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Xenorhabdus spp. has dual life styles: they are pathogenic to insects and mutualistic with *Steinernema* nematodes. The nematodes vector the bacteria from one insect to another. In return, bacteria provide a suitable environment in the insect cadaver for the nematodes to maturate and reproduce. Each *Steinernema* spp. carries one *Xenorhabdus* sp. Contrarily, a *Xenorhabdus* spp. may associate with more than one nematode host. The most promiscuous bacterium is *X. bovienii*, which associates with nine *Steinernema* spp. In this study, we performed a comparative genomic analysis of nine *X. bovienii* strains to depict novel virulence factors. Furthermore, virulence assays were performed considering three different lepidopteran hosts. Results revealed that four *X. bovienii* strains were attenuated, whereas the other five were virulent. The genomic platform MicroScope was used to identify known and candidate genes that contribute to their pathogenicity. Additionally, loci involved in their association with the nematodes were investigated. Two loci were identified as novel candidates involved in the bacterium's ability to interact with both nematode and insect hosts. The first region appears to be specific to interactions with nematode partners. The second region contains a type six secretion system (T6SS), which is known to contribute to bacterial pathogenicity. We hypothesize T6SS may contribute to the bacterium's ability to cause death in a wide range of insect hosts. Further molecular studies are undergoing to expand our understanding on the role of these loci and their mode of action in the dual lifestyle of this bacterium.

Contributed paper. Tuesday, 9:00. **69**

Molecular mechanism of the nematicidal activity of *Photorhabdus luminescens* LN2 against *Heterorhabditis bacteriophora* H06 nematodes

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Photorhabdus luminescens subsp. akhurstii LN2 (Enterobacteriaceae) is a symbiont of entomopathogenic nematodes *Heterorhabditis indica* LN2 and showed nematicidal activity against *H. bacteriophora* H06 infective juveniles (IJs). The LN2 bacteria may secrete unidentified toxic factors lethal for the H06 nematodes. The trans-specific nematicidal activity of the bacteria against the non-symbiotic nematode may have an impact on competitive interactions when one insect host is co-infected by different nematode species. To explore the molecular mechanism of the trans-specific nematicidal activity of *P. luminescens* LN2 against *H. bacteriophora* H06, the complete genome of *P. luminescens* LN2 was sequenced; two mutagenesis libraries of *P. luminescens* LN2 were constructed using Tn5 transposon and rifampicin antibiotic respectively; the mutants from the libraries were tested for nematicidal activity and mutants negative for nematicidal activity were genetically and proteomically characterized. At least 9 putative proteins including DsbA, HlpA, RhlE, RplC, RpoB, NamA, NamB (a protein from T3SS), and 2 hypothetical proteins (similar to unknown protein YgdH and YggE of *Escherichia coli* respectively) were involved in the nematicidal activity of LN2 bacteria against H06 nematodes. This hypothesis was further confirmed by creating insertion-deletion mutants of corresponding genes. It seems that a big network system is involved in this nematicidal activity.

Contributed paper. Tuesday, 9:15. **70**

Natural products from entomopathogenic bacteria: Understanding the interaction of bacteria, insects and nematodes

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Entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively, and together they are able to infect and kill several different insect larvae. We have shown recently by chemical analysis and genome sequencing that these bacteria are able to produce a huge variety of different low molecular weight natural products. These compounds show insecticidal but also antibiotic and anticancer activity and novel bacterial signalling compounds have also been identified.
Recent work indicates that several of the bacterial natural products are addressing different parts of the insect immune system in order to make sure that the bacteria can evade it and kill the insect host. As the nematode immune system shows the same basic principles, it is of high interest how the natural products can differentiate between insect prey and nematode host. We will present our recent finding on natural products and their natural targets as well as ways to improve the production of these – probably also pharmaceutically useful – compounds.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

VIRUSES 3

Contributed paper. Tuesday, 8:00. **71**

Characterization and formulation of a Colombian isolate of *Erinnyis ello* granulovirus (L.) (Lepidoptera: Sphingidae)

