

Contributed paper. Monday, 17:45. **42 STU**

Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey

E. Erin Morris¹ and Ann E. Hajek²

¹Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg 1871, Denmark; ²Department of Entomology, Cornell University, Ithaca, New York 14853-2601, USA.

Address for correspondence: morris@plen.ku.dk

The parasitic nematode *Deladenus siricidicola* is widely used for the biological control of the invasive pine-killing woodwasp, *Sirex noctilio*. The nematode has a unique life cycle where it lives in pine trees, feeding on the symbiotic fungus of *S. noctilio*, the basidiomycete white rot fungus *Amylostereum areolatum*. In the presence of *S. noctilio* larvae, however, the nematode develops into a parasitic form which invades the woodwasp larvae, ultimately leading to sterilization of the host. The fungal-feeding stage of the nematode is used to commercially mass produce it for biological control programs. Previous studies investigating the effect of *A. areolatum* strain on *D. siricidicola* reproduction suggested the possibility of a role reversal where the fungus could eat the nematode. The present study examined the relationships between three species of *Deladenus* nematodes and their associated *Amylostereum* fungi. For *D. siricidicola* and *A. areolatum*, we hypothesized that significantly fewer nematode eggs placed in petri dishes containing potato dextrose agar medium would hatch in the presence of *A. areolatum* fungus than in control petri dishes with no fungus. Results supported this hypothesis. Additionally, light microscopy, fluorescence microscopy, and cryogenic scanning electron microscopy were used to show the ability of both *A. areolatum* and a second species, *A. chailletii*, to penetrate nematode eggs and adult living females of three species of *Deladenus* nematodes.

CONTRIBUTED PAPERS Monday, 16:30-18:30

VIRUSES 2

Contributed paper. Monday, 16:30. **43**

Insect feeding induces transgenerational resistance to NPV in Lepidoptera

Grant L. Olson¹, Judith H. Myers², Jenny S. Cory¹

¹Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; ²Biodiversity Centre, Dept. of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

Address for correspondence: jsc21@sfu.ca

When population density increases, insects experience a number of stresses as a result of crowding including alteration in food quality and quantity. These changes have been shown to alter the resistance of insects to pathogens. Previous studies have tended to investigate the impact of these factors individually: however, in nature density-related factors are likely to interact simultaneously. While changes that occur within a single generation have been well documented, we know less about the transgenerational impact of changes in food quality on disease resistance. We tested the impact and interaction of three factors that are likely to occur in insect populations when density rises using the western tent caterpillar, *Malacosoma californicum pluviale*. Western tent caterpillars exhibit population cycles every 8-11 years, which

are characterized by NPV epizootics at high density. We manipulated food quality, food quantity and the presence of phylloplane bacteria in the parental generation and measured the impact on immunity and resistance to NPV in the offspring. The treatments, particularly the foliar treatments, had clear impacts on the disease resistance of the offspring generation: however, not necessarily in the direction predicted. We discuss these data in relation to how changing levels of susceptibility could influence population cycles in these forest insects.

Contributed paper. Monday, 16:45. **44**

The resistance of *Cydia pomonella* against baculoviruses is provoked by a mutation of the immediate-early *pe38* gene of *Cydia pomonella* granulovirus

Manuela Gebhardt, Karolin E. Eberle, Johannes A. Jehle

Institute for Biological Control, Julius Kühn Institute (JKI), Federal Research Center on Cultivated Plants, Heinrichstraße 243, 64287 Darmstadt, Germany

Address for Correspondence: manuela.gebhardt@jki.bund.de

The *Cydia pomonella* granulovirus (CpGV) (*Baculoviridae*, genus *Betabaculovirus*) is a worldwide used biological agent to control the infestation of pome fruits by codling moth (*C. pomonella* L.). In 2005, first CM field populations resistant to commercial CpGV products containing the isolate CpGV-M (so-called Mexican isolate) were discovered in Europe. These resistant CM populations showed 1,000 – 100,000fold reduced susceptibility to CpGV-M when compared to normally susceptible CM populations. Infection experiments with isolates from different geographical origins showed that various CpGV isolates were able to overcome CM resistance in the genetically homogenous resistant laboratory CM strain. Molecular analysis of these resistance overcoming isolates (-I12, -I07, -S, and -E2) showed that the only genomic difference, which all resistance overcoming isolates have in common, is a single common 24 nucleotide indel mutation coding for eight amino acids within the immediate-early gene *pe38*. Phylogenetic analyses presume that this mutation is an insertion within the genome of CpGV-M.

