

strategies. INBIOSIL explores in detail the recently discovered synergistic effects between entomopathogenic fungi (EPFs), entomopathogenic nematodes (EPNs), and semiochemicals by developing innovative co-formulations, making use of strategies derived from nature. These co-formulations will be based on capsules containing EPFs (*Metarhizium brunneum* or *Beauveria bassiana*) in combination with strains of EPNs (*Heterorhabditis bacteriophora*), or semiochemicals. Additionally, INBIOSIL will develop integrated pest management (IPM) strategies that exploit synergies between these biocontrol agents and semiochemicals. The overall aim of the project INBIOSIL is to optimize the use of biocontrol agents in the soil for more efficacious, low input, control of pests in farming systems of major importance in Europe. New crop protection strategies will be developed that will i) reduce pesticide inputs, ii) provide protection in non-sterile soils, eliminating for soil sterilants, iii) reduce production costs, and iv) result in the production of high-quality and safer crops in accordance with theme priority area (Integrated pest management in farming systems of major importance in Europe).

Poster / Fungi. Wednesday, 16:30. **FU-36**

Oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* growing in very long-chain hydrocarbons

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Broad host range entomopathogenic fungi attack insect hosts via attachment to insect surface, with the subsequent production of degrading enzymes that help penetration through the cuticle. The outermost insect surface is covered by a lipid-rich layer, usually composed of very long-chain hydrocarbons. It is known that *B. bassiana* is able to grow on straight chain hydrocarbons (alkanes) as a sole source of carbon and energy, but it would have to pay a high cost to do so. The aim of this work was to study the oxidative stress levels in alkane-grown *B. bassiana*. For this purpose, we analyzed the gene expression pattern of *sod1*, *sod2*, and *sod3* encoding superoxide dismutases, *catA*, *catB*, *catC*, *catD*, and *catP* encoding catalases, and *gpx* encoding glutathione peroxidase; and the enzymatic activity of SOD, CAT, and GPx in crude homogenates. Fungi grown either in hexadecane (*n*-C16) or octacosane (*n*-C28) showed overlapping but differential gene induction, with a concomitant increment in enzymatic specific activities, compared with controls grown in complete medium. These results confirm that high levels of reactive oxygen species are produced in *B. bassiana* during growth in alkanes, and an antioxidant response is triggered in fungal cells to overcome this drawback.

MICROBIAL CONTROL

Poster / Microbial Control. Wednesday, 16:30. **MC-1-STU**

Fungal strain selection and screenhouse evaluation of the virulent isolate against aphids on crucifer and okra vegetables

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Aphids are major pest problems of crucifer and okra vegetables in sub-Saharan Africa. Biopesticides are now acceptable pest control alternatives to synthetic chemical insecticides. Five isolates of *Metarhizium anisopliae* and three of *Beauveria bassiana* were screened for virulence against the following apterous adult aphids in the laboratory: *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* on kale, and *Aphis gossypii* on okra. *Metarhizium anisopliae* isolates ICIPE 30, ICIPE 62 and ICIPE 69 outperformed the others causing mortality of 85-98%, 83-97%, and 73-77%, in *B. brassicae*, *L. pseudobrassicae* and *A. gossypii*, respectively, at 5 d post inoculation. However, *M. anisopliae* ICIPE 62 had the shortest LT₅₀ values of 2.8, 2.1 and 1.9 d; and the lowest LC₅₀ values of 5.5×10^5 , 8.1×10^4 and 1.7×10^4 conidia ml⁻¹ against *A. gossypii*, *B. brassicae* and *L. pseudobrassicae*, respectively. It also produced significantly higher conidia on cadavers compared to the other isolates, and was therefore selected for screenhouse experiments. In the screenhouse, aqueous and oil formulations of ICIPE 62 significantly reduced aphid population growth rate (*r*_i), *B. brassicae* -0.03 and -0.03 and *L. pseudobrassicae* -0.02 and -0.04 on kale, and *A. gossypii* -0.04 and -0.07 on okra, respectively; compared to the control (0.08 and 0.04 for *B. brassicae*, 0.01 and 0.01 for *L. pseudobrassicae*, and 0.03 and 0.01 for *A. gossypii*, respectively). These results are indicative of the potential of isolate ICIPE 62 in the management of aphids

Poster / Microbial Control. Wednesday, 16:30. **MC-2**

Virulence of fungal spores produced in liquid and solid state media on nymphs of *Trialeurodes vaporariorum*

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Quality of spores of five fungal entomopathogens, produced in liquid and on solid media, was assessed on nymphs of whitefly *Trialeurodes vaporariorum*. Isolates of *Lecanicillium attenuatum*, *L. muscarium*, *L. longisporum* and one unidentified *Isaria* sp. were first passed through larvae of *Tenebrio molitor* to enhance virulence. Three-times subcultured pure colonies were used to inoculate liquid or solid media to produce submerged and aerial spores. The liquid medium production system consisted of 250 mL Erlenmeyer flasks containing a mineral solution with a C/N ratio of 10/1 supplemented with yeast extract, placed in an orbital shaker at 180 rpm and 25°C. The solid medium production system consisted of Petri dishes containing PDA, placed in an incubator at 25°C. Spores were collected and suspensions of 1×10^6 germinable spores were prepared. Five tomato leaves, infected with *T. vaporariorum* nymphs at 2nd-3rd instars, were submerged for one minute in the spore suspensions of each isolate, and maintained in 200 mL water-agar glasses in a growth chamber during ten days. The number of dead nymphs was evaluated six and ten days after inoculation. Control treatments consisted of ten leaves infected with the whitefly nymphs and treated with sterile water. Aerial spores of the *Lecanicillium* spp. isolates caused higher mortality than submerged spores. *L. longisporum* was the least affected by the production system. Contrary to *Lecanicillium*, submerged spores of the *Isaria* isolate killed more nymphs than aerial spores six days after inoculation. The production system should be considered during the screening and evaluation of microbial control agents.

Poster / Microbial Control. Wednesday, 16:30. **MC-3-STU**

Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency?