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Erinnyis ello (L.) is a polyphagous lepidopteran pest that may cause serious annual losses in the rubber industry. The use of granulovirus represents an interesting alternative as a biological control agent for this insect. One Colombian granulovirus isolate (VG010) was obtained from *E. ello* larvae in the field and was characterized at morphological, biological and molecular level. Occlusion bodies showed an oval morphology with a unique nucleocapsid, and a size of 302.9 ± 22 x 181.5 ± 16 nm. The VG010 viral genome size was estimated to be approximately 88.7 kb. Phylogenetic relationships based on selected gene sequences *lef-8, lef-9* and *gran* showed a close relationship between VG010 and another isolate from *E. ello* previously reported (M34-4), suggesting that these isolates are genotypic variants of the same viral specie. The mean lethal dose of VG010 against second instar *E. ello* larvae was 4.3×10^3 OBs/mL and the viral productivity ranged between 2.1×10^9 and 3.8×10^9 OBs/g of larval tissue. With this virus, a wettable powder formulation was

developed which photostabilized viral OBs against UVB radiation and improved shelf life. This product presented an efficacy of 99% for controlling the pest in laboratory and quality control limits for the product were established. This biopesticide constitutes a new tool with high quality and efficacy that needs to be scaled up and evaluated under field conditions in order to confirm its potential for controlling this important pest in rubber crops.

Contributed paper. Tuesday, 8:15. **72**

Production of the *Cydia pomonella* granulovirus (CpGV) in a heterologous host

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The codling moth (CM), *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), is considered one of the most significant pests of apples and pears in the Western Cape, South Africa. Traditionally, control measures have relied heavily on the use of broad spectrum insecticides. *Cydia pomonella* granulovirus (CpGV) has proved to be an effective alternative to chemical application. The main objectives of this study were to identify a novel South African isolate of CpGV and to ascertain the viability and shortcomings of producing CpGV in the heterologous host, false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Initially four field collected isolates were compared genetically to two commercially available products. PCR amplification and sequencing of CpGV *granulin* and *egt* genes as well as single restriction endonuclease digestion of genomic DNA isolated from purified occlusion bodies indicated that the South African isolates were genetically similar to the Mexican strain. A further two isolates have been collected from the Langkloof (Eastern Cape) and Harrismith (Free State) areas in which there is no previous record of commercial virus application. Genetic comparisons are currently being conducted. Initial results indicate genetic variation in the Harrismith isolate when compared to the Mexican strain. Rearing parameters for CM and FCM, including fecundity, percentage hatch, larval developmental times and percentage mortality, were compared. The quantity of CpGV per larval unit was calculated for both FCM and CM. Mortality and virus yields were assessed by inoculating early 4th and 5th instar larvae with eight concentrations of purified CpGV. The mortality data obtained from the virus yield trials were used to establish the concentrations required to conduct surface dose bioassays against both FCM larval larvae. Dose and time response values for 4th and 5th instar FCM larvae were determined and used in establishing a virus production technique. Effective quality control parameters have been established to ensure the integrity of virus being produced, namely bioassay, RE analysis using *Hind* III as there is no recognition site for this enzyme in CpGV DNA and, lastly, development of a set of standards for a qPCR reaction, which can be used to calculate the proportion of CpGV in a mixed virus solution. If this production technique was to be successfully implemented into a mass production programme the cost of producing CpGV could be significantly reduced.