Therefore, the role of *pe38* in overcoming the resistance of CM was analyzed by constructing knockout and rescue pseudoviral mutants based on a CpGV-M bacmid. According to the source of *pe38*, we could show that the pseudoviruses are infective against susceptible larvae only - in the case of *pe38* from CpGV-M - or against both susceptible and resistant larvae - in the case of *pe38* from CpGV-S. Therefore, we conclude that *pe38* is not only an essential factor for the infectivity of CpGV but also the key factor in overcoming CpGV resistance in CM.

Contributed paper. Monday, 17:00. **45**

CpGV-R5 allows replication of CpGV-M in resistant host insect larvae

Benoit Graillot^{1,2}, Sandrine Bayle¹, Christine Blachere-Lopez^{1,3}, Samantha Besse², Myriam Siegwart⁴, Miguel Lopez-Ferber¹

¹LGEI, Ecole des Mines d'Alès, Institut Mines-Telecom et Université de Montpellier Sud de France. 6, Avenue de Clavières, 30319 Alès, France. ²Natural Plant Protection, Arysta LifeScience group, Avenue Léon Blum, 64 000 Pau, France. ³INRA, 6, Avenue de Clavières, 30319 Alès, France.

⁴INRA, unité PSH, Agroparc, 84914 AVIGNON Cedex 9, France

Address for Correspondence: benoit.graillot@mines-ales.fr

In resistant codling moth larvae, CpGV-M replication is blocked at an early step in all tissues. Among others, the CpGV-R5 isolate is able to overcome this resistance. A genetically heterogeneous virus population, containing 1% CpGV-R5, and

99% CpGV-M has been used to infect non permissive host populations. Unexpectedly, this mixture, and the OBs recovered from killed larvae performed 100 times and 2000 times better than CpGV-M used alone, respectively. qPCR analysis using specific markers for each viral isolate was performed. The viral mixture CpGV-R5, 1%, and 99% of CpGV-M was amplified on permissive (Cp_{NPP}) and non-permissive (R_{GV}) populations and their offspring was tested for their respective proportion of each kind of marker. On permissive host, the R/M markers ratio raised to 15/85. On resistant host, a similar R/M ratio (12/88) was obtained, indicating that CpGV-M has been able to perform a complete replication cycle in a non-permissive host. These results suggest that in the presence of a small proportion of CpGV-R5, CpGV-M is able to replicate in resistant hosts. Accordingly, CpGV-R5 seems to act as a helper for CpGV-M genomes. Understanding the mechanism involved in the unlocking of the replication process opens the possibilities of innovative control strategies.

Contributed paper. Monday, 17:15. **46**

Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*

Agata K. Jakubowska¹; Melania D'Angiolo¹; Rosa M. González Martínez¹; Anabel Millán Leiva¹; Arkaitz Carballo²; Rosa Murillo²; Primitivo Caballero²; Salvador Herrero¹
¹Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain; ²Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain
 Address for Correspondence: sherrero@uv.es

Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that did not reach enough viral titer to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In the last years, a large number of viruses causing covert infections in all type of hosts have been identified, mostly due to the revolution in the sequencing technologies. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant losses to agricultural and ornamental plant industries. A comprehensive transcriptome analysis of the larval stage of *S. exigua* revealed the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirales*. In order to characterize *S. exigua* viral complex, we have completed the genomic sequences of three picorna-like viruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their morphology, infectivity, tissue distribution and abundance in the larval hosts. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Contributed paper. Monday, 17:30. **47**

Mixed SeMNPV genotypes comprised transmission capacities and insecticidal properties

Cristina Virto¹, David Navarro^{1,2} M^a del Mar Tellez², Trevor Williams³, Rosa Murillo^{1,4}, Primitivo Caballero^{1,4}
¹Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Ctra. de Mutilva s/n 31192, Mutilva Baja, Spain; ²IFAPA, La Mojonera, 04745, Almería, Spain; ³Instituto de Ecología AC, Xalapa 91070, Mexico; ⁴Departamento Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain

Address for Correspondence: pcm92@unavarra.es

Recent studies have demonstrated that transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) parents to offspring (vertical transmission) is frequent and could contribute to biological control of this pest by causing viral mortality in the pest population in successive cropping cycles. The aim of this work was to study the efficacy of using mixtures of two SeMNPV genotypes that had either high insecticidal properties (SeG25) or the capability to be transmitted through host generations (SeA11). Mixed populations containing 25 and 75% of SeG25 resulted in increased pathogenicity (LC₅₀) compared to the SeA11 genotype. However in terms of virulence (mean time to death) and productivity (OBs/larva), no differences were observed between the individual genotypes or their mixtures. The capacity to induce persistent infections by each genotype and their mixtures was evaluated using qPCR (*DNA-polymerase* gene) in adult survivors of a sublethal dose of the virus. The prevalence of covert infection varied between 70 and 100% in adults that survived inoculation with the vertically transmitted genotype Se-A11. The adult survivors to the mixtures and the SeG25 genotype alone are currently being analyzed to determine covert infection. Finally, field trials were carried out to evaluate the capacity of mixed virus populations to establish covert infections in greenhouse conditions. Adults developed from larvae collected in experimental plots sprayed with either single genotypes or one of the mixtures 75%SeA11+ 25%G25 (75:25) and 25%SeA11 +75%G25 (25:75) are being processed currently. The F₁ offspring from adult survivors of SeA11, 75:25, 25:75, SeG25 and control treatment did not showed differential susceptibility to a 25:75 mixture of OBs. The implications of these findings will be discussed.

Contributed paper. Monday, 17:45. **48-STU**

A novel mode of resistance of codling moth against *Cydia pomonella granulovirus*

Annette J. Sauer, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle
 Julius Kühn-Institut, Darmstadt, Germany
 Address for Correspondence: Annette.Sauer@jki.bund.de

The codling moth (CM, *Cydia pomonella*) is one of the most devastating pests in nearly all pome fruit growing regions. An alternative to the application of chemical insecticides is the application of *Cydia pomonella granulovirus* (CpGV) (family *Baculoviridae*), which is registered as biological control agents in 34 countries worldwide. Since 2005, CM populations with a reduced susceptibility to CpGV products have been reported from about 40 plantations in seven European countries. For many of these CM populations, the resistance could be traced back to a single, dominant allele that is linked to the sex chromosome Z. CpGV-M, the so-called Mexican isolate, was the common agent used in all commercial CpGV products registered in Europe. Currently, resistance management strategies are based on the application of improved CpGV products, containing resistance-overcoming isolates. However, a CM field population, termed NRW-WE showed even resistance to most resistance overcoming CpGV isolates, suggesting a second mode of CpGV resistance. In order to elucidate the inheritance of this type of resistance and after failure of single crossing experiments, successive mass crossings under virus pressure were carried out to establish a genetically homogenous resistant strain of the CM population NRW-WE. Subsequent reciprocal crossing experiments with the resulting CM strain and a susceptible laboratory CM strain (CpS) followed by bioassays fitted to a dominant but autosomal inheritance model. Further analyses of the mode of resistance are under way.

The effects of temperature on *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) in mortality rates of false codling moth larvae *Thaumatotibia leucotreta*

Devon Brits, Jaryd Ridgeway & Alicia Timm
Department of Zoology and Entomology, Rhodes University,
Grahamstown, South Africa
Address for Correspondence: aetimm@gmail.com

False codling moth (FCM), *Thaumatotibia leucotreta* is a major citrus pest in South Africa. *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) has been found to be a successful biological control agent for FCM. South Africa grows citrus in many different geographical areas throughout the country that experience different temperature differences; this in turn could affect the efficiency of the virus upon the larvae. The aim of this study was to determine the effectiveness of the virus on larvae at temperatures ranging between 15-35°C. Unpaired T-tests, one-way ANOVA tests and post-Hoc Tukey's HSD tests were conducted on both virus and control treatments to test for significant differences among different temperatures as well as between the virus and control treatments. The number of deaths between infected and control treatments were significantly different at all temperatures. The differences between treatment mortality times were significantly different for all infection stages except the final death stage (5th stage). The virus was found to be most efficient at higher temperatures since the larvae grow faster at higher temperatures. The virus was found to have very little effect at 15°C. These results should assist with the control of FCM in citrus orchards, and in particular would affect the timing of applications, to ensure that the virus is used at its maximum efficiency.