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Mosquitoes (Diptera: Culicidae) are zoonotic vectors responsible for numerous infectious diseases of medical and veterinary importance such as filariasis, malaria and encephalitis. As part of an integrated vector control, entomopathogenic fungi could be developed as biopesticides in two ways: spores and metabolites recognized as effective virulence factors. Solid-state fermentation enhances spore production and induces the secretion of metabolites quantitatively and qualitatively different from submerged fermentation, which impairs fungal metabolic efficiency. In this context, we showed high spore productivity of solid-state media based on agro-industrial substrates as wheat bran. Spores remained pathogenic, as revealed by classical toxicity tests and electron microscopy. However, the absence of free water makes culture parameter variations difficult to control in large-scale. Recently, we performed a bioreactor design intended for simultaneous spore and metabolite production, combining the technological advantages of submerged and solid-state fermentations. Biofilm fermentation (i.e. growth of fungal biomass on an inert support immersed in a nutrient medium) is a tremendous production system favouring the secretion of insecticidal metabolites in the liquid medium as we showed recently. This is also an interesting tool to provide an overview of the complexity of the metabolic pathways involved in the regulation of extracellular metabolites secretion because corresponding genes are reported to be differentially expressed from classical fermentation systems. Researches in vector control are currently intensified. In this context, the identification of genes and metabolites specifically expressed during biofilm fermentation will help to develop new technologies related both to the design of bioreactor and the production of insecticidal proteins.

Poster / Microbial Control. Wednesday, 16:30. **MC-4**

The basis for rootstock resilient to *Capnodis* species: screening for genes encoding delta-endotoxins from *Bacillus thuringiensis*

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Conventional methods often fail to control the flatheaded borers *Capnodis* spp, major pests of stony fruit trees; the larvae are protected from insecticides and predation because they feed deep in the roots. A potential solution is transgenic trees producing in their roots toxic compounds such as Cry proteins of *Bacillus thuringiensis* (Bt). Toxicities against *Capnodis* larvae were demonstrated by exploiting a recently-designed artificial larval diet and an available collection of field isolated Bt. An isolate of *Bt tenebrionis* (Btt) from commercial bioinsecticide (Novodor) displayed LC₅₀ and LC₉₅ values of 3.2 and 164 mg g⁻¹ respectively against neonates of *Capnodis tenebrionis*, whereas values of the most toxic field isolate K-7

were 1.9 and 25.6 mg g⁻¹ respectively. Weights of surviving larvae after 1 month on diets containing low concentrations of K-7 (0.1 - 1.0 mg g⁻¹) were lower than on Btt or untreated larvae. K-7 was also toxic against larvae of *C. cariosa* and *C. miliaris* and found to harbor genes encoding Cry9Ea-like and Cry23Aa/Cry37Aa binary toxins. Larvae of *Capnodis* spp. are susceptible to Bt Cry toxins. Expressing cry genes active against these pests thus seems a feasible solution toward production of transgenic rootstock trees resilient to the pest

Poster / Microbial Control. Wednesday, 16:30. **MC-5**

Selection of entomopathogenic fungi for the control of *Aegorhynus nodipennis* (Coleoptera: Curculionidae) under laboratory conditions

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Plum weevil *Aegorhynus nodipennis* is one of the most important native pests of blueberry in Chile. The larvae produce severe damage to the root system, by destroying the plant crown, and causing decay and death of the plant within a few years. Adults are long-lived and feed on twigs during the day and oviposit on the crown of the plants, where they hide during the night. Strains of the insect pathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* were evaluated on adults of the Plum weevil under laboratory conditions. 45 strains of *M. anisopliae* and 50 strains of *B. bassiana*, from the Chilean collection of insect pathogenic fungi where screened. Plum weevil adults were exposed to a dose of 1 x 10⁷ conidia / insect and mortality was assessed every day for up to 10 days. Two strains of *B. bassiana* and *M. anisopliae* were selected as the most effective on adults. The *B. bassiana* strain reached 100% mortality and the *M. anisopliae* was only 80% of control. Attributes such as high performance of the spore, stability and virulence will determine the selection of strains to be evaluated in greenhouse and field trials.

Poster / Microbial Control. Wednesday, 16:30. **MC-6**

Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* strain HD1

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The development of insect resistance to *Bacillus thuringiensis* (Bt) appears to involve various mechanisms and to be dependent on the type of insect, toxin, and Bt strain. The aim of this research was to investigate the factors affecting the susceptibility of insects to Bt (protein level, the midgut bacteria, and mutations in the ABCC2 gene), in five Brazilian populations (PC, PA, PX, SBT and BT) and one English population of *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae). The BT population of *P. xylostella* showed a high resistance to the HD1 strain, and therefore was used in the molecular assays. Enzymatic and molecular experiments with the guts of larval populations were also conducted to investigate the factors affecting the susceptibility of insects to

Bt. We analyzed total protein, total protease, protease activity, esterase levels, intestinal bacteria, and exon characteristics. Mutations in the *ABBC2* gene may be related to resistance to Bt in various insects, as deletion of this gene occurs in a resistant strain of *P. xylostella*. The exon that has a known mutation in the (NO-QAGE) Bt-resistant population was sequenced. None of the populations showed this or any other mutations in the exon. Gut bacteria may influence the susceptibility of insects to Bt and all sequences had similarities above 99% for the *Enterococcus mundtii* 16S rRNA gene. The tests performed, both enzymatic and molecular, were inconclusive as to the factors that may influence the susceptibility of *P. xylostella* to Bt and further studies should be conducted to elucidate these factors.