Contributed paper. Tuesday, 8:30. **73**

Post-translational cleavage of P74 of the Helicoverpa armigera single nucleopolyhedrovirus facilitates *per os* infection

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Baculovirus oral infection is mediated by binding and fusing of occlusion derived virus (ODV) with the microvilli of midgut epithelium under alkaline condition. Previous studies showed that ODV attachment protein, P74, of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) undergoes two sequential cleavage events, the primary one being conducted by the endogenous alkaline protease at an unidentified site and the secondary one by host midgut trypsin at amino acids R195/R196/R199. Here we report that Helicoverpa armigera single nucleopolyhedrovius (HearNPV) P74 was first cleaved after translation in the host cell and was not dependent on the endogenous protease during ODVs release. The cleavage produces two subunits which were not associated by disulfide bonding. Judging from the molecular mass of the subunits, the cleavage was predicted at an arginine and lysine (R/K) rich region in the middle of HearNPV P74. A series of site-directed mutants in this region were generated. Feeding experiments showed that the single or multiple mutations significantly impaired *per os* infectivity and mutagenesis of R334Q/R339Q/R344Q/R347Q eliminated the specific cleavage of HearNPV P74. A mutant of the proposed second cleavage site R220Q/R221Q/R224Q was also generated and bioassays showed that the region was essential for oral infection. The results suggested that although there are some differences during the first cleavage, P74 of both AcMNPV and HearNPV undergo two steps cleavage, and the cleavage sites are likely to be conserved in the two viruses. An integrated model of P74 cleavage is provided which sheds lights on the molecular mechanism of ODV entry.

Contributed paper. Tuesday, 8:45. **74 STU**

Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (*Phop*GV)

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The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a major pest of solanaceous crops in sub-tropical and tropical regions worldwide. This pest has developed resistance to many traditional pesticides, thus alternate means of control are required to protect the R2.5 billion (€168 million) potato industry in South Africa. The *Phthorimaea operculella* granulovirus (PhopGV) is considered a promising biopesticide that can be incorporated into integrated pest management programmes. Several PhopGV isolates recovered from geographically different insect populations have been genetically characterised and the full

genome of the Tunisian PhopGV-1346 isolate has been sequenced, providing a reference strain for comparison with novel isolates. This study reports the identification and genetic characterisation of a South African PhopGV isolate recovered from a *P. operculella* colony reared in the laboratory. Sequencing of the *lef*-8, granulin and *egt* genes confirmed the identity of the virus as PhopGV. Phylogenetic analysis of egt sequences grouped PhopGV-SA together with the Kenyan and South American isolates. Virulence evaluation against *P. operculella* larvae using surface dose and egg dip methods are currently underway and the preliminary data indicate that the virus has potential for development as a biopesticide for control of the pest in both the field and storage.

Contributed paper. Tuesday, 9:00. **75**

Genetic and biological characterisation of a novel South African *Plutella xylostella* granulovirus, PlxyGV-SA

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The diamondback moth, *Plutella xylostella* (L.) (Lep, Plutellidae), is a serious world-wide pest of cruciferous crops, with a global estimated cost of control and damage amounting to approximately US$4–5 billion annually. The *P. xylostella* granulovirus (PlxyGV) is considered a promising alternative to synthetic chemical insecticides and *Bt* insecticidal proteins for control due to the development of resistance in pest populations. Several PlxyGV isolates have been genetically and biologically characterised although many of these have not been commercialised as bio-pesticides. This is the first study to describe a novel South African PlxyGV in terms of genotype and biological activity. PlxyGV was recovered from an overcrowded laboratory *P. xylostella* colony established using field-collected insects. Occlusion bodies (OBs) were extracted from diseased larvae and purified by glycerol gradient centrifugation. PlxyGV-SA was genetically characterised by restriction endonuclease (REN) analysis of genomic DNA, and PCR amplification and sequencing of *granulin, ecdysteroid UDP-glucosyltransferase (egt), late expression factor 8 (lef-8) and late expression factor 9 (lef-9)* genes. Comparison of PlxyGV-SA REN profiles with those of PlxyGV-Japan (GenBank accession No. AF 270937.1) and other documented PlxyGV isolates together with sequence and alignment data showed that PlxyGV-SA is genetically unique. Neonate larvae were more susceptible to PlxyGV-SA infection than fourth instars at the same virus concentration. Biological activity determined by surface dose bioassays was estimated to be 3.56×10^5 OBs/ml (LC_{50}), which is comparable with values obtained in similar studies. These results suggest that PlxyGV-SA has significant potential for development as an effective biopesticide for the control of *P. xylostella* in the field.

