



COST Action 850
Biocontrol Symbiosis

<http://www.cost850.ch/index.html>

Working Group 3+4 Biotechnology and Interactions with Field Biota

COST 850 Working Group Meeting:
Use of entomopathogenic nematodes to control
white grubs in turf

Agricultural Research Centre
9820 Merelbeke, Belgium

Sunday 2nd - Monday 3rd May, 2004

PROGRAMME
Sunday, 2nd May 2004

09:30 Organised transport from the hotels to Merelbeke

Use of different EPN species against grubs in turf

10:00 -10:10 **Maurice Moens:** Welcome and opening of the meeting

10:10 -10:15 **Ralf-Udo Ehlers:** Introduction, aim of the meeting

10:15 -11:00 **Ralf-Udo Ehlers:** Ten years of field work with *Heterorhabditis bacteriophora* to control grubs of the garden chafer in Germany: from research to commercial application

11:00 -12:00 **M. A. Ansari, L. Tirry & M. Moens:** Biological control of *Hoplia philanthus* (Coleoptera: Scarabaeidae) with entomopathogenic nematodes and fungi

12:00 -12:30 **Michael Barth, Roger Fischer & Ralf-Udo Ehlers:** Susceptibility of different grub species to *Heterorhabditis bacteriophora* and *Steinernema scarabaei*

12:30 Lunch

Experiences of entomopathogenic nematodes use in the field

14:00 -14:30 **Marek Tomalak:** Susceptibility of June chafer, *Amphimallon solstitiale* to entomopathogenic nematodes

14:30 -15:00 **Lars Stubsgaard:** Commercial trails with entomopathogenic nematodes in Denmark

15:00 -15:30 **Henk Vlug:** Guiding commercial introduction of entomopathogenic nematodes in The Netherlands

15:30 -16:00 **Silvia Hellingmann:** Experimental use of different entomopathogenic nematode species against *Melolontha melolontha*

16:00 Coffee/Tea break

Discussion

18:00 Transport to the hotel in Gent

Conference Dinner

Bus leaves from hotels at 19:30

PROGRAMME
Monday 3rd May 2004

White grub species identification

- 09:30 Organised transport from the hotel to field visit
- 09:30 - 11:00 Visit to field trail of **M.A. Ansari** (Eeklo; 35 km from Merelbeke)
- 11:30 - 12:30 Practical Session "Hands on the microscope" guided by:
A. Peters, M. A. Ansari & H. Vlug:
(meeting room and lab of insect nematology, room 72)
- 12:30 Lunch

Presentation of company representatives

- 14:00 -14:10 **Bavo Schelhout & David Vanderbruggen** Biobest N.V., Belgium
- 14:10 -14:20 **Bart Lievens** Scientia Terrae VZW, Belgium
- 14:20 -14:30 **Michael Barth & Arne Peters** E-nema GmbH, Germany
- 14:30 -14:40 **Jan van Monfrans** Prograss, The Netherlands
- 14:40 -15:00 **Rick van der Pas, Harald Mikkelsen & E. De Baat**
Koppert Biological Systems, The Netherlands
- 15:00 -15:10 **Cyrille Verdun** Becker Underwood Ltd, UK

Discussion and future cooperations

- 15:10 -18:00 Discussion on need for research and cooperation on:
Grub species identification and distribution
Nematode species to control grubs
Virulence and defence mechanisms
Application technology and timing
Sustainable control
Integrated control
The major mistakes
How to make it work - cooperation with users
- 18:00 Transport to the hotel in Gent

Group dinner

Bus leaves from hotel Astoria at 19:30

Ten years of field work with *Heterorhabditis bacteriophora* to control grubs of the garden chafer in Germany: from research to commercial application