Poster / Microbial Control. Wednesday, 16:30. **MC-7**

Sublethal effects of the Cry1Ac toxin of *Bacillus thuringiensis* Berliner in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations

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The diamondback moth (DBM), *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is a key pest of crucifers. Although can be controlled with insecticides, *P. xylostella* can quickly develop resistance to insecticides, such as those of from *Bacillus thuringiensis*. The objective of this research was to analyze the sublethal effects of *B. thuringiensis* Cry1Ac protein in five Brazilian populations of *P. xylostella* (PC, PA, PX, SBT, and BT). Bioassays examining the sublethal effects of Cry1Ac protein in DBM larvae were conducted using concentrations of 0.1, 0.25, and 0.5 µg/mL for the BT population, and 0.001, 0.005, 0.01, and 0.05 µg/mL for the PA, PX, PC and SBT populations. As a control treatment, autoclaved deionized water and 50 µg/mL Triton-X100[®] was used. The period of life from the third instar to pupa, pupal period, pupal weight, sex ratio, survival of from the third instar to pupal stage, survival of from the third instar to adulthood, and leaf consumption by the larvae were all evaluated for sublethal effects. Sublethal effects on the Bt population were most significant in prolonging the larval period, for approximately 2 days with a toxin concentration of 0.05 µg/mL, and the emergence of adults was 44% lower than that in the control. For the PA, PC, SBT, and PX populations, the most significant sublethal effects observed were also in prolonging the larval period and adult emergence. No influence on consumption of the larvae was observed, except with the Bt population, where the consumption was significantly lower at all tested concentrations.

Poster / Microbial Control. Wednesday, 16:30. **MC-8**

Effect of *Bacillus thuringiensis* Berliner on biological characteristics of *Orius insidiosus* Say (Hemiptera: Anthoridae) fed with eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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The Diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is considered the most important pest of Brassicaceae (Cruciferae) worldwide, occurring throughout the year in Brazil, where chemical control is the most widely used method, justified by the convenience, quick action and efficiency. The indiscriminate use of pesticides can affect non-target organisms, and the growing concern for the environment, the high cost of pesticides and frequent cases of resistance in populations of increased interest in the use of other control tactics as entomopathogenic organisms such as *Bacillus thuringiensis* and predators such as *Orius insidiosus*. The objective of this work is to evaluate the action of *B. thuringiensis* (Agree[®]) in biological characteristics of *O. insidiosus*. The predators were fed with eggs of *P. xylostella* treated with distilled water (control) and a suspension Agree[®] (*B. thuringiensis* aizawai CG91), at a dosage of 0.7 g/0.5L. The nymphal period, consumption and nymphal survival rate were assessed, whereas with adults were measured consumption, the number of eggs per female and egg viability. Parameters were also determined for the construction of fertility life tables for eggs treated and not treated with *B. thuringiensis*. The parameters duration of the second instar, nymph consumption and female longevity of *O. insidiosus* are affected by the presence of Agree[®], and females who consume eggs treated have the progeny decreased, resulting in lower population growth rate.

Poster / Microbial Control. Wednesday, 16:30. **MC-9-STU**

Evaluating microbial biocontrol agents: effects of *Metarhizium brunneum* on a non-target arthropod

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The objective of this study was to evaluate the virulence of *Metarhizium brunneum* on non-target arthropods. The strain Met52/BIPESCO 5/F52 is used against various pest insects throughout Europe and North America. However, research related to the ecotoxicology and side effects against non-target organisms are still needed. In this MSc study, a part of the EU supported project INBIOSOIL, we documented that *M. brunneum* had a high virulence against the model insect *Tenebrio molitor* (Coleoptera: Tenebrionidae), whereas it had a much lower virulence against the beneficial arthropod *Atheta coriaria* (Coleoptera: Tachynidae), a soil dwelling predator used for macrobiological control. In addition, the virulence of *M. brunneum* was compared to that of another entomopathogenic fungus (*Beauveria bassiana*). Bioassay results showed notable efficacy of the entomopathogenic fungi against *T. molitor*, both at high and low spore concentrations (respectively 1×10^7 and 1×10^5 conidia/ml). Conversely, infection bioassays carried out on *A. coriaria* showed significantly lower virulence of the fungal isolates at a high spore concentration. These data suggest that this *M. brunneum* strain does not represent a threat to the non-target arthropod *A. coriaria*. Further studies are still needed to evaluate the effects of *M. brunneum* on other non-target arthropods. Nevertheless, based on the results of this study we propose that *M. brunneum* can be considered a 'low risk substance', a novel category of plant protection agents currently considered by the EU Commission.

Poster / Microbial Control. Wednesday, 16:30 **MC-10-STU**

An experimental autoinoculation device to control an invasive Asiatic pest, *Drosophila suzukii*

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Unlike most drosophilids, which typically infest overripe or decaying fruit, it has been observed that *Drosophila suzukii* (Matsumura) also oviposits eggs into the skin of immature and/or ripening fruit through the use of a serrated ovipositor. *Drosophila suzukii* is an important pest of fruit such as strawberry, cherry, blackberry, blueberry, peach, plum, nectarines and grapes. Spotted wing *D. suzukii* was first found in Spain in 2008. Managing this pest is a challenge, and new methods of control are being developed. In our research, the transmission potential of EAMa 01/58-Su *Metarhizium brunneum* strain was evaluated against *D. suzukii* adults in experiment cages, using an experimental autoinoculation device which consists in a plastic mineral water bottle with fermented food as lure, and a tissue with the fungal propagules. *D. suzukii* adults entered and exited the autoinoculation device for the 48 h of exposure and became infected with the fungus with 100.0% mortality followed by mycosis. These results show the potential of the lure and infect as a strategic option for the control of *D. suzukii* using EAMa 01/58-Su strain, with the persistence of the inoculum in the device and the time course evolution of the adult fly infection being actually investigated.

