number of potent invertebrate-active toxins that do not fall within these classes. Crystallography is labour-intensive, requiring large quantities of pure, mono-disperse protein and often proves difficult. Recent developments in the field of *in silico, ab initio* structural modelling allow the generation of models in the absence of related sequences in the protein structure database. This may allow us to predict protein structures and use these predictions to develop testable hypotheses for the modes of action of the toxins. This procedure has been applied to several non-3-domain toxins and toxin-associated proteins. For one such protein, a structure is proposed, consistent with a pore forming mechanism of action. Analysis of secondary structure content is consistent with this model and evidence of pore formation has been produced. Mutagenesis of a region known to be important in structurally-related toxins was shown to eliminate toxicity. While further study is clearly required, modelling, thus, allows us to predict and test hypotheses related to the mode of action of toxins for which experimental structures are, as yet, unavailable.

Symposium. Wednesday, 10:30 **143**

Novel MTX Toxins for Insect Control

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In addition to the conventional 3-domain Cry proteins, the Gram-positive bacteria Bacillus thuringiensis can also harbor other classes of insecticidal toxins with distinct structures, receptors, and modes of action. Among them are a group of proteins that share significant similarities to MTX2/3 toxins at the structural level, but are very divergent at the amino acid sequence level. In this presentation, we will discuss the general features of these MTX toxins, and agriculture applications for the control of insect pests.

Symposium. Wednesday, 11:50 **144**

Insecticidal toxins from *Photorhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins

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Photorhabdus luminescens and *asymbiotica* live in the gut of entomopathogenic nematodes, which invade insect larvae, where they release the bacteria. Here, the bacteria produce toxins, which kill the insects. We studied tripartite Tc toxins from *P. luminescens* and a novel toxin (PaTox) from *P. asymbiotica*.
Tc toxins consist of three components TcA, TcB and TcC, which occur in several isoforms. TcA is responsible for the binding and up-take of the toxin, B is a linker and C carries the biological activity. Recent crystal structure analysis revealed a novel type of syringe-like injection mechanism, which depends mainly on TcA but needs all components (1). We studied the biological activity of TccC3 and TccC5, which are isoforms of TcC. TccC3 ADP-ribosylates actin at threonine148, thereby actin polymerization is enhanced (2). TccC5 ADP-ribosylates Rho proteins at glutamine61, a modification which persistently activates of Rho GTPases. Both modifications of actin and Rho proteins induce clustering of the actin cytoskeleton (2).
The *P. asymbiotica* toxin PaTox glycosylates Rho proteins by attaching GlcNAc at tyrosine32/34 (3). The modification inhibits Rho signaling, because Rho activation and interaction with effectors are blocked. In addition, PaTox harbors a deamidation domain, which activates heterotrimeric G proteins, including Gq/11 and Gi family proteins. Functional consequences of the actions of *Photorhabdus toxins* on actin and GTP-binding proteins are discussed.
References
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Symposium. Wednesday, 12:10 **145**

Molecular basis of parasporin-2 action toward cancer cells

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Parasporin-2 (PS2) is a crystal toxin isolated from parasporal inclusions of *Bacillus thuringiensis* A1547. PS2 has a strong cytocidal activity in liver and colon cancer cells without showing a typical insecticide. Accumulated molecular, cellular and *in vivo* experimental observations on PS2 indicate that the protein form a pore in membrane with a mega size assembly. The crystal structure of active PS2 monomer reveals that the protein elongates like a short rod, comprising almost β-strands. The polypeptide folding is similar to a class of aerolysin-like β-pore-forming toxins while there is no homology to insecticidal Cry toxins. N-terminal domain of PS2 is rich in aromatic residues and forms a groove which could be capable to grapple the target molecule. Amino acid substitutions of PS2 in the region indicate that the residues could be involved in cell-binding. The C-terminal domain contains β-sandwiches and the surface of the protein has a unique extensive track of exposed side chains of serine and threonine where thought be related to PS2 oligomerization and membrane pore formation. Single-particle EM analysis reveals that PS2 oligomer shows a ring shape with the 24nm length, 8 nm diameter and a 4nm pore while a structure of pore-forming aerolysin is the ring-like mushroom structure with a central pore. We would like to introduce current observations on anti-cancer toxin PS2 in vitro and vivo in this symposium.

