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 x 10^8 conidia, and the formulation was applied directly in two concentrations 6 x 10^8 and 1 x 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 ^oC ± 2,0 ^oC 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 x 10^8 and 77% at 1 x 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 x 10^8 and 24% at a concentration of 1 x 10^9.

Financial support: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

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* (Microspordia: 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.

Poster / Microsporidia. Wednesday, 16:30. **MI-2**

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

pg/µl and DNA amplification was completed within 60 min at an optimal temperature of 63°C. The assays detected 6 different geographical isolates of *N. ceranae*, and no cross-reaction was observed with other microsporidia species. The performance of LAMP and PCR was comparable: 100% specific, 100% sensitive, 100% positive predictive value (PPV), and 100% negative predictive value (NPV). In conclusion, the LAMP assay was equally specific but with a shorter detection time when compared to PCR in the identification of *N. ceranae*. The LAMP assay is an easy-to-use method and a promising alternative to conventional PCR for the rapid, cost-effective for specific identification of *N. ceranae* and other microspridia species. LAMP is considered an appropriate technology that could be used in resource-limited laboratories and the field.

Poster / Microsporidia. Wednesday, 16:30. **MI-3**

Permanent level of pathogens within ten bark beetles generations

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During the ten generations of spruce bark beetle *Ips typographus* population densities were monitored for 5-10 trap trees at several study sites in the Czech Republic in 2008-2012. On every of the four debarked section number of entry holes of spruce bark beetle were counted and then converted to density per unit area to the size of the studied sections (length about 0.5 m and about half trunk circumference). During the analysis in the field paternal beetles were collected and then stored refrigerated at -5°C. Total of 3,388 *I. typographus* beetles were dissected and checked for the presence of pathogens. In total four pathogenic organisms were detected: intestinal nematodes in 14.8%, microsporidia *Chytridiopsis typographi* in 9.1%, eugregarine *Gregarina typographi* in 0.3% and larvae of endoparasitoids in 4.9% of studied beetles. Relationship between the infection levels of pathogens and population growth of bark beetles from year to year according to the formula for calculating the rate of growth: R = logNt – logNt-1 was studied. Our research has proven that intestinal nematodes, *Ch. typographi* or *G. typographi*, did not influence the population growth of spruce bark beetle at the studied sites and are not as strong and lethal factor during the spruce bark beetle gradation. In contrast, the coefficient of population growth and the rate of beetle infested by endoparasitoids in the population is positively correlated ($y=4.72+10.38*x$; $r=0.68$; $p<0.01$; $r^2=0.47$). Parasitoids are thus able to respond very effectively to increase of the host population.

Poster / Microsporidia. Wednesday, 16:30. **MI-4**

Microsporidia in beet webworm *Loxostege sticticalis* (Pyraloidea: Crambidae): a survey of 2013

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Local populations of beet webworm in South Western Russia (populations “Slobodka”, adults sampled through May to August, “Chertkovskiy” and “Neklinosvkiy”, larvae sampled in May 2013) and Western Siberia (population “Karasuk”, sampled in June and July 2013) were examined for the presence of microsporidia. In South Western Russia, microsporidia were found only in population “Neklinovskiy”. There were three distinct microsporidian species, as proved by SSU rRNA gene sequences: *Tubulinosema* cf *loxostegi* (35% prevalence rate), *Nosema* cf *granulosis* (4%) and *Nosema ceranae* (3%). The identity of the latter species, a widespread pathogen of honey bees, was established using partial gene sequences of SSU-ITS-LSU and IGS rRNA. Its detection in a lepidopteran host implies a wider host range than though earlier and is logically explained by relatedness of *N. ceranae* to species of *Vairimorpha* which eagerly attack lepidopteran hosts and their hymenopteran parasitoids. In Western Siberia, the same isolate of *Tubulinosema* cf *loxostegi* was detected at the prevalence rates of 3% and 30% in June and July, respectively. All three species of microsporidia were able to infect beet webworm larvae in lab assays. For *Tubulinosema* cf. *loxostegi* vertical transmission to infected beet webworm progeny, experimental infection of *Galleria mellonella* and natural infection of tachina fly parasites (Diptera: Tachinidae) emerged from the microsporidia-infected beet webworm population were also confirmed.