Poster / Microbial Control. Wednesday, 16:30 **MC-11**

Use of a commercial *Metarhizium anisopliae* s.l. formulation to control *Rhipicephalus microplus* ticks in pen study

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The present study evaluated the effect of the commercial product Metarriil® SP Organic of *Metarhizium anisopliae* s.l. plus 10% mineral oil to control *Rhipicephalus microplus* ticks in a pen study. Three groups were formed with six animals each: the first group was exposed to Metarriil® plus 10% mineral oil; the second group was exposed to sterile distilled water plus 10% mineral oil (oil control group) and the third group received no treatment (control group). Fungal formulation contained 1×10^8 conidia mL⁻¹. Each animal was sprinkled with 3L of formulation. Fallen ticks were counted daily and a sample of 20 engorged females per group was incubated for assessment of biological parameters. Throughout the study period, Metarriil® oil-based formulation showed an efficiency ranging from 19.20% to 67.39% in comparison with the control group; and from 8.18% to 61.38% in comparison with the oil control group. Average efficiency of Metarriil® oil-based formulation was 47.74% and 40.89% in comparison with control and oil control groups, respectively. Changes in the biological parameters of *R. microplus* females were observed in the first three days after treatment. There was statistical significant reduction in females' egg mass weight, larval hatching percent, nutritional index and egg production index. We concluded that Metarriil® SP Organic plus 10% mineral oil was efficient against *R. microplus* ticks in

pen studies. Further *in vivo* studies are required in order to increase efficiency of this product aiming establish a protocol for the use of Metarriil® in field conditions against the cattle tick.

Poster / Microbial Control. Wednesday, 16:30. **MC-12**

Two Colombian entomopathogenic fungi are highly efficient on *Ceratomyxa tingomariana*

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The Chrysomelids (Coleoptera: Chrysomelidae) are a limiting soybean pest in Colombia. These insects can affect seeds, nodules, leaves and roots, reducing the yield crop. An amount of 19 species has been registered, but *Ceratomyxa tingomariana* is the most important, due to its high frequency and distribution. This insect is controlled with insecticides (I - II category) and some of them are forbid in USA or Europe. The aim of this work was to select an efficient entomopathogenic fungus on *C. tingomariana*. Seven isolates of *Beauveria bassiana* (Bv) and six isolates of *Metarhizium anisopliae* (Mt) were biological testing on laboratory. In addition, this isolates were tested on different temperatures (5°C, 15°C, 25°C, 30°C and 35°C), pH values (3, 5, 7, 9) and tolerance to UVB radiation (302 nm) by measuring germination (%), radial growth and Colony Formate Unit (CFU). Mt isolates showed efficiency under 50%. Isolates Bv060 and Bv003 showed an efficiency of 100%. In the UVB radiation test, Bv060 reduced the conidia viability between 75% and 80%, and Bv003 reduced the viability between 65% and 66%. At 5 and 9 pH value, the two isolates (Bv003 and Bv060) showed germination higher than 90% and the faster rate of radial growth. Bv003 showed the best growth at 15°C and 25°C and Bv060 at 25°C and 30°C. These results suggested that Bv060 and Bv003 could be use as an active principle for a biopesticide on *C. tingomariana* control in soybean.

Poster / Microbial Control. Wednesday, 16:30. **MC-13-STU**

Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana*

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Pollen beetles are a main pest in oilseed rape (OSR) throughout Europe, able to cause substantial yield loss. The main damage is caused by adult beetles feeding on pollen in spring during bud stage of inflorescences. There is currently no possibility to control pollen beetles in organic OSR cultivation. In addition, increasing resistance of pollen beetles to commonly used insecticides hampers conventional OSR production and further emphasizes the need for alternative control possibilities.

The application of entomopathogenic fungi (EPF) is a promising tool in biological control of pollen beetles (Hokkanen 2008). Several Swiss isolates of the EPF *Beauveria bassiana* showed promising effects in laboratory experiments, causing up to 80% mortality seven days after application (Kuske 2011). Field treatments showed similar results regarding beetle mortality, but did not result in significantly increased yield so far. To improve their efficacy, synergies of EPF and

other natural compounds, such as stone dusts or vegetable oils, are tested. First laboratory results of combined applications of *Beauveria bassiana* spores and vegetable oil have shown a potential increase in beetle mortality due to improved fungal infection. The exploitation of synergistic effects and innovations in formulation technology should result in a better spore persistence under field conditions and a higher efficacy of the fungal treatments against pollen beetles.

References

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Kuske, S., Schweizer, C., Kölliker, U., 2011. Mikrobielle Rapsglanzkäferbekämpfung: Erste Erfahrungen aus der Schweiz. Agrarforschung Schweiz 2(10), 454-561.

Poster / Microbial Control. Wednesday, 16:30. **MC-14**

Pathogenicity and virulence of *Beauveria* spp. against mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytidae)

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The mountain pine beetle (MPB), is a forest pest to western Canada and the United States and causes severe disturbance in lodgepole and other pine forests. We evaluate pathogenicity and virulence of number of *Beauveria* spp. including the two commercial strains of *B. bassiana*, GHA and Naturalis against adult MPB. All the 29 isolates tested in the preliminary bioassay proved to be pathogenic to MPB adults. Mean survival times (MST) of MPB adults when treated with 1×10^6 conidia/ml falls between 4.05 to 8.95 days and the commercial isolate GHA is the most virulent (MST 4.05 d), followed by isolates INRS 211 (MST 4.59 d), and INRS 236 (MST 4.82 d) based on the log rank test. Among the 3 different species tested, *B. bassiana* isolates were highly virulent followed by *B. pseudobassiana*. The *B. brongniartii* isolates used in this study were neither virulent nor supported conidia growth on the cadavers. From this initial screening, seven isolates of *B. bassiana* viz., GHA, Naturalis, INRS 211, INRS 236, INRS CFL-A, L49-1AA, and ARSEF 8150, were selected based on their virulence as well as mycosis/condiosis for further dose-wise bioassay. Based on the LC_{50} values, the commercial isolates, GHA and Naturalis were the most virulent to MPB, however, isolates INRS 236 and INRS CFL-A were the better conidia producer. The result obtained from this study was used in selecting amendable and virulent *Beauveria* isolates to be deployed in managing MPB through classical biological approaches in a trap based auto-contamination-dissemination strategy.

