PS1Aa2 (Cry31Aa2) formed pores in artificial membranes. These pores had several levels of conductance; the most frequently observed in 150 mM KCl solutions were of 11, 16 and 21 pS. Microspectrofluorometric experiments with the Fura-2 probe showed that the presence of PS1Aa2 can induce changes in intracellular calcium levels, most often in the form of calcium oscillations and sometimes as sustained increases. Such responses were observed in the presence and absence of extracellular calcium, with the tumor cell lines HeLa and HepG2, and with the non-tumorous cell line HEK 293. Calcium oscillations have not been described previously for Cry toxins even though some studies have shown that calcium appears to be involved in their mode of action. Our experiments required the use of much higher concentrations of toxin than suggested from the published cytotoxicity results. Despite the presence of fragments previously identified as active, its low efficacy appears to be related to the presence of DNA in the preparations causing the protein to precipitate. Future work aimed at elucidating the origin of these calcium oscillations and their role in toxicity will be greatly facilitated by an improvement in the method of preparation of this toxin.

Contributed paper, 10:00. **86-STU**

***Caenorhabditis elegans – Bacillus thuringiensis* interactions: new insights into mechanisms of host resistance and pathogen virulence**

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Genetically tractable model nematode *Caenorhabditis elegans* has been successfully used in the host-pathogen interaction studies and helped to uncover conserved virulence factors of clinically relevant pathogens. At the same time interactions of this nematode with its natural pathogens are poorly investigated. Bacteria from the genus *Bacillus* are among potential natural pathogens of the nematodes. Therefore, previously we isolated 768 *Bacillus* strains and tested them for the virulence to nematodes. Although only 3% of tested *Bacillus* strains were pathogenic, one strain called *B. thuringiensis* DB27 exhibited extreme virulence to *C. elegans*. Currently we are trying to tackle both sides of this host-pathogen equation and aiming to identify virulence factors of *B. thuringiensis* DB27 as well as *C. elegans* defense mechanisms. First, combining plasmid-curing and genome sequencing, we discovered that novel nematicidal Cry21 toxins with synergistic activity are the main nematicidal factors of DB27. We expressed these novel toxins in *E. coli* and confirmed their activity against *C. elegans*. Importantly, these toxins are also active against other free-living and animal parasitic nematodes, suggesting their potential application against parasitic nematodes. Our parallel work on the host side led to the discovery of *C. elegans* novel innate immunity pathway involved in the defense against pathogens. Specifically, we identified novel function for Dicer in *C. elegans* antibacterial innate immunity and showed that this function is largely associated with microRNA processing. Taken together, our reciprocal studies uncovered a previously unknown role for DCR-1/Dicer in *C. elegans* antibacterial immunity as well as identified novel nematicidal toxins.

SYMPOSIUM 4 (Viruses) Tuesday, 10:30-12:30

Small non-coding RNAs as regulators of insect host-virus

Symposium. Tuesday, 10:30. **87**

Role of cellular and virus-encoded microRNAs in insect host-virus interactions

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MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides which play significant roles in gene regulation at transcriptional as well as post-transcriptional levels. They are produced in almost all eukaryotes and also encoded by some viruses. Besides cellular miRNAs that may participate in anti-viral responses following infection, virus-encoded miRNAs may target host genes to interfere with host survival, proliferation and immunity. Furthermore, virus-encoded miRNAs may regulate replication of virus to avoid over replication and quick demise of the host or facilitate virus entry into persistent infection. The interaction may become more complicated in the presence of third parties, such as microbiota and endosymbionts, that in turn may affect the host's miRNA profile and indirectly disturb virus replication. In the presentation, the role of miRNAs in mosquito-arbovirus interactions with a reference to the effect of Wolbachia as an endosymbiont on the interactions will be discussed.

Symposium. Tuesday, 11:00. **88**

Sensing viral RNA in *Drosophila melanogaster*

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RNA interference plays a central role in antiviral innate immunity in flies. Indeed, flies mutant for the three key components of the small interfering (si)RNA pathway, namely Dicer-2, R2D2 and Argonaute (AGO) 2 are highly sensitive to a wide range of viruses (1). Dicer-2 produces virus derived-siRNAs from viral RNAs throughout its RNaseIII activity. The Dicer-2/R2D2 heterodimer then loads these siRNAs onto AGO2 in the RNA-induced silencing complex, RISC. The RISC complex is then able to target viral RNAs, thus impairing the ability of the virus to successfully replicate. Although *in vitro* and *in vivo* experiments clearly indicate that Dicer-2 can process long double stranded RNA, the exact nature of the viral RNAs sensed *in vivo* in infected cells remains mysterious. We are interested in understanding how Dicer-2 senses viral RNAs, with a particular focus on the contribution of the N-terminal DExD/C helicase domain, which is conserved in mammalian RIG-I like receptors. Indeed, *in vitro* experiments have revealed a critical role of this domain in both processivity of the enzyme and discrimination of the extremities of the template RNA (1,2). To address this question, we take advantage of a combination of approaches including *Drosophila* genetics, next-generation sequencing technologies and bioinformatics analysis.