CONTRIBUTED PAPERS Wednesday, 10:30-12:30

MICROBIAL CONTROL 2

Contributed paper. Wednesday, 10:30 **146**

Evaluation of the non-target effects of *Bacillus thuringiensis* subspecies *israelensis* in standardized aquatic microcosms

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Malaria, one of the most deadly vector-borne diseases in the world, is transmitted by the bite of an infected female *Anopheles* mosquito. *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is a gram-positive, aerobic, spore-forming bacterium that produces crystalline inclusions that contain insecticidal proteins. *Bti* has been shown to be highly insecticidal to larvae of mosquitoes and blackflies, but is considered to have weak insecticidal activity against non-dipteran invertebrates in aquatic environments. Few studies have comprehensively studied the non-target effects of *Bti* under reproducible and standardized conditions. The objective of this study was to evaluate the effects of *Bti* on key non-target invertebrates in a highly reproducible synthetic multi-species system, the

standardized aquatic microcosm (SAM) system. The SAM system is initiated in a chemically defined medium with synthetic sediment and is inoculated with nine different species of photosynthetic microorganisms (PMOs) and different non-target invertebrates. Replicate SAMs were inoculated with a LD_{90} of *Bti* strain HD-522, whereas the control SAMs were not inoculated with *Bti*. Over a period of 2 months, the abundance of PMOs and invertebrates were determined by biweekly sampling. Differences between *Bti*-treated and control SAMs were assessed by statistical analyses of sampling data and biological diversity indices. The contributions of the SAM experiments to our understanding of the non-target effects of *Bti* are discussed.

Contributed paper. Wednesday, 10:45 **147**

Bacillus thuringiensis 00-50-5 strain with high activity against plant-parasitic nematodes and insect pests

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Plant-parasitic nematodes (PPNs) are serious pathogens of many other crops. Yellow cutworm Agrotis segetum is a serious insect pest of vegetable and grains. After liquid fermentation of Bacillus thuringiensis (Bt) strain 00-50-5, the cell-free supernatant (CFS) and the crystal protein toxins (CPT) have high activities against the root-knot nematode (RKN) Meloidogyne incognita and the yellow cutworm A. segetum. The mortality for second-stage RKN juveniles (J2) was 100% as early as 5 hrs after exposure to 0.909μg.mL^{-1} of dried CFS. The LC_{50} values were 0.037- and 0.015μg.mL^{-1} of partially purified Bt exotoxin at 5 hrs and 24 hrs after exposure, respectively. The mortality of A. segetum was 100% for first-instar larvae (L1) after exposure to 10μg.mL^{-1} CPT for 72 hrs. The LC_{50} value for A. segetum L1 was 0.417μg.mL^{-1}. An SDS-PAGE of the purified 00-50-5 CPT resulted in four main proteins with 133-, 60-, 27- and 25 kDa after treatment with 1%SDS, and three proteins with 133-, 60-, and 27-kDa after treatment with 0.1N NaOH. The Bt 00-50-5 has dual nematicidal and insecticidal activities against soil-dwelling pests, such as M. incognita and A. segetum.

