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*

anisopliae under simulated field settings called 'biocosms'. Experimental biocosms were created in plastic boxes where the vertical walls were fitted with sprayed plastic sheeting (blank oil or conidia in oil). A cohort of 300 fly pupae was added to each biocosm; on emergence, the adult flies moved to the vertical surfaces to harden their wings, simulating the exposure likely to occur in the fields. The biocosms were monitored daily for mortality and enumeration of egg laying and egg viability until all adult flies had died. Fungal treated biocosms resulted in 100% mortality within 10-17 days, depending on the fungal species. Treated populations also showed significant reduction in egg viability and life-time fecundity. Furthermore, >20% reduction in basic reproductive rate (B0) was observed in treated fly populations. Together these results demonstrate that application of oil formulations of entomopathogenic fungi could suppress existing fly populations and substantially reduce population growth rates in poultry houses as part of an IPM program. Further studies will focus on evaluating fungal persistence on typical structural surfaces, optimizing application parameters and validating these strategies under actual field conditions in poultry houses.

Contributed paper. Tuesday, 11:00. **93-STU**

Management of *Prostephanus truncatus* (Horn.) on stored maize using *Beauveria bassiana* (Bals.)

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Control of the larger grain borer (*Prostephanus truncatus* Horn) using chemical insecticides is no longer desirable due to environmental and food safety issues. Classical biological control using the Histerid beetle, *Teretrius nigrescens* has been adapted in several locations in Ghana. However, *P. truncatus* is still causing tremendous losses of stored maize. There is a growing interest in using mycopesticides to complement other integrated pest management measures. Recent research in the UK has identified *Beauveria bassiana*, IMI 389521 as a suitable control agent for grain storage pests in the UK. In this study, the pathogenicity of *B. bassiana* IMI 389521 was evaluated against adults of *P. truncatus, Sitophilus zeamais* and *T. nigrescens*. The result obtained from the study indicates that *B. bassiana*, is pathogenic against adults of *P. truncatus* and *S. zeamais. Teretrius nigrescens* was less susceptible to the fungus. To determine the most effective concentration of *B. bassiana* for the control of *P. truncatus* in a semi-field trial, a laboratory dose response experiment using four concentrations of *B. bassiana*, ($1x10^8$ to 10^{11}/kg maize) was studied. Successful control of *P. truncatus* on infested maize was achieved at $1x10^{10}$conidia per kg maize. Semi-field trial to evaluate the efficacy of *B. bassiana*, against *P. truncatus* on maize stored on cobs (dehusked) and on shelled kernels is on-going. The availability and safety of maize will be enhanced, through reduction in the use of chemical insecticides if the isolate is proved effective thereby improving the livelihood of smallholder farmers in Ghana.

Contributed paper. Tuesday, 11:15. **94-STU**

Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* exudates to the aphid *Myzus persicae*

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Insect disease causing *Beauveria bassiana* produces a range of insecticidal metabolites and enzymes such as chitinases and proteases although few have been shown to be toxic simply through contact. Using supernatant from broth cultures of a single strain, *B. bassiana* could cause significant mortality of green peach aphid, *Myzus persicae,* within 24 hours of application. Three day old broth cultures were the most effective, with less insect mortality seen using 6 day old broth. However, aphicidal activity increased again for 7 day old broth. Submerged cultures grew better and produced stronger aphicidal supernatants when cultured in media with start pH above 5.5. Chitinase was produced a day earlier than protease Pr1. The enzymes, however, appeared to have little impact on aphicidal mortality given that their peak production periods do not correlate with the aphicidal activities of 3 or 6 day old cultures. Supernatants treated with EDTA and heat respectively, still killed aphids. High quantities of hydrolytic enzymes produced using insect cuticle medium showed no aphicidal activity. No beauvericin nor bassianolide, two known insecticidal metabolites, were detected in the supernatants. The identities of the key aphicidal components of the *B. bassiana* supernatants thus remain to be resolved.
Keywords: supernatant, *Beauveria bassiana*, chitinase, Pr1, aphicidal, EDTA, beauvericin, bassianolide

Contributed paper. Tuesday, 11:30. **95-STU**

Entomopathogenic fungi for control of false codling moth in South African citrus orchards

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False codling moth, *Thaumatotibia leucotreta* Meyrick (1912) (Lepidoptera: Tortricidae) is a key economic pest of citrus in South Africa causing pre- and post-harvest damage. Soil microbes, such as entomopathogenic fungi, offer an additional means of controlling this pest by targeting the soil-dwelling life stages. Three fungal isolates, two of the species *Metarhizium anisopliae s.l.* and one of the species *Beauveria bassiana s.l.* caused the highest levels of mortality of *T. leucotreta* fifth instar larvae in laboratory bioassays. In addition, these isolates were capable of persisting in a citrus orchard for six months, in sterile soil, whilst still remaining infective towards *T. leucotreta* fifth instar larvae. Since results may differ substantially under field conditions, further research was undertaken to determine whether these isolates remained effective when applied to non-sterile soil beneath the canopy of citrus trees in an orchard. A field trial consisting of one hectare treatment blocks, and a smaller caged trial were initiated to address this issue. Fungal spores were applied via spraying in an aqueous suspension at a concentration of $1x10^{14}$ spores per hectare for the field trial and at three different concentrations for the caged trial. Results of the large scale field trial, four months post application, support the

persistence capability of these isolates and, suggest that, although all three isolates were capable of reducing *T. leucotreta* infestation in comparison to the control block, *B. bassiana* performed best with an 81.33% reduction. It is thus suggested that future trials focus on the performance of this isolate.

