Contributed paper. Monday, 18:00. **49**

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

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False codling moth (FCM), *Thaumatotibia leucotreta* is a major citrus pest in South Africa. *Cryptophlebia leucotreta* 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 tests and post-Hoc 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 (5^{th} 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.

Contributed paper. Monday, 18:15. **50**

Enhancement of insecticidal activity of a nucleopolyhedrovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (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 SfMNPV, 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 betabaculovirus (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 1×10^4 OB/mL to 1×10^8 OB/mL. For each mixture, the median lethal concentration (LC_{50}) 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 LC_{50} 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.

FUNGI 2

Contributed paper. Monday, 16:30. **51**

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 bronginiartii* (3), *Beauveria amorpha* (2), *Beauveria vermiconia* (2), *Beauveria asiatica*, *Beauveria australis*, *Beauveria kipukae*, *Beauveria malawiensis*, *Beauveria sungii* 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.

Contributed paper. Monday, 16:45. **52-STU**

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

chemical may induce the production of cellular reactive oxygen species (ROS), which cause damages to most biomolecules such as DNA, protein, and lipids. Fungal superoxide dismutases (SODs) that detoxify superoxide anions could be putative virulence factors for entomo-pathogenic fungi. Three genes encoding SODs have been identified in the *Beauveria bassiana*: a cytoplasmic Cu/ZnSOD (BbSod1), a mitochondrial FeSOD (BbSod4) and a cell-wall Cu/ZnSOD (BbSod5). During growth, *BbSod4* was weakly expressed compared with other SODs and the deletion of *BbSod4* was lethal. To probe their effects on the biocontrol potential of *B. bassiana,* Δ*BbSod1*, Δ*BbSod5* and three hairpin RNA-interfered (RNAi) mutants were constructed and assayed for various phenotypic parameters in conjunction with Δ*BbSod1*/ *BbSod1*, Δ*BbSod5*/ *BbSod5* and wild-type (control strains). The knockout mutants showed phenotypic alterations, including delayed sporulation and impaired conidial quality, but little change in RNAi mutants. Their mycelia or conidia became more sensitive to menadione or H_2O_2 induced oxidative stress but had little change in resistance to hyperosmolarity and wet-heat stress. Their UV tolerance and virulence was also impaired. Transcriptional changes of five *Sod* genes and other relative genes described try to explain the phenotypic changes among the mutants. Our finding highlight that these three Sods regulate the oxidative resistance in different method, thereby exerting profound effects on the fungal biocontrol potential.

Contributed paper. Monday, 17:00. **53-STU**

Effect of temperature, water activity and UV-B radiation on conidia germination and colony growth of *Beauveria bassiana* isolates from soil and phylloplane

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Have entomopathogenic fungi phylloplane isolates any advantage over soil isolates in terms of environmental competence and virulence?. To address this question, 20 *Beauveria bassiana* isolates from soil and phylloplane of two holm oak ecosystems in Southern Spain pathogenic to medfly *Ceratitis capitata* adults and belonging to different type sequences and genotypes as interfered from EF-1α, Bloc and microsatellites were selected and their comparative response to temperature, water activity and UV.-B investigated. Effect of temperature on germination and colony growth rate was monitored in the range of 15-35ºC, with optimum temperature ranging from 23.8-28.7 ºC. All isolates showed maximum germination values between 1 and 0.996 water activity (a_w). Germination at a_w values lower than 0.928 were not observed for any isolate. Moreover, conidia were exposed to different irradiances (920 and 1200 mWm^{-2}) during 2, 4 and 6 hours, and germination, culturability and mycelia growth were evaluated. These results show that a "recovery" of the fungal propagules could occur after being exposed to UV-B, even if such recovery is lower for longer exposure times (6h) and irradiance (1200 mWm^{-2}). Therefore, the answer may be now addressed: the fungus isolation habitat does not always provide advantage in terms of environmental competence.