(1) Kemp *et al.*, J. Immunol. 2013
(2) Cenik *et al.*, Mol Cell. 2011

Symposium. Tuesday, 11:30. **89**

Small RNA-directed antiviral immunity in disease-vector mosquitoes

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The natural maintenance cycles of many mosquito-borne pathogens require establishment of persistent non-lethal infections in the invertebrate host. While the mechanisms by which this occurs are not well understood, antiviral responses directed by small RNAs are important in modulating the pathogenesis of viral infections in disease vector mosquitoes. Infection of Aedinine vector species with viral pathogens triggers the production of short interfering (siRNAs) and another class of virus-derived small RNAs, ping-pong-dependent piwi- interacting RNAs (piRNAs). Unlike ping-pong-dependent piRNAs that have been described previously, from repetitive elements or piRNA clusters, our work suggests biogenesis in the mosquito soma. Similar to siRNAs, viral piRNAs also appear capable of modulating the pathogenesis of viral infections in mosquito cells. Thus, the non-canonical piRNA pathway present in the soma of Aedinine vector species may provide robustness to the primary siRNA-based antiviral response.

Symposium. Tuesday, 12:00. **90**

Controlling viral infection in in insects

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The paradigm held for a long time that RNAi response in most metazoans does not undergoes an amplification step and only acted cell-autonomously has prevailed in the model system Drosophila melanogaster and it is widely accepted for higher metazoans. The absence of systemic RNAi spread in Drosophila was directly tested in one study that used dsRNA-expressing transgenes in vivo in flies. We challenged this idea by identifying a dsRNA uptake pathway in Drosophila and showing that flies defective in several of the RNAi uptake genes are hypersensitive to virus infection, indicating that RNAi uptake is essential in the process of antiviral defense. In a second area, using a cloning approach to capture small RNAs with a 5' triphosphate group, we show that virus-derived siRNAs (vsRNA) bearing 5' triphosphate group accumulate in Sindbis virus (SINV) infected Drosophila melanogaster, suggesting that secondary vsRNA are produce during infection. Finally, we found that Criket Paralysis virus encoding RNAi suppressor, CrPV-1A specifically interacts with Ago-2 within assembled holo-RISC, without modifying RISC composition, and that this interaction prevents RISC cleavage of target mRNAs. Interestingly, we discovered that CrPV1A recruit an E3 ligase. The implication.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

MICROBIAL CONTROL 1

Contributed paper. Tuesday, 10:30. **91**

Double trouble for thrips: Effective biopesticide combinations to control soil-dwelling stages in chrysanthemums

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Western flower thrips (WFT) are pests of global significance and a constant challenge in greenhouse floriculture. Faced with a lack of conventional control products, Canadian growers have embraced the use of biological control strategies to manage this pest. Soil-dwelling stages of thrips (pro-pupae, pupae) can be targeted with different natural enemies, including biopesticides. *Steinernema feltiae* (e.g., Nemasys®), applied as a drench, is widely used. *Metarhizium brunneum* (formerly *anisopliae*; Met52™) has recently been registered in Canada and the granular biopesicide product is incorporated into potting media. A series of trials were set up to assess compatibility of the two control agents, and the relative efficacy of individual and combined, i.e. nematode plus Met52, treatments against WFT. Fewer *S. feltiae* were recovered from Met52 treated soils over time than from untreated media; this was generally accompanied by a concurrent increase in the number of free-living nematodes recovered. The rice carrier in the biopesticide may have served as a food source for the free-living nematodes, promoting population growth, which may have affected survival of *S. feltiae*. The individual nematode and fungus treatments had a measurable suppressive effect on thrips, but the combined nematode/fungus treatment provided superior control throughout. WFT populations were consistently lower on plants receiving the combined treatment and significantly fewer WFT (< 2 per plant) were found on the plants at the conclusion of the trial (8 weeks). Opportunities therefore exist to enhance the reliability and cost-effectiveness of thrips biocontrol strategies by taking an integrated approach to the deployment of biopesticides.

Contributed paper. Tuesday, 10:45. **92-STU**

Lethal and sub-lethal impacts of fungal biopesticides on house fly populations in simulated field settings of biocosms

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Management strategies for control of house flies in poultry houses include cultural, biological and chemical tactics; however, use of broad-spectrum chemical larvicides and adulticides is the only reliable tool for poultry growers to manage 'fly burst' situations. Our aim was to exploit post-eclosion resting behaviors of teneral flies to evaluate the population control potential (lethal and sub-lethal impacts) of oil formulations of *Beauveria bassiana* and *Metarhizium*