fluorescence. Deletion of the C-terminal 184 aas was detrimental to virus production, and even deletion of the C-terminal 36 aas severely compromised virus spread. Thus almost the entire C-terminus beyond the polymerase domain was required for normal virus replication. Confocal fluorescence microscopy showed this might be due to failure of DNApol nuclear localization. Of several expression plasmids with C-terminal DNApol truncations fused to EGFP, only pBC949, expressing DNApol aa1-949 translocated to the nucleus; shorter truncations remained cytoplasmic, mimicking the results for the same truncations in bacmid constructs. AA sequences in aa804-827 and aa939-948 were consistent with a bipartite and monopartite NLS, respectively. Peptides with either NLS fused to EGFP, independently allowed for strong nuclear localization. However, deletion of either NLS in DNApol:EGFP fusions resulted in only cytoplasmic DNApol:EGFP. A highly conserved C-terminal sequence at aa972-981 was found in all group I alphabaculoviruses. For bacmid constructs with alanine mutagenesis in this region, there was limited spread of GFP fluorescence but only by 144 hpt. Thus DNApol requires both NLSs and even the C-terminal 10 aas for nuclear translocation, viral DNA replication and virus production.

Contributed paper. Monday, 15:30. **23-STU**

Investigations into the role of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0) and *Trichoplusia ni* kinesin-1 in budded virus nucleocapsid egress

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The nucleocapsids (NC) of alphabaculoviruses budded virus (BV) virions are assembled in the nuclei of infected cells, transported from the nucleus, through the cytoplasm and bud from the plasma membrane enabling systemic spread of infection. The AcMNPV viral protein AC141 (EXON0) is required for efficient BV production and has been shown to associate with β -tubulin and potentially directly interact with a Drosophila kinesin-1 TPR domain. The objective of this study was to determine if AC141 can associate with the host lepidopteran kinesin-1. To enable these studies the sequence of *T. ni* kinesin-1 heavy (KHC) and light (KLC) chains were identified from a transcriptome analysis of *T. ni* Tnms42 cells. *T. ni* KLC and KHC cDNAs were subsequently generated and cloned into plasmid expression vectors, and tagged at the 5' and 3' ends with Myc or HA epitope tags, or EGFP. These constructs were used to generate stably transformed High Five (BTI-Tn5B1-4) cell lines. Initial experiments showed that both N- and C-terminal HA-tagged KLC expressed in stable cell lines co-immunoprecipitates AC141 and β-tubulin. In addition, HA-tagged AC141 co-immunoprecipitates with WT KLC. Sequential confocal laser scanning microscopy shows that Myc-KLC in stable cell lines co-localizes with HA-AC141 in regions adjacent to the plasma membrane at 20, 24 and 48 hpi. This technique was also used to examine co-localization of AC141, microtubules and tagged KLC molecules. These studies provide additional support to a model in which the association of AC141 with microtubules plays an important role in anterograde trafficking of BV NCs
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Contributed paper. Monday, 15:45. **24**

The Twist In Baculoviruses

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It has been know for over forty years that baculovirus genomes are supercoiled ds DNA molecules, yet the implications of this fact has had little affect on current explanations of baculovirus replication and hyperexpression. It is now known that negatively supercoiled ds DNA is spontaneously bound by nucleosomes upon entering the nucleus, which is what happens to baculovirus genomes on nuclear entry. Because both replication and transcription require that nucleosomes be slid or removed for these processes to occur, baculoviruses also must be able to regulate chromosome remodeling. Two of the four major classes of chromasome remodelers, INO80 and SWI/SNF, contain actin as an essential subunit. If either or both were necessary for transitioning from late to very late gene expression, the observed transient dependence on polymerizable actin for the period of transition would be explained. Moreover, it is now known that replication of covalently-closed circular DNA in eukaryotic systems requires topoisomerase II (topo 2). Topo 2 makes double-strand breaks (DSBs) and DSB's are considered to be among the most deleterious of DNA lesions. Their occurrence could explain the induction of the DNA damage response during baculovirus replication. SWI/SNF complexes facilitate topo 2 positioning for dsDNA cleavage, hence polymerizable actin is also required. An SV40-based model of baculovirus replication will be presented.