Contributed paper. Monday, 17:15. **54**

Non-target aquatic arthropods testing of *Metarhizium* strains and their crude extracts produced by solvent extraction and nanofiltration technology

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Strains of insect pathogenic fungi within the genus *Metarhizium* have increasingly been developed for the control of pest species. Risk assessment studies are a prerequisite before the fungus can be registered as a plant protection product. In this work we determined the risks posed by preparations of secreted metabolites and viable conidia to two aquatic, ecological toxicity indicator species, *Artemia salina* and *Daphnia pulex.* Organic solvents (OS) are normally used to extract the metabolites but they pose a risk to human health and the environment. Nanofiltration (NF) is an environmentally responsible technology that can be used to extract the metabolites as an alternative to the OS. Since risk assessment of each secondary metabolite produced by EPF could be a long and expensive process, the RAFBCA-REBECA decision scheme proposes evaluation of the risks posed by crude extracts. Therefore, three fungal strains (BIPESCO5, ARSEF 4556, and ARSEF 3297) were produced in three different culture media [Czapek-dox + peptone, Czapek-dox + yeast, and 10:1 (C:N ratio)], and their metabolites extracted by OS and NF methods. The chromatographic profiling of all the products was determined and their toxicity tested against *A. salina* and *D. pulex.* Concomitantly, the pathogenicity of the strains was tested against these non-target arthropods. At a relatively high dose (10^8 conidia ml^{-1}), the conidia could cause 69% and 75% mortality in *A. salina* and *D. pulex* respectively. Both arthropods were sensitive to metabolites. Mortality depended on the fungal strain, extraction method, and test organism. Our study showed that *A. salina* and *D pulex* mortality was due to the combination of *Metarhizium* conidia induced stress as well as secreted metabolites.

Contributed paper. Monday, 17:30. **56-STU**

Development of analytical methods for the analysis of *Metarhizium brunneum* metabolites in crop matrices

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The main secondary metabolites produced by the entomopathogenic fungus *Metarhizium brunneum* are destruxins (dtxs), cyclic hexadepsipeptides, which exhibit a wide variety of biological activities. Overall they are best known for their insecticidal and phytotoxic activities. Since the fungus is used for biological control of insect pests there are some concerns regarding whether the produced secondary metabolites entail risks to humans and the environment. To asses if the major secondary metabolites secreted by *M. brunneum* enter the food chain a two-step sample preparation protocol, consisting of the sample extraction by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method followed by the sample purification by offline solid phase extraction on a reversed phase material was established. For the analysis and quantification of dtx congeners a fast and selective UHPLC-DAD/TOF-MS method based on a previously developed method was optimized. It

turned out that the QuEChERS-method is an efficient way to extract dtxs from different crop matrices. Using offline SPE for the clean-up of the samples analytes can be separated from disturbing matrix compounds and quantified by the UHPLC-DAD/TOF-MS method.

Contributed paper. Monday, 17:45. **57-STU**

α-1, 2-mannosyltransferase ktr1, ktr4 and kre2 regulate positively growth, conidiation, viability, virulence, and multi-stress tolerances in *Beauveria bassiana*