Poster / Microbial Control. Wednesday, 16:30. **MC-15**

The Use of Microbial Plant Protection Agents for Insect Control in Germany

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Micro-organisms play an important role in biological plant

protection in Germany. By the Directive 2009/128/EC on the sustainable use of pesticides, biological control measures are proposed to be enforced in order to reduce the application of chemical pesticides in Europe. To obtain information about the scale of application of biological control agents in Germany, we have performed a survey on their use. Two baculoviruses are registered for tortricid control in Germany. The most important one is the *Cydia pomonella* granulovirus, which is used on about 30% of apple plantations in Germany. Three *Bacillus thuringiensis* (Bt) subspecies (*kurstaki*, *azawai* and *tenebrionis*) are in use and play an important role in organic farming and integrated pest management (IPM). So far, there is no entomofungal product registered as plant protection agent in Germany. However, some strains of *Beauveria bassiana*, *B. brongniartii* or *Metarhizium anisopliae* have been used for research purposes or for restricted use with a specific legal allowance. The data are presented in the Status Report Biological Plant Protection, which is published every five years by the Julius Kühn Institute and represents an indicator of the National Action Plan to monitor the use of plant protection products.

Poster / Microbial Control. Wednesday, 16:30. **MC-16-STU**

Synthesis and secretion of volatile organic compounds by *Triatoma infestans* infected with *Beauveria bassiana*

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Physically disturbed *Triatoma infestans* adults secrete volatile organic compounds (VOC) with alarm and defense function. It is still unclear whether infection with entomopathogenic fungi changes or not the profile of these volatiles. The aim of the present research was to study the effect of *B. bassiana* on secretion of VOC by *T. infestans* and to study the expression of genes potentially involved in the biosynthesis of these volatiles in triatomines infected or not. Volatiles released by *T. infestans* on different periods after treatment (1-4, 6-10, 11-15 days) were quantified and identified employing capillary gas chromatography coupled to mass spectrometry. The expression pattern of *Ti-brnq* and *Ti-bckdc* was analysed by real-time PCR, 4 and 10 days after treatment. Isobutyric acid was the most abundant VOC found (70 to 78% of the total) with no significant effect of the progress of infection on quantitative secretion of this compound. Secretion of propionic acid, however, was highest in the beginning ($18.6 \pm 5.8\%$) and decreased distinctly with the progress of infection and at this time did not differ from values found for the control. Highest expression of both genes was found on insects 4 days after treatment. Significant difference was found in *Ti-brnq* expression, with 1.3 ± 0.5 and 3.0 ± 0.4 fold induction over the controls in insects treated with 1×10^6 and 1×10^8 con/ml, respectively. Similar results were observed for *Ti-bckdc* expression, resulting in 1.9 ± 0.3 and 2.5 ± 0.4 fold induction, respectively. The results help to understand better the impact of fungal infection on the chemical ecology of *T. infestans*.

Poster / Microbial Control. Wednesday, 16:30. **MC-17**

Preliminary studies of entomopathogenic microorganisms present in Latvian population of horse-chestnut leaf miner *Cameraria ohridella*

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The first record of horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae) in Latvia was made in summer 2002. In recent years *C. ohridella* has spread across all territory of Latvia. The aim of the study was to acquire preliminary data on mortality factors of horse-chestnut leaf miner *C. ohridella* and identify present entomopathogenic fungi and bacteria. Since 2010 *C. ohridella* population dynamics are monitored in two sampling plots. This work provides information about causes of mortality of *C. ohridella* larvae and pupae and gives first record about bacterial and fungal microflora of collected larvae. Observed larval mortality, caused by pathogens was low (0.2-1.6%). Specimens with symptoms of infection were used for pathogen isolation. Twelve species of entomopathogenic fungi were isolated from collected dead specimens. A pilot experiment to test virulence of fungal isolates *Beauveria bassiana*, *Isaria fomesorozeus* and *Metharizium anisopliae* on *C. ohridella* larvae and hibernating pupae was performed. Bacteria were isolated from insects by using standard methodology - dissecting insect and preparing homogenates. Individual bacterial isolates 16S rRNA genes were amplified and sequenced. Results showed that bacterial community is relatively simple and it's similar to composition found in other insect species described by the same methodology. Community was dominated by proteobacteria - *Pseudomonas* sp. and *Pantoea* sp.

Poster / Microbial Control. Wednesday, 16:30. **MC-18**

Toxicity of *Bacillus thuringiensis* BERLINER Cry toxins in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations

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Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), the diamondback moth (DBM), is a major insect pest of crucifers (Brassicaceae) worldwide. The most common insecticides used to control *P. xylostella* are based on the entomopathogenic bacterium *Bacillus thuringiensis* (Bacillaceae) (Bt). Although many studies have focused on the action of Bt on various agricultural pests, such as DBM, many doubts still persist, particularly regarding the toxicity of Bt proteins. We analyzed the virulence of Cry proteins in Brazilian populations of *P. xylostella*. Bioassays of susceptibility in five Brazilian populations (PC, PA, PX, SBT, and BT) of *P. xylostella* and Cry1Ac, Cry2Aa, and Cry1IE *B. thuringiensis* proteins, estimating the virulence of the toxins, were performed. Seven concentrations, ranging from 0.001 to 1.0 µg/mL, for the PA, PC, PX, and SBT populations, and 0.1 to 2.5 µg/mL for the BT population, were used to calculate the values of LC₅₀. Five replicates were performed, with each replicate being a petri dish containing 20 larvae, totaling 100 insects per concentration for each population. The Cry2Aa and Cry1IE toxins caused no mortality in larvae from any of the populations; therefore, tests were performed only with Cry1Ac. The PC, PA, PX, SBT, and BT *P. xylostella* populations exhibited different levels of susceptibility to the Cry1Ac toxin. The PA, PC, and SBT populations showed LC₅₀ values of 0.02, 0.04, and 0.04 µg/mL. The LC₅₀ estimate for the BT population was 0.78 µg/mL, while that for PX it was

0.01 µg/mL. The LC₅₀ estimated for the BT population was 78 fold greater than that for the PX population.

