp. has rather complicate systematic thus a biomolecular analysis based on DNA is necessary for more specific identification and to understand whether the nematode is autotochtone or was brought with this new invasive Coleopteran. Further studies are necessary before this nematode can be considered as a biological control agent of the *A. chinensis*.

Susceptibility of the tomato leaf miner, *Tuta absoluta* to entomopathogenic nematodes

Ana Morton, Laia Batalla-Carrera, Fernando García-del-Pino 417-419

Abstract: The tomato leaf miner, *Tuta absoluta*, is a new imported pest of tomato crops in Europe. Although chemical pesticides are quite effective, factors such as insect resistance or environmental pollution limit their use for management of *T. absoluta*. Biological control with entomopathogenic nematodes can provide a good control of this pest. The efficacy of different entomopathogenic nematode strains of the families Steinernematidae and Heterorhabditidae was tested against last instar larvae and pupae of *Tuta absoluta*.

Natural occurrence of entomopathogenic fungi and nematodes in hazel orchard soils from Catalonia (N.E. Spain)

Sergi Santamaria, Ana Morton, Fernando García-del-Pino..... 420-422

Abstract: The occurrence of entomopathogenic fungi and nematodes has been studied sampling 295 hazel fields in Catalonia (N.E. Spain). Fungi were recovered from 133 of 295 fields, which represent 45.1% of the samples. *Beauveria bassiana* and *Metarhizium anisopliae* were the most often collected fungi, and both were recovered in all types of crops analyzed (integrated, conventional, ecological, abandoned, and wild hazels). Nematodes were recovered from 15 (5.1%) of the sites sampled. The most common family of entomopathogenic nematodes isolated was *Steinernematidae*, which made up 93.3% versus 6.6% of *Heterorhabditidae*. The nematodes were present in four of the five types of crops sampled, lacking only from conventional hazel orchards.

Soil Insect Pests

The relationship between *Agriotes* click beetle (Coleoptera: Elateridae) wireworms and environmental factors

Carly Benefer, Rod Blackshaw, Mairi Knight, Jon Ellis 425

Abstract only

Predicting the size of leatherjacket populations in grassland: a pilot study using water traps

Rod P. Blackshaw 426-429

Abstract: Leatherjackets, *Tipula paludosa* larvae, are sporadic pests of a range of crops. There are indications that they may potentially be controllable with biocides, but these need to be applied in the early autumn when estimating populations is all but impossible. This paper reports an investigation into the use of water traps to catch adults as predictors of subsequent larval populations. It is concluded that female trap counts will be a better predictor than male or total crane-fly counts. It is also noted that there appears to be a spatial dimension to correlations suggesting that predictions may be specific to the immediate vicinity of the traps.

Isolation of bacteria pathogenic on wireworm larvae *Agriotes* spp. (Coleoptera: Elateridae)

Zihni Demirbağ, Mehtap Yakupoğlu, Remziye Nalçacioğlu, İsmail Demir..... 430

Abstract only

Biological control of the European cockchafer: Does *Beauveria brongniartii* have effects on fungal community structures in soil microcosms?

Jürg Enkerli, Kaspar Schwarzenbach, and Franco Widmer 431-435

Abstract: Investigation of non-target effects is an important aspect for risk assessment of biological control agents (BCA). *Beauveria brongniartii*, which is commercially applied to control the European cockchafer, *Melolontha melolontha*, was used to assess effects of a fungal BCA on soil fungal communities. The experimental system consisted of six soil microcosm treatments with and without *M. melolontha* larvae and included BCA- and carbofuran-based chemical control agent (CCA) treatments. Quantitative real-time PCR analysis of a specific microsatellite marker was used to quantify *B. brongniartii* in soil and fungal ribosomal internal spacer analysis (RISA) was applied to assess changes in soil fungal communities over a period of 91 days. Strongest and most significant changes in soil fungal communities were detected for treatments containing larvae that had died from either control agent. The BCA alone revealed much smaller and transient effects, while CCA effects were also small but significantly increased at the end of the experiment. The study has shown that both control strategies induced relatively

small effects on soil fungal communities and it demonstrated that molecular genetic techniques are efficient tools for monitoring and effect assessment of fungal BCAs.