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Entomopathogenic fungus *Beauveria bassiana* is a mycoinsecticide against arthropod pests. Three α-1, 2-mannosyltransferase proteins (Ktrp) named Bbktr1, Bbktr4 and Bbkre2 are responsible for extension of the second and third mannose residues on secretory protein. Here, we characterized the role of three Ktrp in *B. bassiana* and found that they were positive, but differential, regulators of the growth, conidiation, multi-stress tolerance and virulence of the entomopathogenic fungus. The three disruptions accompanied with their corresponding complement Δ*Bbktr1/ktr1*, Δ*Bbktr4/ktr4*, Δ*Bbkre2/kre2* and wild-type were constructed. Δ*Bbktr4 and* Δ*Bbkre2* grew 50–83% slower on nutrition-rich and limited media while Δ*Bbktr1* show similar colony sizes on all the tested media. Their conidial yields on a standard medium were reduced by 31–96%, accompanied with abnormal germination. All the mutants became significantly less tolerant to most stresses of cell wall perturbation, high osmolarity, oxidation, wet heat and UV-B irradiation during colony growth. Furthuremore, the Ktrp mutants were altered in cell wall structure and composition, which contributed to the thickness of cell wall, increased sensitivity to lyase, the low conidial hydrophobicity and cell surface carbohydrate epitopes. Coincidentally, the attenuated cell wall in Ktrp mutants also brought out the more protoplast to release. Remarkably, insect bioassays revealed decreased virulence in Δ*Bbktr4*, Δ*Bbkre2* for 18% and 1.2-fold with topical application, and 31% and 26% with intrahemoceol injection. Our findings revealed that Ktrp plays a central regulatory role in *B. bassiana*.

TUESDAY - 5 August

SYMPOSIUM 3 (Fungi) Tuesday, 8:00-10:00

Fatal attraction: Fungi and Odours in Deadly Combinations for Pest Control

Symposium. Tuesday, 8:00. **58**

Conifer - bark beetle - fungus interactions

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Tree-killing bark beetles such as the spruce bark beetle (*Ips typographus*) have huge economic and ecological impacts in conifer forests worldwide. Just in the last 25 years the spruce bark beetle has killed millions of cubic meters of Norway spruce (*Picea abies*) in Europe. Trees are killed by a combination of pheromone-mediated mass-attacks and infection with phytopathogenic bluestain fungi vectored by the beetles. *Ceratocystis polonica,* the most virulent fungal associate of the spruce bark beetle, can kill healthy trees in the absence of beetle attack if it is experimentally inoculated into the bark at high densities. Norway spruce protects itself against combined beetle-fungus attacks by multiple preformed and inducible defense mechanisms. Structurally diverse mixtures of mono-, sesqui- and diterpenes are central components of these defenses. Preformed terpenes stored in resin ducts in the bark and sapwood may repel or inhibit initial attacks. Terpene levels increase tremendously following induction by e.g. fungal infection or application of methyl jasmonate (a defense-inducing plant hormone). This induced terpene response reduces pheromone emission by the spruce bark beetle and inhibits tree colonization in a dose-dependent manner. However, fungal associates of the spruce bark beetle can greatly reduce monoterpene levels in the tree by biotransforming them to oxygenated monoterpenes. In addition, the fungi also produce different metabolites which may play multiple roles in bark beetle host finding and colonization. These observations demonstrate the complicated interactions between conifer-bark beetle-fungi.

Symposium. Tuesday, 8:20. **59**

Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi

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The larvae of soil dwelling insects use carbon dioxide gradients, established by growing roots, to orientate towards their host plants. This long distance orientation cue is complemented by other volatile cues to finally accept a host plant for feeding. Previous application strategies using entomopathogenic fungi for soil pest control were using high concentrations of spores per m^2, set against competing microorganisms in the rhizosphere. In the attract and kill approach the strategy is turned upside down: larvae voluntarily make their way to the spores, contained in capsules emitting CO_2. When near to these capsules, probability of larval infestation with spores is higher. However, to make this strategy work, the capsules need to fulfill several prerequisites, such as building up a gradient significantly higher than the background CO_2 concentration in the soil, maintained for at least several weeks, and the larvae need to be attracted to the capsules to feed on them. In lab experiments we assessed the larval behavior of corn rootworms and wireworms towards these artificial CO_2-capsules. Both pest larvae were attracted by the capsules, but only stayed for short periods at these sites. Thus, additional compounds need to be incorporated into these capsules to increase their attractiveness for the larvae. In German field experiments these capsules, combined with *M. brunneum*, were used in potato fields for wireworm control. Treatments resulted in significantly lower tuber damage in some, but not all fields. Necessary improvements of the attract and kill strategy for anapplication in the field are discussed.