Ralf-Udo Ehlers

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First results about the use of EPN against the garden chafer *Phyllopertha horticola* were published by Peter Smits from the Netherland (Smits, 1992: Control of White Grubs with Nematodes, In: Use of Pathogens in Scarab Pest Management, T.A. Jackson and T.R. Glare [eds.], Intercept, Andover, Hampshire, pp. 229-235). In field trails the control did not surpass 49%. The results had been evaluated three weeks after application of 1 or 3 million *Heterorhabditis megidis* per m² (Smits, pers. communication). Although these results were disappointing, Sulistyanto and Ehlers (1996, Biocontrol Science & Technol. 6, 247-250) tested *H. bacteriophora* and *H. megidis* in the field in Germany. Their results surpassed those of Smits reaching a maximum of 83% with 1.5 million *H. bacteriophora* after 98 days. The nematodes had been applied in May during an infestation with the dung beetle *Aphodius contaminatus*. The discrepancy between the results could only be explained after results were available of a field trail testing *H. bacteriophora* against *P. horticola* at 0.5 million/m² in August and recording of the control every two weeks. After 2 weeks control reached 30%, after 4 weeks 60% and after 6 weeks > 90%. Additional field results recording up to 77% control underlined the potential of EPN to be used to control grubs in turf, resulting in the commercial nematode product nema-green[®] to control grubs of the Garden Chafer *Phyllopertha horticola* in turf in 1997 by e-nema GmbH. Results from five years of field testing revealed significant control between 70% and 92%. In order to test the persistence of the nematodes, soil samples were taken from golf courses in 1999 and in 2002. In 1999, at least one soil sample of 80% of the golf courses contained *H. bacteriophora*, whereas all controls were negative. In 2002, at least 20 samples/fairway were taken from 13 different golf courses. Of these 712 samples, 19% contained *H. bacteriophora* and 47% of the fairways were positive. From half of the golf courses, which had used nematodes in 1998, nematodes were reisolated, from 75% of those that had applied in 1999, 18% of those that treated in 2000 and 57% of those that treated in 2001. The low establishment in 2000 was probably related to the low humidity in August during that year which could have prevented nematode recycling and migration to yet uninfected hosts. Commercial applications were monitored for success. Failure could in most cases be attributed to nematode application on dry soil, not sufficient water during application or other mistakes during application. *H. bacteriophora* can successfully control grubs of the Garden Chafer and also of other scarabaeid larvae which have a life cycle of a single year. The presentation will discuss the impact on environmental conditions on the control potential and recycling of the nematodes in the host. An overview on the potential against other grub pests and tipulids will be given.

Biological Control of *Hoplia philanthus* (Coleoptera: Scarabaeidae) with entomopathogenic nematodes and fungi

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Hoplia philanthus Füssly (Coleoptera: Scarabaeidae) is an economically important pest of sports turf, lawns, pastures and ornamentals crops in Belgium. The beetle has a 2 years life cycle, during which the larvae stays in the soil and feed on the roots of grasses and ornamental plants. Currently, mainly chemical insecticides are used to control the grubs, but these often prove to be inadequate; moreover, concern about user safety and environmental considerations are growing. Therefore, the development and demonstration of alternative biological control are needed. The overall objective of this research was the search for an alternative control method for this pest. The strategy we used along this research was to search for two biological control agents (i.e. entomopathogenic nematodes and fungi) to learn more about their activity and mode of action when they are applied alone. In addition, it was also interest to determine whether the combined application of both agents could result in a better control of *H. philanthus* larvae.

In view of all these, several serious of laboratory, greenhouse and field experiments were conducted. In a first series, the nematode species, *Heterorhabditis megidis*, *Steinernema feltiae* and *S. glaseri* are tested against 3rd-instar *H. philanthus* in order to select a virulent nematode species for further study. In a dose response experiment, 0, 1, 10, 100, 1000 and 10,000 infective juveniles (IJs) were inoculated in wells of 24-well plates containing sand and a single *H. philanthus* larva. *S. glaseri* was most effective (LC₅₀ = 4.6 and LC₉₀ = 79.3 IJs/larva after 7 days exposure) compared to *H. megidis* (LC₅₀ = 9.7 and LC₉₀ = 511.8 IJs/larva). The lethal concentration of *S. feltiae* could not be determined because of the strain's low virulence against *H. philanthus*. In a second experiment, the pathogenicity of the symbiotic bacteria *Photorhabdus luminescens*, *Xenorhabdus bovienii* and *X. poinarii* was studied. Three microliters of a bacterial suspension (containing 0, 25, 250, 2500 or 25,000 cells) were injected into the hemocoel of *H. philanthus* and late instars of *Galleria mellonella*. *P. hotorhabdus luminescens* and *X. bovienii* killed 100% of the larvae of both species after 72 h; *X. poinarii* caused lowest mortality for both insect species. In a third experiment, 3 µl of a cell-free filtrate of *P. luminescens*, *X. bovienii* and *X. poinarii* were injected into the hemocoel of *H. philanthus* and *G. mellonella*. The filtrates of *P. luminescens* and *X. bovienii* caused 100% mortality after 24 h to *H. philanthus* and *G. mellonella*. The *X. poinarii* filtrate was least toxic to both insect species. In pot trials, *H. megidis* and *S. glaseri* caused more than 80% mortality of *H. philanthus* larvae infesting potted perennial ryegrass (*Lolium perenne*) 42 days after application of 2.5-7.5 billion nematodes/ha. The mortality was greater than the grub mortality caused by either *S. feltiae* (16%) or the control (10%).