Poster / Microbial Control. Wednesday, 16:30. **MC-19**

***Bacillus thuringiensis* isolation from Brazilian soil samples: molecular characterization and biological activity against *Plutella xylostella* (Lepidoptera: Plutellidae)**

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Plutella xylostella L. (Lepidoptera: Plutellidae) is one of the most important pests of crucifer worldwide, and farmers usually control this pest with pesticides that favors the population resistance development, depletion of natural enemies and environmental pollution. Studies on biological control agents such as the entomopathogenic bacterium *Bacillus thuringiensis* must be carried out aiming to minimize or even replace the pesticides in the field. This research was carried out to isolate *B. thuringiensis* from 40 soil samples, to characterize them by Polymerase Chain Reaction (PCR) and mortality bioassays were performed to verify the *B. thuringiensis* biological activity of each isolate against 100 *P. xylostella* second instar larvae. 50 *B. thuringiensis* isolates were obtained from soil samples. No isolate amplified genes *cry1Ab*, *cry1Ac*, *cry1Ea*, *cry1Eb*, *cry1Fa*, *cry1Fb*, *cry2Aa*, *cry2Ab*, *cry2Ac*, *cry9A*, *vip1*, *cyt2B* and *cyt2Ba* but isolates named LCMA04, LCMA05 and LCMA29 amplified gene *vip2* and the isolates LCMA06, LCMA20, LCMA45 and LCMA46 amplified gene *cry1C* and *vip2*. These isolates were pathogenic to *P. xylostella* second instar larvae but the mortality range from 38,0% to 55,5%. This mortality is too low to consider these isolates as promising ones to *P. xylostella* management. This isolation is ongoing to find isolates with high virulence to *P. xylostella*.

Poster / Microbial Control. Wednesday, 16:30. **MC-20-STU**

Effect of endophytic *Beauveria bassiana* on herbivore defence in *Arabidopsis thaliana*

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The entomopathogenic fungus *Beauveria bassiana* can live as an endophyte by colonizing plant tissues without causing disease symptoms. Recent studies in different crop plants indicated that endophyte presence can have a negative effect on herbivorous insects. However, whether this was due to induced plant defence responses has not been reported. We established *Arabidopsis thaliana* as a model plant to find out whether *B. bassiana* colonization increases herbivore resistance by activating/priming the jasmonic acid (JA) or salicylic acid (SA) defence pathways. Three *B. bassiana* strains were applied as conidial suspension to *Arabidopsis* using root dipping. Colonization was assessed through plating on selective medium and through PCR based detection using *B. bassiana* specific SCAR markers. The endophyte was recovered from leaves and inflorescence confirming systemic colonization throughout the plant. Bioassays were carried out to test the effect of endophyte presence on caterpillars of *Plutella xylostella* and the aphid *Myzus persicae*. Endophyte presence did not have any antagonistic effects on the growth of *P. xylostella* and the fecundity of *M. persicae*. The re-

isolated fungus caused 100% mortality when applied topically on caterpillars. This correlated with the finding that JA levels were only induced by caterpillar feeding but were not influenced by the presence of the fungus. No effect by either treatment was found on endogenous SA levels. In conclusion, our results do not confirm that endophytic *B. bassiana* induces plant defences against the selected herbivore species. Further studies are planned to assess the plant's transcriptomic response to the presence of this endophytic entomopathogen.

Poster / Microbial Control. Wednesday, 16:30. **MC-21-STU**

Pathogenicity of *Beauveria* and *Metarhizium* to the two stink bug species *Nezara viridula* and *Piezodorus guildinii* (Hemiptera: Pentatomidae) in laboratory and semi-field

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The stink bug species *Nezara viridula* and *Piezodorus guildinii* are troublesome pests of common bean (*Phaseolus vulgaris*). The aim of this study was therefore to determine the pathogenicity of two *Beauveria* isolates (Bb-1 and Bb-18) and two *Metarhizium* isolates (Ma-11 and Ma-30) from Cuba to these two stink bug species. Each fungal strain was tested in the laboratory against adults of the two stink bug species. Further, in a pilot semi-field experiment the two stink bug species inoculated on bean plants with pulses in cages were sprayed with the same four fungal strains. In the laboratory experiment Ma-30 and Ma-11 caused 100% mortality in both stink bug species. The *Beauveria* strains resulted in a lower mortality, however, and Bb-1 caused 85% mortality in both stink bug species, while Bb-18 caused 85 % mortality in *P. guildinii* and 95% mortality in *N. viridula*. In the semi-field experiment the Ma-30 strain caused the highest mortality and 73% of the *N. viridula* was killed by this fungus while only 68% of the *P. guildinii* was killed. The Ma-11 strain caused 65 % mortality in *N. viridula* and *P. guildinii* while Bb-18 and Bb-1 caused 41% and 54% mortality respectively in both stink bug species.

Poster / Microbial Control. Wednesday, 16:30. **MC-22-STU**

Evidence for synergies between *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) and *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) in western corn rootworm control

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The western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelida), is one of the most deleterious pests of maize worldwide, and is commonly controlled by chemical insecticides. Currently neonicotinoid-dressed maize seeds are banned in the European Union which highlights the importance of intensified research into suitable alternative control strategies. Field trials using a blend of entomopathogens in conjunction with chemical insecticides were carried out to determine the effect on survival and development of the WCR as well as on grain yield. The entomopathogens included the nematode species

Heterorhabditis bacteriophora Poinar (Heterorhabditidae) and the fungus *Metarhizium brunneum* Petch (Clavicipitaceae). The agents were applied in two naturally heavily WCR-infested maize fields in the province of Styria, Austria, in 2013. Neither the abundance of larvae nor the number of adults showed significant differences between the treatments. However, when both *H. Bacteriophora* and *M. brunneum* were used in combination with untreated seeds, the grain yield was almost equivalently high compared to treatments using neonicotinoid-dressed seeds. The two entomopathogens possibly interact synergistically and could provide a powerful alternative strategy to chemical insecticides for the larval control of *D. v. virgifera*. Nonetheless, a repetition and extension of the trials in 2014 is essential to further evaluate the efficacy of the different agents for WCR control.