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Poster / Microsporidia. Wednesday, 16:30. **MI-5**

Microsporidia from larvae of different lepidopteran species in Bulgaria

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Thirty-five lepidopteran species in 12 families were investigated for the presence of microsporidia in Bulgaria from April 2009 to June 2012. Infections caused by microsporidia in the genera *Nosema* and *Endoreticulatus* were identified in *Tortrix viridana, Operophtera brumata, Archips xylosteana, Orthosia cerasi, Orthosia cruda* and *Eilema complana.* The prevalence of *Nosema* spp. was low in host species: 0.3% for *T. viridana,* 2.1% for *O. brumata*, 2.4% for *O. cerasi*, 2.7% for *Archips xylosetana* and 3.3% for *O. cruda,* respectively. Spores of *Endoreticulatus* sp. were observed in 13.5% of collected *E. complana.* The spores of *Nosema* in *O. brumata* were localized in host fat body and phylogenetic studies showed that this microsporidium is relatively distantly related to .*Nosema wistmansi*, and the genera *Orthosomella* and *Cystosporogenes*. It is, however, closely related to *Nosema thomsoni. Nosema* sp. found in *Orthosia cruda* was detected in the silk glands of host larvae. Phylogenetic analysis confirmed that the microsporidium observed in the gut epithelium of *E. complana* belongs to the genus *Endoreticulatus;* however, it is not identical to other *Endoreticulatus* spp. described from Lepidoptera.

Poster / Microsporidia. Wednesday, 16:30. **MI-6**

Ultrastructural characterization of a new microsporidium (Opisthokonta: Chytridiopsida) from the pigeon feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea)

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Only about 20 species of microsporidia have been described from mites. All except one species produce typical spores with a long polar filament and a polaroplast. We present the first

study of an atypical microsporidium infection in a feather mite (*Falculifer rostratus).* The infection is restricted to the *colon epithelium where it* leads to hypertrophy of the concerned cells. During sporogony multinucleate plasmodial aggregates are formed within a sporont. The sporonts are in direct contact to the host cell cytoplasm. Merogonial stages were not present. Spores are tiny (3.6 x 2.6 µm), broad ovoid in form and monokaryotic. The spore wall of mature spores has a thickness of about 240 nm and consists of a three-layered endospore and a thin, electron-dense exospore. The polar filament is anisofilar and arranged in 3–4 coils. In cross-sections it has a star-like appearance since the electron-dense core forms rounded compartments for lucent material at its surface. In grazing sections this results in a honeycomb-like pattern. A polaroplast is missing. The life cycle features and atypical spore structures clearly classify the species from the feather mite as a member of the order Chytridiopsida. Its affiliation to one of the known genera is discussed.

Poster / Microsporidia. Wednesday, 16:30. **MI-7**

Infectivity of a *Thelohania* like microsporidian isolated from *Phthonandria atrilineata* to the silkworm, *Bombyx mori*

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The pebrine of the silkworm, *Bombyx mori*, is a disease caused by infection with the microsporidium *Nosema bombycis*, also can be caused by cross-contamination of microsporidium from wild insects. We have isolated a *Thelohania* like microsporidian (TMPA) from the *phthomndria atrilineata* in the silkworm rearing region of Zhjiang province, China. The mature spores of TMPA were cylindrical or ovocylindrical in shape with a strong diopter and glossy surface. The spore size of TMPA was 3.27±0.14×2.03±0.16 µm with a length/width ratio of 1.61±0.11 µm, similar to those of *N. bombycis*. Therefore, the spores of TMPA were hardly distinguished from the spores of *N. bombycis* under light microscope. In TMPA spores formative stages, sporont produced pansporoblast including 8 nuclears by meiosis, and later 8 spores were formed in pansporoblast. Infection was systemic with mature spores produced in muscular tissue, epithelial cell of trachea, fat body, middle and posterior silkgland, fore and middle intestine, malpighian tubule and germ gland, most extensivest in muscular tissue and epithelial cell of trachea, but not in dermal cells, nerve cells, fore silkgland, posterior intestine and hemocyte cells. The IC_{50} value of TMPA to newly-hatched silkworm larvae was 1.55×10^4 spores/ml, 700-fold higher than that of *N. bombycis*, suggesting a weakly infectiousness. TMPA have transovarian transmissibility in silkworm, the rate of transovarian transmission was 1.74%, which was significant lower than that of *N. bombycis*.