Second series, in order to select virulent fungal species for further study, 34 isolates from the genera *Metarhizium*, *Beauveria* and *Paecilomyces* were tested in bioassays by dipping larvae in 1 × 10⁷ conidia/ml suspensions. Two isolates of *M. anisopliae* (CLO 53 and CLO 54) caused maximally 90% mortality 10 weeks post-inoculation while other isolates only caused mortalities between 10 and 62%. The

virulence of *M. anisopliae* CLO 53 was further tested by exposing *H. philanthus* larvae to conidial serial concentrations of 10^4 to 10^9 conidia/g sandy soil for up to 11 weeks at 15, 20 or 25°C. Mortality was dependant on the fungal concentration, exposure time and temperature. Eleven weeks after inoculation, the LC₅₀ values for this isolate ranged from 1.3 to 4.0×10^6 , 1.0 to 3.2×10^5 and 2.5×10^4 to 10^5 conidia/g soil at 15, 20 and 25°C, respectively. The LT₅₀ values for this isolate ranged from 3.5 to 21.7, 2.4 to 18.7 and 2.9 to 16.1 weeks at concentrations of 10^9 and 10^4 conidia/g soils at 15, 20 and 25°C, respectively. In glasshouse pot experiment with perennial ryegrass, the isolate CLO 53 caused mortalities of 50 and 88% of *H. philanthus* larvae 10 weeks after application of 1×10^4 and 1×10^6 conidia/cm² soil surface, respectively. The results suggest that the Belgian isolate CLO 53 has excellent potential for biological control of *H. philanthus*.

Third series, in order to understand antagonism and biological potential of nematode symbiotic bacteria *P. luminescens* and *X. poinarii* against entomopathogenic fungi were investigated. Combining entomopathogenic nematodes with fungi would result in synergistic interaction that would enhance the potential for biological control of *H. philanthus*. However, interaction between entomopathogenic nematode and fungi can also be antagonistic. Dual culture assay on NBTa medium (nutrient agar supplemented with bromothymol blue and triphenyltetrazolium chloride) revealed that *P. luminescens* was antagonistic to *M. anisopliae*, *B. bassiana*, *B. brongniartii* and *P. fumosoroseus* and inhibited their growth and conidial production, whereas the fungal growth was not inhibited by *X. poinarii*. In second laboratory experiment, crude extract produced by *M. anisopliae* was tested for their secondary metabolites activity against *P. luminescens* and *X. poinarii*. The crude extract was extracted with AR dichloromethane from the supernatant of 8 day old *M. anisopliae* cultures in Czapek Dox broth. They were antibacterial to *P. luminescens* and *X. poinarii* at 1000µg/ml and inhibited their growth on culture medium in Petri plate, but had no effect at low concentrations (100 or 10µg/ml). In third laboratory experiment, a bioassay was developed to determine whether crude extract influence dispersal behaviour of *H. megidis* and *S. glaseri* on SDA plates. Results showed that crude extract had no toxic effects on the dispersal behaviour of IJs even at highest concentration (1000µg/ml). It is therefore tentatively concluded that the pathogens antagonism we investigated are likely to minimize their combined application only at higher concentrations.

Fourth series, in studies targeting other insect pests, synergistic interactions have been observed from certain combinations of entomopathogenic nematodes with other pathogens. In this study, the interaction between *M. anisopliae* CLO 53 and *H. megidis* or *S. glaseri* against *H. philanthus* was studied. Larvae were exposed to various concentrations of both *M. anisopliae* and nematodes and larval mortality was assessed weekly. Nematodes were added simultaneously or 2, 3 and 4 weeks after application of *M. anisopliae*. Throughout the experiments, the combined application of *M. anisopliae* with nematodes increased larval mortality either in an additive or in a synergistic way. To achieve strong synergistic effects, larvae had to be exposed to *M. anisopliae* for at least 3 or 4 weeks before the addition of nematodes. We observed this interaction between *M. anisopliae* and both nematode species. The nematode reproduction in insect larvae exposed to the *M. anisopliae*-*H. megidis* combination was not significantly higher than in larvae exposed to only *H. megidis*. The combination of higher concentrations of *M. anisopliae* with *H. megidis* resulted in an antagonistic effect on nematode reproduction. In the greenhouse trial, *H. philanthus* larvae were placed in 3-litre pots with perennial ryegrass treated with *M.*

anisopliae, the nematodes *H. megidis* or *S. glaseri*, or the combination of fungus and nematode. Combinations of *M. anisopliae* and nematodes generated a strong synergistic effect only at higher concentrations (2×10^{12} and 2×10^{13} conidia/ha). This effect was observed when *M. anisopliae* was applied first and followed by the nematodes 4 weeks later.