Contributed paper. Wednesday, 11:00 **148**

Investigations on residues of *Bacillus thuringiensis* on tomato

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After an incidence of diarrhea in 2012, high concentrations of presumptive *Bacillus cereus* (including *B. thuringiensis* (*Bt*)) were found in German lettuce samples. Because of this incidence, in Germany a discussion about the risk of *Bt* residues started and is still ongoing.
To proof the degradation of *Bt* spores in glasshouses, experiments were conducted on tomato under laboratory, experimental field station and professional grower conditions. For all experiments the *Bt* product XenTari® was used.
In the glasshouse experiment with five applications of XenTari® applied in a weekly interval the concentration of *Bt* spores on tomato fruits ranged in all experiments between $4.9x10^4$ und $8.5x10^4$ cfu/ g fresh weight. For single application of *Bt* a max. spore concentration of $4.7x10^4$ cfu/g fresh weight was measured corresponding to the laboratory experiments and the experiments at a commercial farm. To proof the degradation *Bt* spores over time samples were taken after the last application over one week. Over all experiments the concentration of *Bt* spores was reduced up to only 46 to 77 % of the initial spore concentration within one week. A distinct reduction of *Bt* spores on fruits was achieved by modifying the application strategy. When only the upper parts of the tomato plant were treated with XenTari, a maximum concentration of *Bt* spores of 3.3×10^3 cfu / g fresh weight was recorded.

Contributed paper. Wednesday, 11:15 **149**

Biological control of western corn rootworm larvae (*Diabrotica virgifera virgifera*) with Dianem® (*Heterorhabditis bacteriophora*)

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The biocontrol product dianem® contains entomopathogenic nematodes, *Heterorhabditis bacteriophora.* It is officially registered in Austria as a plant protection product to control larvae of the Western Corn Rootworm (*Diabrotica virgifera virgifera*). Field results from Hungary, Austria and Italy applying 2×10^9 nematodes per ha obtained equally high control like chemical seed dressings with neonicotinoides or application of granular insecticides containing the pyrethroide Tefluthrin. Adapted application technology has been developed to apply nematodes with 200 ltr. of water/ha directly on the seeds. Although the insect larvae occur approximately a month later, the nematodes persist long enough to control the pest. Insects penetrate into the roots where they are not easily reached by insecticides, whereas nematodes follow the insects into the galleries and kill the larvae 2-3 days after infestation. Latest field results, which have used the novel application technology, will be presented. Since product costs reach almost the same level like chemical insecticides and since the seed treatment with neonicotinoides was banned by the European Commission in 2013, the product dianem® is in commercial use for the first time on larger scale against this invasive maize pest.

Contributed paper. Wednesday, 11:30 **150**

Evaluation of Ten Plant Extracts as Ultraviolet Protectants for *Spodoptera littoralis* nucleopolyhedrovirus

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Ten plant extracts were tested as ultraviolet protectants to improve the persistence of *Spodoptera littoralis* multiple embedded nucleopolyhedrovirus (SpliMNPV). In an initial test, the SpliMNPV alone or in combination with 10 plant extracts, each at a concentration of 1% was exposed to ultraviolet B (UV-B) for one hour. Among them, five plant extracts, viz. cloves, henna, green tea, pomegranate and grape showed a high rate of virus protection with original activity remaining

(OAR) at 100 %, 97 %, 91 %, 90.6 %, and 77 %, respectively. However, lemon, kiwi, olive, dates and beetroot extracts provided lower protection with OARs of 71 %, 58.4 %, 53 %, 21 %, and 18 %, respectively. Using the same UVB source, secondary screening was carried out on the five best additives from primary screen, and tested at a concentration of 0.5% and using an exposure timing of 5 hours. Clove and henna showed the highest rate of protection with OAR of 96.6% and 76.5%, respectively. In addition, absorption spectra and the obtained protection rate were correlated. These laboratory findings are very encouraging and that field studies are underway.