NEMATODES

Poster / Nematodes. Wednesday, 16:30. **NE-1**

First release of the mermithid *Strelkovimermis spiculatus* in *Culex pipiens* mosquito populations in Argentina

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Mermithids have proved to be effective in parasitizing natural populations of mosquito larvae. However nothing is known about the inoculative introduction of this nematode in natural populations of culicids in our country. We report the results of the first field release of *S. spiculatus* in Argentine. Study area was constituted by house drainage ditches, breeding site of the mosquito *Culex pipiens* where this nematode was not present. The number and stage of mosquitoes were recorded pretreatment. *Strelkovimermis spiculatus* was introduced as second-stage juveniles (J2) obtained from laboratory cultures maintained at CEPAVE laboratory. Release was done in November 2012 (spring). A dose of 10,000 J2 per meter was applied (over a total area of 17 x 0.5 m). The number of J2 was based on previous results. Mosquito larvae were sampled 24 hs post-treatment once a week during a year, to corroborate the presence of nematode by microscopic dissection and emergence from fourth instars larvae. Parasitism by *S. spiculatus* began to be observed at third day post-application (3%). Values ranged between 0.01% and 86.3%. The highest value was recorded at 8 months post-release. This environment remained dry or without larvae during a period of four months. Nevertheless a parasitism of 45.2% was observed after this period during the first larvae collection and reaching levels between 4.8% and 86.3%. Only in three occasions was not observed infected larvae throughout the year of sampling. *Strelkovimermis spiculatus* was able to establish itself in this habitat and cause high levels of infection in *Culex pipiens* larvae.

Poster / Nematodes. Wednesday, 16:30. **NE-2**

Increased infectivity in Steinernema websteri IJ after development in desiccation-stressed hosts

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This study investigates the effect of desiccation during development on entomopathogenic nematode (EPN) infectivity. *Galleria mellonella* hosts infected with *Steinernema websteri* A10 were allowed to air-desiccate in an environmental chamber set at 23^0C for up to 31 days post-infection (DPI) resulting in a host weight loss of approximately 64%. Host carcasses were re-hydrated using reverse-osmosis (RO) water and placed in White traps to collect emergent infective juvenile populations (IJ). IJ were pooled over a three-day time period for time points on days 10, 17, 24, and 31 DPI, respectively. For a randomly chosen sample of 100 IJ for each time point, sine wave movement (number of oscillatory motions completed in one minute) and IJ morphometrics, were measured. To evaluate IJ efficacy, plexiglass “bulls-eye” traps with screens dividing sections into quadrants of specific radii were loaded using sterile soil. Twenty hosts were placed in each quadrant in the outer ring only. A dose of 10,000 IJ from each time point was placed in the center ring. Host mortality was measured over 132 hour time period. Results demonstrated that IJ collected from desiccation-stressed hosts at days 17 and 24 post-infection were significantly smaller while exhibiting greater oscillation compared with controls ($\alpha \leq 0.5$). Furthermore, efficacy experiments using bulls-eye traps demonstrated that the same desiccation-stress IJ populations killed approximately 70% of hosts between 60-72 hours post load as compared 30% mortality between 72-84 hours post load for controls. This study has implications for host delivery systems in field applications.