Contributed paper. Tuesday, 11:45. **97-STU**

Wireworm control with entomopathogenic fungi and plant extracts

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Wireworms, the soil-dwelling larvae of click beetles, cause severe damage in arable crops and vegetable production. Currently, no registered and effective insecticides are available. The development of alternative control approaches including botanicals or insect pathogenic organisms are demanded and favoured by EU legislation (Directive 2009/128/EC). Limited efficacy of already tested entomopathogenic fungi (EPF) could be improved by synergistically acting botanicals. In the present study repellency of botanicals towards the wireworm species *Agriotes lineatus* and potential interactions of the most effective repellent with a wireworm-infecting fungus strain (*Metarhizium brunneum)* was investigated. Behaviour and mortality of wireworms were assessed in two-dimensional terraria (40cm x 50cm x 0.6cm) with a peat-sand substrate in a choice test for up to three weeks. Wireworm location was recorded and locomotion trails were manually traced, photographed and trail length determined on the treated and untreated half of the terrarium. We found that the garlic extract R3 repelled wireworms at rates of 1.2 g/L substrate, while this concentration hardly reduced efficacy of the EPF strain. Thyme oil was comparably repellent, but also strongly antifungal. The EPF strain was not repellent. Potential synergies between EPF and efficacy enhancing botanicals will be discussed for a biological control strategy.

Contributed paper. Tuesday, 12:00. **98-STU**

Long-term persistence of *Beauveria brongniartii* BIPESCO 2 used for cockchafer control in the Euroregion Tyrol

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The fungus *Beauveria brongniartii* (Sacc.) Petch has been used to control the European cockchafer *Melolontha melolontha* L. for more than two decades. The goal of this study was to assess persistence of the applied *B. brongniartii* strain in the soil of 20 cockchafer infested sites in East, North and South Tyrol. The sites have been treated with Melocont® Pilzgerste (BIPESCO 2) at different frequencies and time points during the last 20 years. At all sites the denistiy of *M. melolontha* larvae decreased from high levels at the start of treatments to levels below the damage threshold at the time point of sampling in 2012. A selective medium was used to determine *B. brongniartii* density and recover *B. brongniartii* isolates from soil samples. Collected isolates were subjected to genetic analyses to discriminate the applied strain from naturally occuring strains. Highest densities of *Beauveria* spp. (up to 6.8 x 10^5 CFU g^{-1} soil dry weight) were detected in soils which have been treated with Melocont® Pilzgerste at least three times during the last three years (3 sites) prior to the sampling date. BIPESCO 2 was detected in 7 sites of which one was treated for the last time15 years prior to sampling. *Beauveria* spp. density varied strongly among and within fields and in 71% of the 162 soil samples no *Beauveria* was detected. Results suggest that periodic applications of the *B. brongniartii* biological control agent increase density and persistence of the fungus in soil and support a long-term control of *M. melolontha*.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

DIS. OF BENEFICIAL INVERTEBRATES 1

Contributed paper. Tuesday, 10:30. **99**

The Curious Case of the PaV1 in Adult Caribbean Spiny Lobsters

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The pathogen PaV1 (Panulirus argus Virus 1) exacts a heavy toll from juvenile Caribbean spiny lobsters with an estimated 24% in Florida dying of it before they reach maturity and recruit to the fishery. Prevalence is also high among adult populations, especially in the northern Caribbean (e.g., Puerto Rico – 17%). However, PaV1 manifests differently in adults. They may act as "carriers" because adults rarely develop visible infections and do not seem adversely affected by the pathogen. Infected adults are not avoided by healthy conspecifics, as occurs among juveniles. Moreover, adult females with subclinical PaV1 infections are often captured from the wild with a spermatophore or fertilized eggs, indicating that males are willing to mate with them. Adults with subclinical infections of PaV1 are not infectious to other adults or to the more susceptible juveniles. Although postlarval lobsters infected with PaV1 occur in the nearshore waters of Florida, experiments indicate that vertical transmission of PaV1 from females to embryos is not the mode of transmission. Instead, postlarvae acquire PaV1 shortly after arriving inshore from the oceanic plankton. These recent results suggest that PaV1 may be of little consequence to adult lobsters in contrast to its major effect on juvenile ecology and population dynamics. Just how adult lobsters retain subclinical infections of PaV1 remains a mystery.