Observations on natural mortality factors in wireworm populations and evaluation of management options

Lorenzo Furlan, Christian Bonetto, Bruno Costa, Andrea Finotto, Luca

Lazzeri.....436-439

Abstract: Wireworms (*Agriotes sordidus*, *Agriotes ustulatus*) have been reared in rearing cages placed into fields over a five year period (2004 – 2008) in order to evaluate the effect of rotation on larval development and the potential role of biocidal plants (*Brassica juncea* var. ISCI 99) to reduce wireworm populations in comparison with other mortality factors. Wireworm populations in cages planted with different rotations (**continuous rotation**, very short interruption between different crops; **discontinuous rotation**, long periods with bare soil between crops, meadow) were estimated by using bait traps at monthly intervals. Soil with continuous vegetation cover allowed more larvae to survive. Biocidal plant root systems did not cause significant larval mortality. The incorporation of the above ground material of *Brassica juncea* sel. ISCI 99 at a dosage of 55 t ha⁻¹ of fresh matter corresponding to about 290 μmoles of GLs l⁻¹ of soil, significantly reduced wireworm populations. In the end of the rotation period the larvae were placed in vials inspected twice per week in order to establish larval mortality. According the cause of dead, dead larvae were divided into the following groups: *Metarhizium* spp., *Beauveria* spp., Nematodes, Uncertain. The percentage of larvae found dead because of insect pathogen infection was low and did not differ between larvae coming from different rotations.

Bioprotection for management of soil dwelling pests

Trevor A. Jackson441-444

Abstract: Soil dwelling pests are a major challenge for farmers and pest managers. In New Zealand, a bioprotection strategy has been developed to reduce damage from the endemic grass grub *Costelytra zealandica*. The strategy makes maximum use of knowledge of the insect's biology for prediction of outbreaks of the target pest and uses a natural pathogen for control. A bacterium (*Serratia entomophila*) isolated from diseased larvae has been incorporated into the commercial product Bioshield™, which is marketed in New Zealand for control of the pest. The strategy utilises knowledge of the insect's biology to predict application timing for best effect to minimise damage. Control of the grass grub could be improved by better predictive systems and formulations to increase persistence in the soil. Extension of bioprotection strategies to other soil-dwelling pest species will depend on the discovery of suitable microbial control agents.

Efficacy evaluation of the entomopathogenic fungus *Beauveria bassiana* strain

ATCC 74040 against wireworms (*Agriotes* spp.) on potato

Edith Ladurner, Uwe Quentin, Sergio Franceschini, Massimo Benuzzi445-448

Abstract: In 2008, the efficacy of the entomopathogenic fungus *Beauveria bassiana* strain ATCC 74040 was evaluated in three field trials, one conducted in Germany and two in Italy. The *B. bassiana* strain ATCC 74040-based product, when applied both alone and in an IPM strategy, always significantly reduced potato tuber damage compared to the untreated control. Mean efficacy values ranged from 54 to 94% and were comparable to those of the clothianidin- and fipronil-based reference strategies (77-84%), and higher than those of the chlorpyrifos-based strategy (<35%). The fungus strain can therefore be considered a valuable tool for the control of *Agriotes* spp. in both organic farming and integrated pest management.

Field trials on the use of *Beauveria brongniartii* against *Melolontha* spp. white grubs in forest plantations in Poland – a case study of Kozenice