Poster / Microbial Control. Wednesday, 16:30. **MC-23**

Evaluation of the effectiveness of the entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat

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Wireworms, the larval stage of Elaterid beetles are serious soil dwelling pests of small grain, corn, sugar beet and potato crops. *Limonioides californicus* (Mannerheim) and *Hypnoidus bicolor* (Eschscholtz) are the predominant wireworm species infesting wheat in Montana, particularly in north-central Montana. Currently available insecticides provide only partial control, and no alternative management tools exist. At two field locations (Ledger and Conrad, MT) in 2013, the fungi, *Metarhizium brunneum* F52, *Beauveria bassiana* GHA, and *Metarhizium robertsii* DWR 346, were evaluated in seed coat, in-furrow granular and soil drench applications, in addition to imidacloprid seed treatment, which is currently being used by growers. Wireworm damage in various treatments was evaluated as standing plant counts, wireworm population survey, and grain yield production. The three fungi applied as formulated granules or as soil drenches, resulted in significantly higher plant stand counts and yields at both locations, than fungus-coated seed treatments and the untreated control. Significant difference was detected among the application methods instead of species of the fungi. All three fungi applied as granules in furrow and in soil drench were paramount to seed-coating treatments in wireworm control, and provided an efficacy comparable or superior to imidacloprid. The fungi used in the current study provided significant plant and yield protection under moderate wireworm pressure, indicating their potential utility in the integrate management of this pest.

Poster / Microbial Control. Wednesday, 16:30. **MC-24-STU**

Using the combination of entomopathogenic fungi and extracts improves control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Both the virulence and the insecticidal activity of the crude extracts of 26 isolates of the mitosporic ascomycete entomopathogenic fungi *Metarhizium* sp. and *Beauveria* sp. (Ascomycota, Hypocreales) were determined against the second-instar *S. littoralis* larvae (Boisduval) (Lepidoptera, Noctuidae), which is considered a very harmful polyphagous insect pest. All isolates were pathogenic for second instar *S. littoralis* larvae by immersion on fungal suspension, but only four isolates of *Beauveria* (EABb 01/33-Su, EABb 01/88-Su, EABb 01/103-Su, and 3155) and one isolate of *Metarhizium* caused more than 50% mortality of larvae. EABb 01/33-Su and EABb 01/88-Su isolates caused the higher mortalities with 78.33% and 75.00%, respectively, and their average survival time (AST) values were 9.67 and 8.73 days, respectively. The LD₅₀ and LT₅₀ values were 5.69x10⁶ conidia ml⁻¹ and 6.76 days for EABb 01/33-Su and 1.05x10⁷ conidia ml⁻¹ and 7.02 days for EABb 01/88-Su. On the other hand, the crude extracts obtained from the isolates EAMb 09/01-Su and EAMa 01/58-Su caused the highest mortality rates, 80.00 and 66.66%, and the lowest AST values, 5.13 and 4.43 days, respectively. Topical application of the crude extracts did not cause any mortality. Combined treatments of fungal suspensions of isolates EAMb 09/01-Su and EAMa 01/58-Su and their extracts caused higher mortality rates than the single ones, in a dose-dependent manner, with mortality rates reaching 100% for EAMb 09/01-Su isolate and its extract at 1 mg ml⁻¹ and 76% mortality for EAMa 01/58-Su, and its extract at 1 mg ml⁻¹. The combination of the fungus EAMb 09/01-Su at 10⁸ conidia.ml⁻¹ and the crude extracts had a synergistic effect on larvae resulting in 100 % mortality to concentrations 1 mg protein ml⁻¹ and the combination of the fungus EAMb 01/33-Su + extracts EAMb 09/01-Su to concentration of 10⁷ and 10⁸ conidia ml⁻¹ to 1 mg protein ml⁻¹ crude extract also had a synergistic effect on larvae resulting in 93.33 and 100% mortality. The AST ranged between 4.08 and 5.77 days at 10⁸ conidia.ml⁻¹. These results show the potential of using the combination of entomopathogenic fungi with crude extracts for an integrated *S. littoralis* management strategy targeting larvae.

Poster / Microbial Control. Wednesday, 16:30. **MC-25-STU**

Wireworm control with fungus colonized barley kernels in cover-crops

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Insecticide treatments to control wireworms in cover-crops have been a successful strategy to prevent wireworm damage in sensitive crops planted in the following season. One example was the application of Fipronil as a seed treatment of summer oat preceding potatoes. This way, the wireworm population was reduced below the damage threshold already before planting of potatoes.

We tested a similar strategy in a semi-field pot experiment, replacing the insecticide with *Metarhizium brunneum* ART2825, formulated as fungus colonized barley kernels (FCBKs). Pots were treated with four different doses of FCBKs in August 2013 during sowing of summer oat. In addition, pots were artificially infested with *Agriotes obscurus* larvae. In April 2014, potatoes were planted into these pots. Establishment of the fungus in the pots was evaluated by counting colony forming units per g of substrate. Numbers of recaptured wireworms and the percentages of wireworms dying from mycosis were used to estimate efficacy of the treatments. Finally, effect on yield will be as estimated by counting wireworm holes on harvested potatoes.

Preliminary results are promising: The fungus successfully

established in the substrate after a few weeks and up to 70% of wireworms were killed by the treatments, depending on FCBK doses used for application. Results suggest that treating cover-crops with *Metarhizium*-inoculated FCBKs may be a useful tool for biological control of wireworms in potatoes.

Poster / Microbial Control. Wednesday, 16:30. **MC-26**

A resource efficient method to test non target effects of new biocontrol agents in vitro

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As part of the EU supported project INBIOSOIL we developed a protocol to test the non-target effects of microbial biocontrol agents and their formulations. For this purpose we selected four beneficial - predatory arthropods that are widespread and naturally occurring in Europe and are all also commercially available for biological pest control: *Aphidoletes aphidimyza* (Insecta, Diptera, Cecidomyiidae), *Atheta coriaria* (Insecta, Coleoptera, Staphylinidae), *Orius majusculus* (Insecta, Hemiptera, Anthocoridae) and *Geolaelaps aculeifer* (Acari, Mesostigmata, Laelapidae). These arthropods have different life cycles, prey, and most important, they inhabit different strata of the plant and soil in the field. The protocol allows a quick assessment of the potential side effects of microbiological biocontrol agents and their formulation components on these representatives of beneficial arthropods – and therefore should be considered standard tests to be done before further resource and time demanding testing in the field.