CONTRIBUTED PAPERS Monday, 14:00-15:30

FUNGI 1

Contributed paper. Monday, 14:00. **25**

A new mycopesticide developed especially for the control of the citrus greening vector *Diaphorina citri* (Hemiptera: Liviidae)

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The citrus greening also known as Huanglongbing or yellow dragon disease is one of the most serious citrus diseases in the world. This disease has devastated millions of hectares of citrus crops throughout Brazil and the United States. Considering that once infected the plant has no cure, the primary control strategies currently employed requires intensive use of chemical insecticides against the vector, *Diaphorina citri*. We have developed a new suspension concentrate formulation based on *Isaria fumosorosea* for controlling this pest. The product is effective against adults and nymphs of *D. citri* but it can also contribute to the management of other citrus pests such as the black citrus aphid, *Toxoptera citricida*, the citrus blackfly, *Aleurocanthus woglumi*, and the snow scale, *Unaspi citri.* The *I. fumosorosea* isolate used presented UV tolerance up to two times higher than other fungal isolates tested, and it is compatible and can be tank mixed with most chemicals sprayed in citrus

(pesticides, foliar fertilizers, adjuvants) except for the fungicides. Field sprays (n= >15) on adults confined in voile bags on commercial citrus groves using 60mL of suspensions (2.5-5.0 x 10^6 conidia/mL) per m^3 of leaf area in the citrus canopy caused total mortality ranging from 60-96%. Transmission of the fungus from *D. citri* and *T. citricida* cadavers to uninfected *D. citri* were effectively demonstrated in laboratory and semi-field conditions. The mycopesticide is currently in preparation for commercial registration.

Contributed paper. Monday, 14:15. **26**

Effectiveness of biorationals and *B. bassiana* against tomato fruitworm in Sinaloa

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During autumn-winter 2012 was conducted a field trial with applications of biorational products *B. thuringiensis* (Versa™), Pyirethrins (Abatec™), *S. carpocapsae* (Capsanem™) and native strains of *B.bassiana* and water as control, against neonate larvae of tomato fruitworm *Heliothis virescens* (Fabricius) in a tomato crop cultivated in Guasave Sinaloa, México. The variables evaluated were larvae mortality (LM), fruit damage and yield. The better treatments were: *B. thuringiensis* 39.6%, Pyirethrins 32.3 % and *S. carpocapsae* 23.33%, while the native *B. bassiana* strains ($2.1x10^6$ spores/ml) had 6.3 to 6.6%, and the control 2.66% of LM after 72h. Not statistical differences were found in fruit damage between Versa and Abatec, but they were found in the control and Bb1 strain; in the yield, neither were founded differences between biorational products, these also showed the highest fruit yields, followed by Bb strains. These results indicated a lower field efficacy of fresh native Bb strains at this spore concentration, respect to the other products against *H. virescens*.

Contributed paper. Monday, 14:30. **27**

Evaluating Metarhizium brunneum F52 Microsclerotia Applied in Hydromulch for Control of Asian Longhorned Beetles