Contributed paper. Monday, 18:15. **50**

Enhancement of insecticidal activity of a nucleopolyhedrovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) by coinfection with granulovirus

Paola Cuartas, Laura Villamizar
Centro de Biotecnología y Bioindustria (CBB), Corpoica,
Bogotá, Colombia
Address for Correspondence: lvillamizar@corpoica.org.co

Spodoptera frugiperda is a polyphagous pest with wide geographical distribution. Biological control of this pest has included the use of its nucleopolyhedrovirus SfMNPV, which has shown high potential as biopesticide with efficacies higher than 80% but with some disadvantages related with cost production and time of action. In this sense, other viruses as betabaculovirus (GV) may act as synergists, increasing the insecticidal activity of NPVs. In this work, a Colombian granulovirus isolated from *S. frugiperda* larvae (VG008) was mixed with two different NPVs samples, one corresponding to a wild virus NPV003 and other corresponding to a pure genotype variant obtained from NPV003 (NPV003-A). Each mixture was evaluated in different proportions and in five different concentrations since 1×10^4 OB/mL to 1×10^8 OB/mL. For each mixture, the median lethal concentration (LC₅₀) and mean time of mortality (MTM) were determined by laboratory bioassay in second instar larvae of *S. frugiperda*. Majority of mixtures between the VG008 and NPV003 showed a higher biological activity compared with each individual isolate, confirming the coinfection enhancement effect. The mixture corresponding to 2.5% of VG008 and 97.5% of NPV003, showed the highest enhancement of the NPV insecticidal activity with a decrease of 9.92 times in the LC₅₀ and 4 days (96 hours) in the MTM. This virus mixture was selected and will be used as an active ingredient for the development of a new biopesticide based on both viruses in order to improve NPV efficacy for controlling the pest in the field.

FUNGI 2Contributed paper. Monday, 16:30. **51**

Rapid and simple method for overnight development of strain-specific markers: A case study with the commercial *Beauveria bassiana* strain, GHA.

George Kyei-Poku, Shajahan Johny, Agathe Roucou and
Debbie Gauthier

Canadian Forestry Service, Great Lakes Forestry Centre,
Natural Resources Canada, 1219 Queen Street East, Sault
Ste. Marie, Ontario, Canada P6A 2E5

Address for Correspondence: gkyeipok@nrcan.gc.ca

Genetic markers have proved useful for assessing taxonomy and identifying specific-strains of entomopathogenic fungi. We targeted *Beauveria bassiana* commercial strain, GHA to develop a new reliable, simple, specific, sensitive and cost effective method that allows specific detection and discrimination of GHA from other *Beauveria* strains. We applied a combination of software with intrinsic manipulations to design GHA strain-specific primers by exploiting available *Bloc* nuclear intergenic sequences of GHA and other *Beauveria* strains. The generated primers were used in PCR assays to probe strains of *B. bassiana* (50), *Beauveria pseudobassiana* (13), *Beauveria bronginiartii* (3), *Beauveria amorpha* (2), *Beauveria vermiconia* (2), *Beauveria asiatica*, *Beauveria australis*, *Beauveria kipukae*, *Beauveria malawiensis*, *Beauveria sungii* and *Beauveria varroae*. In the specificity test, we amplified the expected target gene and ~300-bp-fragment from *B. bassiana*, GHA DNA. All other tested strains/isolates reacted negatively with the exception of four out of fifty *B. bassiana* strains that produced positive signals. In addition, the designed primers were highly sensitive; capable of detecting ~20 pg/μl of GHA genomic DNA. For operational feasibility, the newly designed marker would be used for studying the ecology, persistence and monitoring autodissemination of post-released GHA in the environment. To date, our methodology and associate protocol could be considered the simplest with high sensitivity and specificity, and most cost effective strategy for strain-specific marker design in the highly heterogeneous *Beauveria* species complex. Our approach provides a general framework that can be readily or easily adapted for designing strain-specific markers targeting any organism of choice.

Contributed paper. Monday, 16:45. **52-STU**

The functions of two Cu/Zn-superoxide dismutases and a Fe-superoxide dismutase in regulating the growth, antioxidation, UV tolerance and virulence of *Beauveria bassiana*

Fang Li¹, Zheng-Liang Wang², Han-Qing Shi¹, Sheng-Hua Ying¹, Ming-Guang Feng¹

¹ Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China; ² College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang, People's Republic of China.

Address for Correspondence: mgfeng@zju.edu.cn;
lifang870910@163.com

The biocontrol potential of filamentous entomopathogenic fungi, such as *Beauveria bassiana*, depends not only on the virulence of a candidate strain to target pests but also on its tolerance to high temperature and solar UV irradiation often encountered in the field. The stress of UV, heat, drought, or