Contributed paper. Tuesday, 9:15. **76-STU**

Comparative transcriptome analysis of CpGV-M in susceptible and resistant codling moth Cydia pomonella

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The *Cydia pomonella* granulovirus (CpGV) is commercially widely used and a cornerstone in the control of codling moth, *C. pomonella* L. (CM), in both organic and integrated pome fruit production. Recently, nearly 40 CM populations resistant to products based on the Mexican isolate CpGV-M have been located in Europe. So far, new CpGV isolates overcoming this resistance were identified and are applied in orchards with resistant CM populations. However, only limited information on the infection process of CpGV is available. To gain a better understanding of the interaction between CpGV-M and its host microarray analyses of the transcription of CpGV-M genes in the midgut of susceptible and resistant CM individuals was performed. Therefore, CM larvae were infected with CpGV-M and RNA samples were taken from midguts between 0 and 120 h post infection. Microarray analysis of the susceptible CM strain resulted in a detailed overview of the temporal transcription of all 143 CpGV-M genes. Four representative gene clusters were identified by performing a k-means clustering. Some correlation between the promoter motif and the course of the infection pattern could be observed. Thereby, it was also possible to group uncharacterized CpGV-M genes according to their transcriptional profile. In contrast, a delayed and limited transcriptional activity of CpGV-M genes was observed in midguts of CM strains resistant to CpGV-M. This indicated that CpGV-M is able to enter the midgut in resistant CM and start the viral transcription. This truncated infection does not result in a permissive infection of the host. In addition, the transcription of the resistant CM strain infected with the resistance overcoming isolate CpGV-I12 was followed by qPCR to proof if a successful infection of a resistant CM strain leads to the same course of infection as seen as in susceptible CMs. Six representative genes (*ie-1 lef-8, mcp, pe38, f-protein* and *granulin*) were chosen for this analysis. All of them showed the same course of infection in the resistant CM strain as seen in the susceptible CM strain.

Contributed paper. Tuesday, 9:30. **77**

Transmission of mixtures of insect pathogenic viruses in a single virion: towards the development of custom designed virus insecticides

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Alphabaculoviruses (lepidopteran nucleopolyhedroviruses) have a characteristic physical structure that facilitates the transmission of genetic diversity. We demonstrate that coinfection of *Spodoptera exigua* larvae by SeMNPV and a deletion genotype of SfMNPV resulted in the production of mixed virus occlusion bodies (OBs) containing both the parental viruses. This also occurred when phylogenetically more distant viruses were used: SfMNPV and AcMNPV coinfections in *S. frugiperda* larvae also resulted in mixed virus species OBs. Approximately half the virions present in OBs produced following coinfection with mixtures of different alphabaculoviruses contained both viruses, indicating that the viruses coinfected and replicated in a single cell, and were co-enveloped within the same virion. Serial passage experiments revealed that both viruses persisted in the mixed-virus population by coinfection of insects during several rounds of insect-to-insect transmission. These results have dramatic implications in alphabaculovirus evolution and ecology. This mixed virus production technology is the subject of a PCT (patent) and opens the way to the development of custom-designed insecticides for control of different species of caterpillar pests on crops.

Contributed paper. Tuesday, 9:45. **78**

Improvement of UV-resistance of Baculovirus by displaying the Nano-material binding peptides on the Polyhedron Envelope

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Baculoviruses are sensitive to UV radiation and this characteristic causes the control efficacy of viral insecticides unsteady in the fileds. The polyhedron envelope of baculoviruses, which is composed of carbohydrate and phosphorylated protein (PEP), is the first barrier against the disadvantageous environment. We found that orthologs of *Autographa colifornica multiple nucleopolyhedrovirus* (AcMNPV) PEP*,* such as *Helicoverpa armigera nucleopoly-hedrovirus* PEP, *Cydia pomonella granulovirus* Cp20 or Cp22 might not repair the absence of polyhedron envelope in the *pep*-knocked-out AcMNPV construct. The C-terminal (168~252aa) of AcMNPV PEP might deliever GFP to be expressed on the surface of polyhedron. Consequently, we had constructed the AcMNPV recombinants in which the C-terminal of PEP was fused with the peptides which might specifically bind melanin or nano-scale ZnO. These results may lay a foundation for developing intensive UV-resistant viral insecticides.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