Alicja Sierpiska449-452

Abstract: *Melolontha hippocastani* Fabr. and *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) larvae are the most noxious soil insect pests in forest plantations and nurseries in Poland. In 2007-2008 several field trials were carried out to estimate *Beauveria brongniartii* wheat colonized kernels efficacy in the reduction of damages and control of *Melolontha* spp. larvae in forest plantations and nurseries in Poland. Two of the trials described in this paper were projected as a part of a dossier to be submitted for the registration of the used product. They were located in Kozenice Forest District (a coniferous forest growing on sideric soil, pH of the soil 3.5-5.0) in pine *Pinus silvestris* L. and oak *Quercus robur* L. plantations. A defined portion of colonized kernels have been put into the planting hole and mixed with a soil. Three doses were

tested: 60, 120 and 240 kg/ha. The survival rate of seedlings was calculated (after 6 months for pine and after 13 months for oak), the white grub population density was checked, white grubs collected in experimental plots were reared in the laboratory and soil samples were taken to recover *Beauveria* spp. isolates by insect baiting using *Galleria mellonella* larvae. Results suggest that depending on seedling species and white grub population density the time of *B. brongniartii* application to the forest soil should proceed one or two years the forest planting and the application should be repeated in the following years. The *B. brongniartii* strain used to the colonizing of grains is probably not well adapted to the low pH values, typical for most of the forest soils in Poland.

Movement of the Western corn rootworm (*Diabrotica virgifera virgifera*) adults in a trial with Bt and non-Bt maize plots

Kristina Stanikova, Ioan Rosca, Ludovit Cagan453-456

Abstract: In spring 2008 a trial with 32 plots was initiated at Borovce (near Piestany in district Trnava) in Slovakia. The trial included 8 maize hybrids randomly arranged in 4 repetitions. Three repetitions and half of the fourth repetition were sown in plots with a preceding crop different than maize and the other half of the fourth repetition was sown in plots with maize as preceding crop. The results indicate that high numbers of the Western Corn Rootworm (WCR) adults were found at damaged plots on July 8. After their emergence, the adults seem to not move from their site of emergence. On September 16, there were no differences between the plots damaged or not damaged by larvae. This suggested that in August and in September the WCR adults may have moved between plots. WCR-resistant maize genotypes strongly influenced the level of the WCR damage on the maize plants (there were no lodged plants on the plots with MON 88017).

Optimised protocol for wireworm rearing

Ursula Kölliker, Werner Jossi, Stefan Kuske457-460

Abstract: Wireworms for bioassays are usually collected in the field. This can be quite tedious since wireworm infested fields may be difficult to be found and wireworm infestations can be difficult to predict. An optimised protocol has been developed to rear wireworms of the species *Agriotes obscurus*, *A. lineatus* and *A. sputator* in the glasshouse. Adults are collected in the field in May and transferred to plant pots in the glasshouse for egg laying at 20 to 25°C. In January, 20 to 200 larvae large enough to be used in bioassays may be retrieved per pot. Each May the rearing is restarted by collecting adults in the field. Using this method, a sufficient number of larvae can be produced for bioassays with limited effort and without having to rely on field collections.

New challenges for European cockchafer control in Swiss alpine valleys

Stefan Kuske, Ursula Kölliker, Christian Schweizer461-462

Abstract: The European cockchafer (*Melolontha melolontha* L.) is a major insect pest of alpine grasslands in several regions of Switzerland. The larvae extensively feed on roots of almost all grassy and herbaceous plants in meadows and pastures where they do repeatedly cause increased or total yield loss combined with soil erosion and infrastructural damages. In 2009, more than 700 hectares of infested alpine grasslands will be treated against this pest. Since the 1980ties area wide control of cockchafers was achieved in many alpine valleys throughout Switzerland by sowing barley seed inoculated with the entomopathogenic fungus *Beauveria brongniartii* into grassland soils. In most cases, a single application has been successful to achieve a long-term control of the cockchafer population in treated areas. In recent years, increasing pest populations infested grassland areas previously not attacked by the pest and infestations repeatedly reached up to higher altitudes. Moreover, increasing numbers of larvae tend to complete their common three-year life cycle already within two-years, causing new challenges for the established biological pest control practice.

Slugs and Snails

The effects of slugs on the plant communities in upland hay meadows.