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The entomopathogenic fungus Metarhizium brunneum F52 (Hypocreales: Clavicipitaceae) is able to produce environmentally persistent microsclerotia. Incorporating these desiccation-tolerant microsclerotia (Mb MS) granules into hydromulch [a mixture of water + wheat straw mulch + psyllium tackifier], represents a novel, easy-to-use and environmentally-friendly mycoinsecticide that can be sprayed onto the trunks of forest or orchard trees to control insect pests. Hydromulch holds moisture that allows microsclerotia to germinate, and the production of conidia in turn, causes lethal infections in Asian longhorned beetles, Anoplophora glabripennis Motschulsky (Coleoptera: Cerambycidae). To test how quickly beetles could be killed, moist and dry bark pieces and filter paper were sprayed with a low dose (~9 Mb MS/cm^2) of microsclerotia in hydromulch. Median survival times of beetles exposed to moist bark and filter paper were 17.5 d and 19.5 d, respectively. Beetles exposed to dry bark died significantly slower. In an attempt to kill beetles faster, moist bark pieces were sprayed with three doses of microsclerotia in hydromulch: low (6-9 Mb MS/cm^2); medium (10-19 Mb MS/cm^2) and high (20-30 Mb MS/cm^2). At high doses, 50% of beetles died in 12.5 d but at lower doses it took significantly longer to kill beetles (16.5 d-17.5 d). In a two week oviposition period, total beetle fecundity was highest in high-humidity controls, females produced 18.5 viable offspring compared to high-humidity hydromulch treatments that significantly reduced fecundity to 7.9 viable offspring. This however was not significantly different from the low-humidity hydromulch (8.1 viable off spring) or that associated control (9.1 viable offspring). Outdoor spore production by microsclerotia, using moist bark pieces, sprayed with a high dose of hydromulch (20-30 Mb MS/cm^2) and attached to trees in the woods was quantified. There was a significant increase in spore production over 24 days and in the second replicate (another 24 days) spore production was significantly higher than in the first replicate ($P \leq .0001$). Importantly, rainfall was significantly correlated ($P \leq .0042$) to this increase in spore production. Environmental moisture plays a big role in the spore production by microsclerotia and will subsequently affect the level of insect mortality.

Contributed paper. Monday, 14:45. **28-STU**

Management of entomopathogenic fungal disease in rearing mealworms, *Tenebrio molitor* as animal feed

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Mealworm, Tenebrio molitor L. (Coleoptera: Tenebrionidae) has high and safe protein contents, which enables it to be animal feed. However, occurrence of entomopathogenic fungi in mealworms is one of the limitations for mass production. In this work, we investigated relationships between abiotic conditions and occurrence of fungal pathogens and established an effective control method using fungicides. In virulence assay, third instar mealworm larvae were sprayed by six entomopathogenic Beauveria bassiana isolates and kept under high relative humidity; B. bassiana ERL1575 isolate had highest virulence. Under normal humidity, ERL1575 conidian showed different virulence between spray (~0% virulence) and digestion (~80% virulence) method. Furthermore, mealworms, which digested conidia, were exposed to various temperature (20-35°C) and humidity (1-3 ml distilled water spray/35 mm diam. dish) conditions for 5 days. All the treatments showed ~90% virulence except 35°C incubations (~20% virulence), but irrespective to the humidity conditions. Forty chemical fungicides were assayed against conidial germination and hyphal growth of ERL1575. Fluazinam and mancozeb showed strong inhibition of conidial germination at standard application dose (SD), 1/2 SD and 1/5 SD; besides, fluazinam showed strong inhibition of hyphal growth. When fluazinam and mancozeb were applied to the fungal conidia-inoculated wheat bran, most of mealworms were alive after 3 days post application. However, high mortality rate (~100%) were observed in the conidia-inoculated wheat bran without any fungicides. In conclusion, this work suggests that B. bassiana isolates could be pathogens at <30°C when they were digested by mealworms, and fluazinam and mancozeb would be used as effective control agents against the pathogen.