BACTERIA 2

Contributed paper. Tuesday, 8:00. **79**

***Yersinia entomophaga* MH96 (Enterobacteriaceae) BC subcomplex of the Yen-Tc ABC toxin is able to induce toxicity independent of the A subcomplex**

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A novel gram-negative, rod-shaped, non-spore-forming bacterium, *Yersinia entomophaga* MH96 (Enterobacteriaceae), was isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Y. entomophaga* produces a proteinaceous toxin complex (Yen-Tc) that is responsible for mortality in a range of insect species, mainly within the Coleoptera and Lepidoptera. The Yen-Tc is made up of two chitinase subunits (Chi1 and 2) and five Yen subunits (A1, A2, B, C1, and C2). The TcA, B, and C subunits are related to members of the Toxin complex (Tc) toxin family, with orthologs identified from several other bacterial species including *Serratia entomophila* and *Photorhabdus luminescens*. Characterization of Yen-Tc pathology has revealed a progressive deterioration of the midgut epithelium of susceptible insects. Although the specific mechanism of Yen-Tc remains unknown, cellular and molecular work has begun shedding light on how the Tc family functions. The current model proposes that the TcA component binds to the cell surface and forms a pH-triggered channel that allows translocation of the TcBC subcomplex into the cytoplasm. Once in the cytoplasm the carboxy-terminus of the TcC subunit dissociates and becomes active, which causes toxicity in both insect and mammalian cells. A major component of this model is the requirement of an intact toxin complex in allowing TcBC to be transported into the cell. Based on our investigations of Yen-Tc, the YenBC subcomplex and the YenC subunit do not necessarily require full complex assembly to trigger cell toxicity. We will present and discuss our findings in relation to the current model.

Contributed paper. Tuesday, 8:15. **80**

Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures

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Bacillus thuringiensis larvicidal Cry toxins are currently known for their strong host specificity; which is mainly due the presence of specific toxin binding sites on midguts of susceptible insect larvae. Meanwhile Cry toxins can also bind to compounds in the peritrophic matrix (PM) of several insects (*Rees *et al.* 2009; Valaitis and Podgwaite 2013). In *G. mellonella* infected with toxin alone, we observed structural modification of the peritrophic matrix but no evidence for the biochemical explanation for this modification is found so far. Knowing that "mucus" is along with chitin the main components of PM and that mucus is commonly found in several organisms, we aim to investigate the capacity of Cry1Ab to bind to several mucus rich structures. Indeed, our hypothesis is that the heavily glycosylated proteins (peritrophins and mucins) and proteoglycans shared by both vertebrate and invertebrate mucus may bind Cry toxins, therefore questioning on the "specificity" of these toxins used in GMO crops. Using, commercial pork stomach mucins, mice intestinal mucus, vertebrate cell-culture mucus and PM and peritrophins from *G. mellonella,* we then deeply analyzed Cry1Ab-mucus interactions. The presentation will deal with results from far western blot studies, ELISA binding experiments, inhibition ELISA with sugars, lectins or anti-Cry1Ab monoclonal antibodies. Identification of the interacting structure by LC/Ms/Ms analysis and resulting toxicity using insect and cellular models will be also shown.

*J Invertebr Pathol. 2009 Mar; 100(3):139-46; J Invertebr Pathol. 2013 Jan; 112(1):1-8.

Contributed paper. Tuesday, 8:30. **81-STU**

Pore formation helping ability and binding affinity of BmABCC2 and BtR175 against Cry1A toxins.

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By *in vitro* toxicity assay using Sf9/Baculovirus expression system, we previously provided a novel evidence that *Bombyx*