Sarah Barlow 465

Abstract only

Slug control in iceberg lettuce heads using slug parasitic nematodes

Albert Ester, Klaas van Rozen, Leendert Molendijk 466-471

Abstract: Young iceberg lettuce plants (*Lactuca sativa* var. *capitata*) are very susceptible to slug damage, which may cause yield loss as seedlings are destroyed. During the vegetation period, slugs have a preference to hide inside the lettuce head, resulting in quality reduction due to feeding damage and faecal contamination. Just before or immediately after planting iceberg lettuce, bait pellets are frequently used to prevent this problem by controlling slugs. The use of bait pellets during the growth of iceberg lettuce is not allowed, because of potential product residue in the head. In the Netherlands in many cases the grey field slug *Deroceras reticulatum* is the responsible slug species causing these problems in iceberg lettuce. *Phasmarhabditis hermaphrodita* is a slug parasitic nematode and is already used to control the grey field slug in Brussels sprouts and green asparagus. This biological control agent was thought to be a good alternative for bait pellets in iceberg lettuce production. The nematodes can be applied during the growing season, without risk of residuals in the final product. In 2002 and 2003 several semi field trials were conducted in Lelystad, using one square meter bordered plots. The aim of the first trial was primarily to determine the application method and the potential of different dosages, assessing the capability of *P. hermaphrodita* to control *D. reticulatum*. The second trial focussed on the number of slugs to be used per plot. Based on these semi field trials it was concluded that the slug parasitic nematode, *P. hermaphrodita*, has good potential in controlling slugs.

Investigation into effects of sewage sludge application on populations of terrestrial molluscs

Chris Hall, Stewart Rhind, Michael J. Wilson 472

Abstract only

Phylogeny of nematodes associated with terrestrial slugs inferred from 18S rRNA sequences

Jenna L. Ross, Elena S. Ivanova, Sergei Spiridonov, Graeme Nicol, Michael J.

Wilson 473-475

Abstract: The present paper proposes a phylogeny of nematodes associated with terrestrial slugs using 18S rDNA sequences from nematodes isolated from slugs in the Belgium, Chile, Norway, Slovenia, UK and USA. Interpretation of these data will elucidate the relationship between these distinct taxonomic groups of nematodes and attempt to solve several unanswered questions including the number of times the acquisition of parasitism in terrestrial molluscs has occurred throughout history and the phylogenetic relationship between known groups of mollusc-parasitic nematodes.

Prospects for microbial molluscicides to control slugs

Sally Howlett, Upali Sarathchandra, Gabriela Burch, Sandra Young, Nigel Bell,

Trevor Jackson 476-479

Abstract: Current agronomic practices in commercial agriculture and climatic conditions favour the growth of slug populations and there is the need for better management of these pests. Sustainable production requires greater use of biological approaches to pest control which could be used alone in protected crops or included in an integrated system to complement existing options in a broadacre setting. This paper discusses prospects for a microbial approach to slug control, focusing on the niche such a product could fill in the molluscicide market and the characteristics it would require. The need for an economic appraisal of the damage caused by slugs is highlighted along with the role of education in promoting uptake of biocontrol technology.

The role of parasite release in invasion of the USA by European slugs

Jenna L. Ross, Elena S. Ivanova, Paul M. Severns, Michael J. Wilson 480-483

Abstract: Previous surveys have shown that European slug species are invasive in the USA, threatening native species and damaging agricultural and horticultural crops. A possible explanation for the success of these invasive slug species is parasite release. This is a process where invading species are released from their co-evolved natural enemies during invasion into their new habitat. To test this hypothesis we collected European slugs in part of their native range (United Kingdom) and in the USA. We then compared the prevalence, distribution and species richness of their nematode parasites. All slugs were dissected and examined for the presence of nematodes. Nematodes were then fixed directly into hot ~60°C 5% formaldehyde and mounted on

slides for morphological identification. Results showed that in the UK, nematodes were present at 93% of study sites and 16.4% of all slugs examined were associated with nematodes. In the USA the respective figures were 34% of sites and 5.4% of slugs. Twelve species of nematode were found in the UK, whereas only nine were found in the USA, indicating our data supports a role for parasite release during the invasion of the USA by European slugs.