Contributed paper. Wednesday, 10:45 **151**

Interactions among Fungal and Viral Pathogens and Parasitoids

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The majority of studies of pathogens are conducted with one pathogen species or even strain and one host species, although in nature numerous pathogens and other parasites form a community attacking a host, and even attacking the same host individual. We conducted studies of natural enemy interactions using *Lymantria dispar* larvae, the fungal pathogen *Entomophaga maimaiga*, the viral pathogen *Ld*MNPV and parasitoids, all of which have been introduced to North America. Studies in low density populations were conducted in central New York State over 16 years and high density populations were studied at 59 sites in the mid-Atlantic region in 2009, when an outbreak population was crashing. We found very different interactions at low versus high host population densities. At low host density, *E. maimaiga* and parasitoids were both fairly abundant and *Ld*MNPV infections were almost nonexistent. At virtually all higher density sites the emergent *E. maimaiga* was most abundant. Virus infection was positively associated with host density while *E. maimaiga* and parasitoids were both frequency dependent. *E. maimaiga* and parasitoids co-occurred in the same larvae less than expected and *Ld*MNPV and parasitoids co-occurred in the same larvae more than expected while the fungus and virus reproduced in the same cadaver as expected, suggesting little interaction. This pattern of co-occurrence suggests that the two semelparous natural enemies (fungus and parasitoid) seldom successfully share a host larva while the iteroparous virus was more successful in co-inhabiting with either *E. maimaiga* or parasitoids.

Contributed paper. Wednesday, 12:00 **152**

Oryctes rhinoceros* population diversity and potential implications for control using *Oryctes nudivirus

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The discovery of *Oryctes nudivirus* (OrNV) in the 1960s by Dr Alois Huger enabled the successful management of coconut rhinoceros beetle (*Orytes rhinoceros*) populations through classical biocontrol release in the Pacific, SE Asian, and Indian Ocean regions. OrNV continues to be an important biocontrol agent for the control of *Oryctes rhinoceros* in both coconut and oil palm growing regions. Augmentative release of OrNV is commonly used to enhance the natural spread and ensure its continued presence within palm growing regions and surrounding areas. For over four decades after the distribution of the virus, *O. rhinoceros* was not reported to have established in any new regions. However, in 2007 the beetle was discovered in Guam and the population has now established with a highly damaging outbreak such as those not seen for 40+ years. Initial attempts to introduce OrNV into the Guam population were unexpectedly unsuccessful. This has raised the possibility the *O. rhinoceros* population that invaded Guam is less susceptible to OrNV, or potentially resistant. Furthermore, near the end of 2013, a population of *O. rhinoceros* was detected in Hawaii, although it is not believed to have established yet. The discovery of new *O. rhinoceros* invasions within the Pacific region linked with the possibility of a virus tolerant population suggests the beetle may again be on the move. To assist efforts in identifying the source populations for the Guam outbreak, a simple PCR-RFLP method has been developed to distinguish Guam *O. rhinoceros* from other populations. Analysis of several *O. rhinoceros* populations has demonstrated that the Hawaiian beetles are of the same haplotype as those found in Guam. We will discuss current results in relation to what is known about these new invasions and potential implications for the future.

Contributed paper. Wednesday, 12:15 **153**

The Control of Fungi Using with Liposomal Formulation of Essential Oil of *Satureja hortensis* and its cell viability assay

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Satureja hortensis is an annual herb used as nostrum in Eastern Anatolia region of Turkey for the treatment of different infectious diseases and disorders. It can also be utilized as a biopesticide against greenhouse pests. The aim of the present study was to control *Candida albicans* with liposomal formulation of essential oil of *S. hortensis* incorporated into an ointment. Also toxicity of the liposomal essential oil of *S. hortensis* was investigated by MTS assay analysis on L929 mouse fibroblast cell lines. The liposomal formulations were designed using thin film technique and liposomes were properly incorporated into the ointment. The chemical composition of the essential oil obtained from *S. hortensis* was determined by GC and GC-MS analysis. The liposomal essential oil of *S. hortensis* was tested against *Candida albicans* with disc diffusion assay and micro-well dilution assay. Then the toxicity of liposomal essential oil on mammalian cells was determined with MTS analysis. The results of antifungal tests showed that the essential oil of *S. hortensis* incorporated into the ointment and liposomal essential oil formulation have potential antifungal activity against *Candida albicans*. MTS assay results showed that a concentration of 10^{-7} % liposomal essential oil formulation is the safe dose for L929 mouse fibroblast cells. This liposomal formulation dramatically increases antifungal activity by improving cellular intake without side effects on mammalian cells.