Poster / Microbial Control. Wednesday, 16:30. **MC-27**

Ultrastructure of midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) after consumption of prey with the *Bacillus thuringiensis* strain HD1

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The interaction of Cry toxins from *Bacillus thuringiensis* in the midgut of some insect larvae determines their efficacies as insecticides, due to the expression and availability of the sites of action of the toxins in the midgut. Research has highlighted cases of resistance to Cry toxins due to alterations in the binding sites in columnar cell membranes. We analyzed the effects of spraying a *B. thuringiensis* var. *kurstaki* (HD1 Strain) suspension at a concentration 3 × 10⁸ spores/mL, onto leaves that were then offered to the larvae of *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) and subsequently offered as prey to the predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae). We examined the ultrastructure of the midgut of predators. *P. nigrispinus* adults, 3 h after consuming prey with the HD1 strain were used for dissection and extraction of the midgut. The villi present in the midgut of the predator were observed in both cross section and as longitudinal sections. At the apex of the intestinal cells, the microvilli were seen. Also visible were remarkable muscle fibers in the lumen of the intestine; these fibers are perceptible only in the anterior and middle intestine, suggesting that they move when moving food into the large intestine during

digestion. The results showed that there were no adverse effects on the predator when the larvae of *P. xylostella* had previously ingested the HD1 strain of *B. thuringiensis*.

Poster / Microbial Control. Wednesday, 16:30. **MC-28**

Control of sugarcane borer, *Diatraea saccharalis*, with formulations of *Beauveria bassiana* and *Metarhizium anisopliae*

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The control of sugarcane borer (*Diatraea saccharalis*), the most important pest of this crop, with entomopathogenic fungi has already been reported in Brazil. However, have been used the pure conidia, which can decrease the efficiency of control due to environmental factors such as temperature and level of ultraviolet radiation. The objective of this study was to evaluate, in laboratory, encapsulated formulations containing *Beauveria bassiana* and *Metarhizium anisopliae*, against this pest. It was used pure conidia of the isolates IBCB 66 (*B. bassiana*) and IBCB 425 (*M. anisopliae*) and the formulation in sodium alginate. The fungi, were applied in two ways, powdered and sprayed, at the concentration 6×10^8 conidia, and the formulation was applied directly in two concentrations 6×10^8 and 1×10^9 . The caterpillars were evaluated at the 7^o and 14^o day after the application. The jars with insects were kept in air-conditioned room at 25.0 ± 2.0 °C and relative humidity around 70%. The bioassay was done with 30 caterpillars per treatment and 5 repetitions. To pure conidia of *B. bassiana*, in the 14^o day, the mortality of caterpillars was 96% in sprayed application, while in powdered 87%. In the formulation, the mortality was 57% at the concentration of 6×10^8 and 77% at 1×10^9 . As for the *M. anisopliae*, the mortality of caterpillars in the 14^o day, in the sprayed treatment was 47%, and in the powdered 27%, while the mortality in the formulations were 4% at the concentration of 6×10^8 and 24% at a concentration of 1×10^9 .

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Poster / Microbial Control. Wednesday, 16:30. **MC-29-STU**

Identification and functional analysis of two ABCC family genes in *Helicoverpa armigera*

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Bt toxins are widely used for insect control and resistance to *Bt* toxin is a problem that has been presented in recent years. Midgut receptors have been reported as binding proteins for *Bt* toxins and play important roles in toxicity. Recently, mutations in the ABCC2 transporter were reported to take key roles in *Bt* resistance of several species of insects. In this study, we cloned two ABCC genes from *Helicoverpa armigera*, and sequence analysis showed that these genes were quite homologous to ABCC2 and ABCC3 genes from other lepidopteran insects, so were named HaABCC2 and HaABCC3 respectively. Tissue specific expression and instar specific expression analysis showed that the two ABCC genes were mainly expressed in midgut and later instar larvae. RNAi was

done to silence these ABCC genes by feeding dsRNA to *H. armigera*. Bioassays showed that silencing of HaABCC2 in *H. armigera* larvae resulted in increased survival and pupation rates with normal eclosion rate on Cry1Ac toxin-incorporation diet, while silencing of HaABCC3 had no effect. Our research proved that ABCC2 play important role in Cry1Ac toxin pathological mechanism in *H. armigera*.

MICROSPORIDIA

Poster / Microsporidia. Wednesday, 16:30. **MI-1**

Decline of native bumblebees (*Bombus*) and *Nosema* (Microsporidia: Nosematidae) infections associated with introduction of the European bumblebee in Northern Japan

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The European bumblebee, *Bombus terrestris* (L.), has been widely established throughout a broad range of Hokkaido, northern Japan since its introduction for pollinating agricultural products in 1991 and has been suggested to cause the decline of native bumblebee species. Recent invasions of *B. terrestris* into the eastern Hokkaido have been reported in 2007. The Notsuke Peninsula is covered with the species-rich maritime grassland that extends along the coast. This region is also one of the restricted distribution ranges of a rare native species, with a highly diverse bumblebee species. Given the features of the geographic region and the species involved, the invasion of *B. terrestris* into the Notsuke Peninsula is assumed to have devastating influence on native bumblebees. Here, we conducted a multi-year survey of bumblebee species to examine the population dynamics of introduced and native bumblebees. We also investigated the prevalence of *Nosema* spp. which may play an important role in the declines of native bumblebee, as well as genetic variation of the *N. bombi* rRNA ITS region for comparison with the European and North American isolates.

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Development and application of a loop-mediated isothermal amplification method for rapid detection of *Nosema ceranae*

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Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification method, was developed for the rapid detection of the major honey bee microsporidia disease, *Nosema ceranae*. The LAMP method amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions using a set of four specially designed primers and a DNA polymerase with strand displacement activity. In this study we designed primers for LAMP assays to detect *N. ceranae* protein coding gene for DNA dependent RNA polymerase II largest subunit (RPB1) and methionine aminopeptidase type 2 (MetAP2), and evaluated the specificity and sensitivity of these assays. The detection limits for both assays was ~200