strains of *B. thuringiensis* suspension to the tomato seedlings in pot, and transplanted the treated tomato seedlings to FOL infested soil, after 4 weeks the development of wilt symptoms and wilting score become less than control, especially *B. thuringiensis AS17 japonensis* and *AS20 CR371-H*. Also, this study proved that *B. thuringiensis* strains are PGPR. PGPR (Plant growth promoting rhizobacteria) are beneficial bacteria which have the ability to colonize the plant roots and either promotes plant growth through direct action or via biological control of plant diseases. Six strain of Insect Pathogenic *Bacillus thuringiensis* were tested for PGPR effect. Culture filtrates of six strains had remarkable plant growth promotion activity in tomato and alfalfa plants; in each plant after treatment of culture filtrates, both of seed germination rates and the fresh weight were increased compared with control treatment.

Contributed paper. Monday, 15:45. **16**

Vibrios pathogenic for oysters are found associated to plankton species. What possible consequences on pathogen transmission to oysters?

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Vibrios cause major losses in shellfish farming and are associated to recurrent mortalities of oysters. However, to date, the role of plankton species in the transmission of pathogenic vibrios in oyster *Crassostrea gigas* is largely unknown. The main objective of the present study was to identify *in situ* and *in vitro* the interactions of pathogenic Vibrios with local species of planktons from different sites of Thau lagoon, an important region for shellfish farming in south of France. Quantitative-PCR was used to monitor *Vibrio splendidus* and *Vibrio aestuarianus* over the year 2013 at two sites of the Thau lagoon. Out of the oyster farm area, *V. splendidus* was found from May to July and from June to August associated to 5-180 µm and >180µm plankton fractions, respectively. *V. aestuarianus* was also detected in fraction 5-180 µm in May and >180µm in August, before and after the warmer months of the year. For the farm oysters point, *V. splendidus* was found in January and June associated with the 5-180 µm plankton and with the >180 µm fraction in spring and winter. *V. aestuarianus* was not detected. In laboratory controlled conditions, by using a GFP-expressing *V. splendidus* LGP32 and epifluorescence microscopy, we showed that *V. splendidus* LGP32 exhibits strong interactions with copepods of the *Acartia* and *Paracartia* genius as well as with microalgae of the *Alexandrium* genius. Altogether, our data show that vibrios pathogenic for oysters can establish close associations with plankton species, which may enhance the transmission of pathogenic vibrios to oysters.

VIRUSES 1

Contributed paper. Monday, 14:00. **17**

Investigation of Baculovirus RNA Polymerase Subunit Protein-Protein Interactions with *in vivo* Bimolecular Fluorescence Complementation Assays

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Baculovirus transcription utilizes two different DNA-directed RNA polymerases (RNAPs): the insect host RNAP transcribes early genes while a virus RNAP transcribes late and very late genes. The virus RNAP consists of four proteins: P47, LEF-4, LEF-8 and LEF- 9. Conserved motifs in LEF-8 and LEF-9 suggest that the interface of these subunits forms the catalytic site of the RNAP, while LEF-4 has RNA capping-associated enzymatic activities. No specific function has yet been demonstrated for P47. To investigate the *in vivo* intracellular localization and interactions of these proteins, two individually non-fluorescent fragments (V1 and V2) of the Venus yellow fluorescent protein were fused with the N-termini of each RNAP subunit in plasmid expression vectors. We also constructed similar fusions with two components of the virus replisome complex, LEF-3 and P143, and of the host *Spodoptera frugiperda* TATA binding protein. Bacmids, expressing each of these fusion proteins, were constructed and used to generate recombinant viruses expressing each of the V1- or V2-tagged protein subunits. Protein-protein interactions of these subunits were investigated using bimolecular fluorescence complementation assays. Co-infections were used to investigate the interactions of these subunits in the presence of the full complement of virus proteins. Reciprocal co-transfections of the original plasmid constructs were performed to investigate the potential for these proteins to form homo-oligomers, as well as their ability to interact with heterologous partners in the absence of any other viral proteins. The results of co-transfection and co-infection assays will be presented.

Contributed paper. Monday, 14:15. **18-STU**

Characterization and Quantitative Analysis of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) FP25K Localization and Aggregate Formation During Cell Infection

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Localization of AcMNPV FP25K was previously studied by western blot using fractionation. This study, however, was not quantitative. By inactivating the endogenous *fp25k* gene through passage of the AcBacmid in Sf21 cells and subsequent insertion of an *fp25k-egfp* fusion gene at the *polyhedrin* locus, we investigated FP25K localization during infection. Western blot confirmed the 53-kDa FP25K-EGFP fusion protein from infected cells. By using a nuclear stain, we were able to assess and quantify the nuclear to cytoplasmic localization of FP25K-EGFP during Hi5 and Sf9 cell infection through confocal microscopy. During late phase of infection, small aggregates were formed and FP25K-EGFP was found exclusively in the cytoplasm. However, during very late phase of infection, larger aggregates were observed in both the

cytoplasm and nucleus and about 1% of FP25K-EGFP localized to the nucleus. In addition, bioinformatic analysis of FP25K predicts a highly conserved coiled-coil domain at the N-terminus. We hypothesize that this coiled-coil domain may be responsible for the formation of these amorphous aggregates in the cytoplasm and nucleus. Therefore, disruption of the coiled-coil domain will disorder aggregate formation. Quantifying FP25K localization and studying aggregate formation may help to understand the role of FP25K aggregates in infection and polyhedrin promoter activities.

