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.

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

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The Cydia pomonella granulovirus (CpGV) (Baculoviridae, genus Betabaculovirus) is a worldwide used biological agent to control the infestation of pome fruits by codling moth (Cydia 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 (-112, -107, -12, 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 pseudoviruses 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.

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

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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 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.
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 (CpM) and non-permissive (R5) 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

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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 Illaviridae 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

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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 fitness of mixtures of two SeMNPV genotypes that had either high insecticidal properties (SeG25) or the capability to be transmitted through host generations (SeAll). Mixed populations containing 25 and 75% of SeG25 resulted in increased pathogenicity (LC50) compared to the SeAll 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%A11 + 25%G25 (75/25) and 25%A11 + 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

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The codling moth (CM, Cydia pomonella) is one of the most devastating pests in nearly all apple 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.
False coding moth (FCM), *Thaumatotibia leucomela* is a major citrus pest in South Africa. *Cryptophlebia leucomela* 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 and tests 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.

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

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_Spodoptera frugiperda_ is a polyphagous pest with wide geographical distribution. Biological control of this pest has included the use of its nucleopolyhedrovirus SIMNPV, 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 betaculoviruses (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 x 10^4 OB/mL to 1 x 10^5 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.

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

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The virus was found to be most efficient at higher temperatures as well as temperatures between 15°C and 35°C. Unpaired T-tests, one-way ANOVA and 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.

**Fungi 2**

**CONTRIBUTED PAPERS**

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

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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 brongniartii_ (3), _Beauveria amorpha_ (2), _Beauveria vermiconia_ (2), _Beauveria asiatica_, _Beauveria australis_, _Beauveria kipukae_, _Beauveria malawiensis_, _Beauveria sugii_ 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.

**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.**

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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...