Contributed paper. Monday, 15:00. **29**

Use of *Beauveria bassiana* (Bals) in the management of larger grain borer, *Prostephanus truncatus* (Horn.) (Coleoptera: Bostrichidae) on stored maize in Tanzania

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Maize (*Zea mays* L.) is important for livelihoods in sub-Saharan Africa as it is the major staple food for the majority of people. In Tanzania 82 % of all farms, 4.5 million farmers in total, produce maize. The greater proportion of the maize (98 %) is produced by resource poor farmers, on an average of 0.8 hectares, in remote villages with poor road networks and post- harvest storage facilities which often make them incur high post-harvest losses. Grain loss in Africa due to insect pests' damage in storage systems is estimated at 20 to 30 %. The larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), a native to meso-America, is known to cause considerable economic losses of up to 48% dry weight. While satisfactory control of LGB has been obtained by use of synthetic pesticides in Tanzania, since its accidental introduction in the late 1970s, their adverse effects on environment, possible development of resistance and residues in food have motivated the search for safer alternative methods. One such strategy is the use of biological control using entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill. The current paper presents the findings of an ongoing laboratory study to evaluate the efficacy of a formulation (8.65×10^8 CFU g^{-1} spore powder) of *B. bassiana*, isolate IMI 389521 against unsexed adult LGB and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in Tanzania.

Contributed paper. Monday, 15:15. **30**

Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi

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Western flower thrips (WFT), *Franklinella occidentalis*, is a major pest of ornamentals. Mycotized millet grains with entomopathogenic fungi applied to soil of potted marigold plants was tested to target pupating thrips. Two experimental fungal isolates, (*Beauveria bassiana* [ARS7060] and *Metarhizium anisopliae* [ERL1171]), were compared with the registered *B. bassiana* strain GHA [commercialized as BotaniGard®] and untreated controls in greenhouse caged trials. Mycotized millet grains were mixed into the upper surface of the potting soil in pots of flowering 'Hero Yellow' marigolds (4 g/pot). One week after application five mated WFT females were released onto each plant (four plants per cage). At 8 wks post-infestation, the mean total number of thrips per plant was 81 and 90% less in the ERL1171 and ARS 7060 treatments, respectively, than in the controls. The mean numbers of thrips per plant for the control and GHA treatments were not significantly different. Plant damage was 60% less on plants treated with the experimental fungi than the control and GHA treatments. At 10 wks post-application, 75-90% of WFT collected from the treatments were infected with the experimental isolates. These results demonstrate that soil applications of entomopathogenic fungi can reduce WFT populations significantly and prevent damage.

SYMPOSIUM 2 (Microsporidia) Monday, 16:30-18:30

Microsporidiology: Advances in Europe

Symposium. Monday, 16:30. **31**

A new intracellular parasite is a missing link between fungi and microsporidia

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Intracellular obligate parasitism results often in extreme adaptations, whose evolutionary history are difficult to understand, because intermediate forms are hardly ever found. Microsporidia belong to an early-diverging clade of fungi, which evolved extreme physiologic and genomic simplification as well as exceptionally high rates of molecular evolution. They possess the smallest eukaryotic genomes with very few introns, short intergenic regions and bacterial-sized ribosomal genes. As observed in other eukaryotic intracellular parasites, mitochondria in microsporidia have degenerated into small double-layered organelles called mitosomes, which have lost the genome and cannot produce ATP anymore. Instead, they steal it from their hosts. We describe the evolutionary history of a gut parasite of the crustacean *Daphnia* with remarkable morphological similarity to the microsporidia, but genomic features of ancient fungi. This parasite, which we formally name *Mitosporidium daphniae* gen. et sp. nov., possesses mitochondria, genes for oxidative phosphorylation and an infection apparatus typical for microsporidia. Phylogenomics places *M. daphniae* together with the microsporidia in a clade that also includes the most ancient fungi, the Cryptomycota. Comparative genomics further supports the missing link status of *M. daphniae* highlighting both its microsporidian and fungal like characteristics, and reveals the intermediate evolutionary steps that led to extreme metabolic simplification. The new species demonstrates that the extreme reduction in energy metabolism genes as well as the loss of introns in microsporidia was preceded by a reduction in the machinery controlling cell cycle, DNA recombination, repair and gene expression that may have contributed to the characteristically accelerated rate of microsporidia evolution..