Contributed paper. Monday, 14:30. **19 STU**

Bracovirus-derived genes in the genome of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) and their role in host susceptibility to pathogens

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The association between parasitic wasp, its polydnavirus and the lepidopteran host can represent an interesting model to study the horizontal transfer of genes since three different genomes are simultaneously in contact. In this context, the transcriptome of *Spodoptera exigua* revealed the presence of eight unigenes with high homology to bracovirus genes. All of them encoded for lectin-like proteins except one coding for a protein with homology to proteins of unknown function, which we named *gasmin*. Sequence analysis of the genomic region of *gasmin* and of one of the bracovirus lectin-like proteins (*Se-BLL2*) confirmed their integration into the *S. exigua* genome. Gasmin as well as the lectins were mainly expressed in the hemocytes which indicate their possible role in the interaction with the parasitic wasp and insect's immune response. Functional analysis of gasmin revealed that this protein interacts with the cellular actin inhibiting its polymerization. This inhibition leads to a drastic reduction in the capacity of hemocytes to phagocytise bacteria. Moreover, high expression of gasmin reduces the multiplication and the production of baculovirus particles in cell culture experiments. Analysis of the bracovirus-derived lectins revealed that they respond to gram-positive and gram-negative bacteria in addition to baculovirus infection. Remarkably, *Se-BLL2* responds to all tested pathogens. Further characterization of Se-BLL2 showed that it recognizes and agglutinates gram-negative as well as gram-positive bacteria. The results obtained suggest that the insect has domesticated the viral genes to cope with infections by pathogens.

Contributed paper. Monday, 14:45. **20**

Entry of *Bombyx mori* nucleopolyhedroviruse (BmNPV) into BmN Cells by Macropinocytic Endocytosis

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Abstract: *Bombyx mori* nucleopolyhedroviruse (BmNPV) is a serious virus pathogen of silkworm, *Bombyx mori*, and no drugs or specific protection is available presently, whereas knowledge on BmNPV entry, a remarkable target for the development of protection target, is still limited. Here we used BmNPV virus combined with different drugs and subcellular analysis to investigate BmNPV entry mechanism. Results indicated that BmNPV entry into BmN cells was clathrin- and caveolar/lipid raft- indpedent endocytosis pathway, but actin-, microtube-, PKC-, Rac1- and PI(3)K-depedent, and virus entry mediated by cholesterol in a dose dependent manner, these results suggested that BmNPV entry into BmN cells by macropinocytic endocytosis, which was further confirmed by TEM and live image analysis. Our study suggested that BmNPV take a different mechanism to invade host cell that was different with that of AcMNPV..

Contributed paper. Monday, 15:00. **21**

Nuclear translocation of *Autographa californica* nucleopolyhedrovirus ME53

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The baculovirus early/late *me53* is conserved in all sequenced lepidopteran baculovirus genomes. If AcMNPV *me53* is deleted, DNA replication is normal but virus replication and spread is severely compromised. The 449 amino acid AcMNPV ME53 is a nucleocapsid-associated protein colocalizing with the major envelope glycoprotein GP64 at putative "budding" foci on the cell membrane. However, ME53 also localizes to the nucleus. In the absence of an easily identifiable nuclear localization signal we wished to identify ME53 sequences responsible for its nuclear translocation. To that end we generated a series of HA- or GFP- tagged N and C-terminal and internal deletions of ME53 as well as internal ME53 peptides through a baculovirus bacmid intermediate. Localization of the tagged ME53 variants following bacmid transfection, was monitored by confocal fluorescence microscopy. An HA-tagged ME53 lacking aa83-152 was excluded from the nucleus while an internal HA-tagged aa83-152 peptide showed nuclear localization. Further N-terminal deletions up to aa107 (or carboxy terminal deletions up to aa250) showed nuclear localization of GFP-tagged ME53, while N-terminal deletions up to aa121 did not. Among several internal deletions tested, the aa107-121 deletion lacked nuclear localization. Overlapping that region was an alphahelical domain aa107-133. However alanine mutagenesis of some of the basic residues (E121A, R122A, K126A and even double E121A/R122A) predicted to destroy the alphahelix, failed to prevent nuclear localization. As the aa83 to 152 peptide on its own showed nuclear localization we predict the ME53 nuclear localization domain to begin between aa107 and 121 and end upstream of aa152.

Contributed paper. Monday, 15:15. **22**

Nuclear localization and other domains of *Autographa californica* nucleopolyhedrovirus DNA polymerase

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The baculovirus *dnapol* is a core gene essential for viral DNA replication. The 984 aa AcMNPV DNApol has polymerase and 3'-5' exonuclease domains spanning aa189-750. To determine if these domains were sufficient for viral DNA replication and virus production we generated a series of bacmids with DNApol C-terminal deletions. Virus spread and DNA replication were monitored following transfections using GFP

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