Miscellaneous

Biocontrol of *Ostrinia nubilalis* and *Sesamia nonagrioides* by *Bt* maize in South

Western France: Search of biological indicators by a model-based approach for managing mycotoxin risks

Laurent Folcher, Marc Jarry, Alain Weissenberger, Nathalie Eychenne, Marc

Delos, Catherine Regnault-Roger487-490

Abstract: The aim of this study was to assess the consequences of the biocontrol of two major maize insect pests, *Ostrinia nubilalis* Hüb. and *Sesamia nonagrioides* Lef. by *Bt* maize (MON 810 event) on *Fusarium* spp. mycoflora and mycotoxins trichothecenes (DON), fumonisins B1 and B2 and zearalenone (ZEA). Field trials were conducted during summer 2005 in South Western France. *Bt* maize induced a significant reduction for both Lepidoptera, *Fusarium* spp. mycoflora and fumonisins. But difference was noted following mycotoxin families. The characterization of biological indicators by model-based approach showed that Lepidoptera and *Fusarium* spp. biocontrol could be considered to be biological indicators for the model fumonisins B₁ and B₂, although the model trichothecenes appeared to be linked only to *Fusarium* spp. Both results suggest the hypothesis of a competition of mycoflora between *Fusarium* spp. producing fumonisins and *Fusarium* spp. producing trichothecenes.

Entomopathogenic *Rickettsiella* bacteria: From phylogenomics to biology of infection

Andreas Leclerque491-494

Abstract: The application of molecular techniques to the phylogenetics of “rickettsias of insects” has triggered a recent controversy on the most appropriate taxonomic organization of the bacterial genus *Rickettsiella*. We have made use of the first whole genome sequence available from this taxon – that of the pathotype ‘*Rickettsiella armadillidii*’ – to establish its phylogeny beyond the 16S rRNA gene level. Employing a combination of phylogenetic reconstruction and likelihood-based significance testing over a basic core set of 211 single-copy orthologous gene (SCOG) families we demonstrate that ‘*R. armadillidii*’ is correctly assigned to the gamma-proteobacterial order *Legionellales*, but that its 16S rRNA-based current assignment to the family *Coxiellaceae* is not positively supported by genomic data. Consequences of these phylogenetic findings for an experimental approach to the biology of infection by *Rickettsiella* bacteria are discussed.

Infection of *Lymantria dispar* (Lep.: Lymantriidae) larvae with the midgut pathogen

Endoreticulatus schubergi (Microsporidia) has little influence on growth and development of the host

Manana Kereselidze, Daniela K. Pilarska, Andreas Linde, Gernot Hoch.....495-498

Abstract: We studied the effects of infection with the microsporidium *Endoreticulatus schubergi* on growth, development and consumption of *Lymantria dispar* larvae in the laboratory. While rearing temperature (21° or 26°C) significantly affected most parameters at least in female insects, infection with *E. schubergi* had very little effects. Neither fresh mass of pupae, nor weight gained during larval stage (four and five) and food consumption was significantly altered by infection.

Potential of mites, fungi and microsporidia for biological control of *Pityokteines*

spinidens

Milan Pernek499-501

Abstract: *Pityokteines spinidens* is the most abundant and most important pest species on Silver fir in Croatia. Due to a lack of knowledge about the potentials of their natural enemies, emerging beetles from incubated stem sections were examined for phoretic mites and pathogens. A total of 8 mite species was documented as associates of *P. spinidens* of which *Ereynetes scutulalis* could have some potential for biological control. The fungus *Beauveria bassiana* is a common pathogen of bark beetles and was often found in breeding galleries of *P. spinidens* (13% mortality). Furthermore, three microsporidia and one gregarinan species were observed: *Canningia spinidentis*, *Menzbieria* sp., *Chytridiopsis* cf. *typographi* and *Gregarina* sp. Data presented in this

study show possible candidates for further tests to utilize pathogens as microbial control agents against fir bark beetles in the future.

