<u>SCIENTIFIC REPORT FROM STSM – 12. 7. - 11. 8. 2004</u>

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Title of the mission:
Evaluation of the pathogenetic and immune defence interactions of entomopathogenic nematodes (*S. scarabaei*, *S. glaseri* and *H. bacteriophora*) and scarabaeids (*Amphimallon* sp. and *Hoplia* sp.).

This mission maked possible to transfer known techniques for the investigation of cellular and humoral defence mechanisms in insects against entomopathogenic nematodes (EPNs) to a new pathogen-target system. Scarabaeidae are very resistant to EPNs except *Steinernema scarabaei*, a recently described specie from the USA. Interestingly this nematode has a low effectivity against *G. mellonella*, an insect which is highly susceptible to most known EPNs. During my short term mission the pathogenesis of *S. scarabaei* against scarabaeids (*Amphimallon* sp. rsp. *Hoplia* sp.) and *G. mellonella* was compared to that of other EPNs (*Heterorhabditis bacteriophora*, *Steinernema feltiae*). The work was focussed on detection of encapsulation. The number of replicates in the different experiments was kept low in order to maximise the number of host-EPN and dose combinations. It was tested how applicable are the techniques for the well known *G. mellonella*-EPN system for a scarabaeid-EPN system.

The following experiments with encapsulation responses was carried out:

The encapsulation response of host insect to the EPNs was investigated by exposing the insects to nematode dauer juveniles (DJs, dose 50 or 25 DJs / 1 larva or grub) and by injecting DJs (10 DJs / 1 larva or grub, according to Peters & Ehlers 1997) directly into the hemocoel. The encapsulation was searched microscopically after 24 hours incubation when larva (or grub) was dissected. The encapsulation response was presented as percentage of susceptible

invasion. Larval mortality, condition of EPNs (recovery or not), percentage of susceptible invasion after 24 hours and also after 48 hour using homogenization method was evaluated. All three species of EPN used were successfully cultivated on *G. mellonella* larvae.

Main results:

Encapsulation was detected after natural invasion only in *G. mellonella* with *H. bacteriophora* (Fig. 1A, 2). After injection of EPN encapsulation was detected in the same combination - *G. mellonella* and *H. bacteriophora* and in *Amphimallon* after injection of *H. bacteriophora* and *S. scarabaei* (Fig. 1B, 3). No encapsulation was observed in *Hoplia* sp. If encapsulation was detected, only app. 10 % of EPNs was encapsulated. Higher values (app. 20 %) of encapsulated EPNs were obtained in *Amphimallon* after injection. The encapsulation response varied considerably between individual insects. Always only partly encapsulation was detected, never whole EPN was encapsulated. It was published that symbiotic bacteria can suppress the immune response since they adhere and kill the hemocytes before ending of the capsule (Dunphy & Webster 1988). Encapsulation was most often initiated at the tail region of the EPN, detected capsules were disappearing, not stabilised. Only cellular encapsulation in *G. mellonella* and grubs was detected, no melanized capsules were observed.

A/ natural invasion - encapsulation YES / NOT

	G. mellonella	*Amphimallon* sp.	*Hoplia* sp.
H. bacteriophora	YES	NOT	NOT
S. glaseri	NOT	NOT	NOT
S. scarabaei	NOT	NOT	NOT

B/ injection - encapsulation YES / NOT

	G. mellonella	*Amphimallon* sp.	*Hoplia* sp.
H. bacteriophora	YES	YES	NOT
S. glaseri	NOT	NOT	NOT
S. scarabaei	NOT	YES	NOT

Fig.1: Encapsulation detected after natural invasion (A) or injection (B) of EPN.

Fig. 2: Encapsulation detected in *G. mellonella* after natural invasion of *H. bacteriophora*.

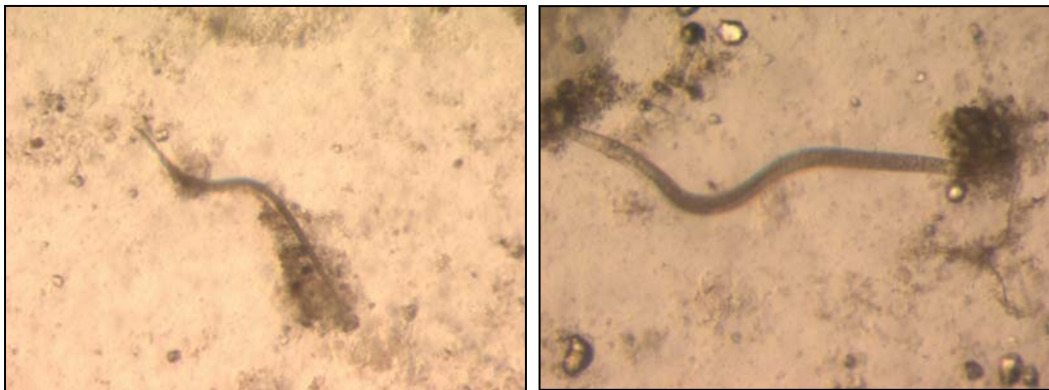

Fig. 3: Encapsulation detected in *Amphimallon* sp. after injection of *S. scarabaei.*

S. glaseri was not encapsulated after natural invasion and injection in all insects, this species is also non-encapsulated in *P. japonica,* where two surface coat proteins were shown to reduce hemocyte numbers and one of these also markedly reduced melanization and the ability of hemocytes to phagocyte (Dowds & Peters 2002). Dauer juvenile recovery of all EPNs tested after natural invasion and injection in *G. mellonella* was higher than 90 %. In grubs the values were lower, mainly because of the small amount of replications (during my STSM was too late for grubs in this year, I used totally only 37 grubs of *Amphimallon* sp. and 39 grubs of *Hoplia* sp.). Mortality of *G. mellonella* and grubs 20 - 24 hours after injection of *H. bacteriophora* was higher than 37 % and after injection of *S. glaseri* and *S. scarabaei* was 100 %. Many DJs were dead after natural invasion or injection, mainly in *Hoplia* sp. (14 – 73 %), so other immune reactions than encapsulation are certainly activated!

Collected hemolymph from *G. mellonella* or *Amphimallon* sp. was used for in vitro experiments with *H. bacteriophora.* Encapsulation was found in *Amphimallon*, but others EPN were not tested.

I tried to isolate symbiotic bacteria (*Photorhabdus* / *Xenorhabdus*) from host hemolymph 24 hours after invasion using plates with BTB or TSA agar, but withouth success. The reason

should be that insects were not good dried in a sterile air flow after sterilisation in 70 % alcohol or bacteria were just adsorbed by insect, thus I was unable to detect the presence in the hemolymph. Dunphy & Thurston (1990) reported low density of symbiotic bacteria within the first 24 hours after infection as well.

Following experiments:

This work will continue in Masaryk University Brno with more replications to make possible statistical analysis of obtained data. There are many possibilities to improve the experiments. The differencies between injection of surface sterilized and non-surface sterilized EPN can be tested. Other doses of EPN can be applied for natural invasion and injection. Injection of bacteria and subsequent injection of EPN should be tested to detect the role of the symbiont on encapsulation. Other species of grubs should also be tested.

References:

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Confirmation of host institution:

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