Use of insect pathogens in Ukraine: Currant status and future perspectives

Tatyana Stefanovska.....502-504

Abstract: Commercial use of microbial biocontrol agents has a long and rich history in Ukraine, which is closely linked with other countries of the Former Soviet Union. The use of insect pathogens against Ukrainian pests, particularly *Cydia pomonella* L. has been practiced. Entomopathogenic nematodes have generated some interest for future use. We consider here the potentials and pitfalls to commercial production of insect Finally, we identify steps that should be taken for growing of microbiological pesticides market.

Pathogen and nematode occurrence in the spruce bark beetle *Ips typographus* (Col., Scolytidae) in two different region of Georgia

Medea Burjanadze505-508

Abstract: Occurrence of pathogens and nematodes in the spruce bark beetle *Ips typographus* was investigated. Beetles were collected in 2008 from two different regions of Georgia: The Shovi resort (1370 m a.s.l) of the Caucasian Mountains (Northern region) and Daba (950 m a.s.l) at the Borjomi–Bakuriani gorge (Southern region). Various pathogens and nematodes were recorded: *Gregarina typographi* (18.9-49.5%) and *Chytridiopsis typographi* (6.2% and 15%) were found in adults from both regions. *Nosema* sp. (like *Nosema typographi*) was observed in 5.3% of the beetles from the Daba district. The entomopathogenic fungus *Beauveria bassiana* (0.9%) occurred at the same place. Two species of parasitic nematodes, *Contortylenchus diplogaster* and *Bursaphelenchus* sp. were found in bark beetle populations of both regions (40.7–50%).

Microbial antagonists of the codling moth, *Cydia pomonella* L., diagnosed from 1955 to 2008

Regina G. Kleespies, Alois M. Huger, Gisbert Zimmermann509-512

Abstract: At the Institute for Biological Control in Darmstadt, Germany, more than 20,000 specimens of the codling moth, *Cydia pomonella* L., were diagnosed for insect pathogens from 1955 to 2008. Altogether, 87 accessions were received for diagnosis, mostly from Germany (79), but also from Switzerland (4), Austria (2), Italy (1), and Israel (1). Fifty accessions (57.5%) with larvae and with adults of field catches by light and pheromone traps revealed high infection rates up to 70% by the microsporidium *Nosema carpocapsae*, causing a chronic disease process. In 21 accessions (24.1%), the fungus *Beauveria bassiana* was identified most frequently on old and diapausing larvae and on pupae, but also *Isaria farinosa* (syn. *Paecilomyces farinosus*), *Hirsutella* spp., *Lecanicillium* spp., *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*), *Metarhizium anisopliae*, as well as different fungi assigned to saprophytes were found. The *C. pomonella* granulovirus (CpGV) was identified in eight accessions (9.2%) of larvae and pupae of rearings or pertinent field experiments. Several bacteria, mostly undetermined, were also diagnosed. The results and possible contributions of the most important pathogens to natural control of codling moth populations are briefly discussed.

Fungi, microsporidia and nematodes in *Gastrophysa viridula* (Col., Chrysomelidae) from Eastern and Central Europe

Rudolf Wegensteiner, Cezary Tkaczuk, Bernard Papierok513-516

Abstract: As a result of a comparative study on the occurrence of pathogens and parasites on the beetle *Gastrophysa viridula* in Austria and Poland in 2007 and 2008, an entomophthoralean fungus from the genus *Zoophthora* was found on adults and larvae as well, but only from locations in Poland. Microsporidia (*Nosema* sp.) were found, in a relatively high proportion of beetles from all Polish sites, but only in some few individuals from one Austrian site. Nematode larvae (Mermithidae) were found in beetles from some Austrian sites only.

Genetic engineering of baculoviruses for biocontrol: Dead end or bright future

Just M. Vlak.....517-518

Abstract only

Gut gene expression profiles of *Heliothis virescens* larvae fed *Bacillus thuringiensis* toxins

Omaththage P. Perera, Anaïs Castagnola, Juan Luis Jurat-Fuentes..... 519

Abstract only