Finally, these interactions were also tested in field conditions, in order to see their future implementation. Three field experiments were conducted in September to November 2003 against natural populations of *H. philanthus* consisting of late second and early third-instars. The combination of *M. anisopliae* with *H. bacteriophora* resulted in only additive effects. The combination treatments caused significantly higher mortality than the pathogens alone in experiment 1 but results were more variable in experiment 2 and 3. We concluded that the *H. bacteriophora* and *M. anisopliae* combination we investigated are likely to improve control of *H. philanthus* larvae. Further study will be needed to confirm synergistic interaction with other nematodes species, rates, and timing of application.

In conclusion, the combined use of nematodes with *M. anisopliae* may offer an integrated approach to increase the efficacy of entomopathogenic nematodes for *H. philanthus* control and perhaps other insect pests. In addition, these combinations may not only relieve of the use of broad-spectrum chemical insecticides but also provide between season controls because of their ability to recycle in field populations.

Susceptibility of different grub species to *Heterorhabditis bacteriophora* and *Steinernema scarabaei*

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The entomopathogenic nematode *Heterorhabditis bacteriophora* is the most widely used nematode for the biological control of white grubs in Turf. Although it controls the most common species in Europe, the garden chafer, *Phyllopertha horticola*, sufficiently, it provides lower control for other white grub species such as *Hoplia philanthus* and *Aphodius contaminatus*. Better control is obtained with applications in spring or summer than with September applications. Recently *H. bacteriophora* provided good control of the June beetle *Amphimallon solstitiale* when certain conditions were met. A new nematode species isolated in New Jersey named *Steinernema scarabaei* has shown highly virulent against many European white grub species. The LD₉₀ of this nematode for *Amphimallon solstitiale* and *Melolontha melolontha* was 64 and 137 infective juveniles per grub respectively. Even *P. horticola* is more susceptible to *S. scarabaei* than to *H. bacteriophora*.

Susceptibility of the June chafer, *Amphimallon solstitiale*, to entomopathogenic nematodes

Marek Tomalak

Department of Biological Pest Control and Quarantine, Institute of Plant Protection,
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The June chafer, *Amphimallon solstitiale* may cause extensive damage to turf and young trees in nurseries and newly forested areas. Chemical control of this pest is difficult as most grubs are highly resistant and the use of toxic compounds is undesirable in recreational areas, and in the forest. The success against other scarab species made entomopathogenic nematodes to be considered as potential candidates for effective control of *A. solstitiale*. Our laboratory and field studies conducted since 1996 have revealed, however, that *A. solstitiale* cannot be easily controlled by this group of bioagents. Among 17 strains and isolates of 12 nematode species tested in the laboratory heterorhabditids (i.e. *Heterorhabditis megidis*, *H. indica*, *H. zealandica* and *H. bacteriophora*) proved to be most effective. Their efficacy at the application rate of 10^6 IJ m⁻² was, however, less than 60% after 21 day exposure in the soil. The most effective steinernematid (i.e. *Steinernema glaseri*) controlled only less than 40% of exposed grubs. In laboratory experiments L3 grubs of *A. solstitiale* were more susceptible to nematode infection than L2 grubs, and pupae were clearly more susceptible than grubs. This could be related with modifications of spiracles in the insect pupae. Surprisingly, the most effective against the insect pupae was *S. arenarium*. It controlled almost 95% of pupating insects. There were significant differences in susceptibility of non diapausing and diapausing grubs collected in the field during spring and late fall.

A field trial conducted in urban lawn against a population of mostly L3 grubs gave rather poor results. After 2 months the population of *A. solstitiale* was reduced by 36 and 27% in plots treated with *H. megidis* and *S. glaseri*, respectively. In spite of the limited efficacy against *A. solstitiale* the nematodes applied to the field persisted well in the soil. They apparently recycled in infected grubs and in curculionid (i.e. *Phyllobius vespertinus*) larvae present in the experimental plots at the rate of about $2-4 \times 10^2$ individuals m⁻².

Commercial trials with entomopathogenic nematodes in Denmark

Lars Stubsgaard

Borregaard BioPlant ApS, Denmark

The product “Nemagreen” containing *Heterorhabditis bacteriophora* nematodes against the garden chafer *Phyllopertha horticola*, was introduced in Denmark by Borregaard Bioplant ApS in 1997. Since then there has been done several commercial and scientific trials with the product. Scientific trials have shown no significant effect at all. Commercial trials have with a few exceptions shown equally bad results, even though they have been carried out under many different conditions. Practical use by greenkeepers has also given insufficient results and this has led to a decrease in the professional use of “Nemagreen” on Danish turf areas. Grubs isolated from turf and treated in their natural soil under laboratory conditions are easily infected by *H. bacteriophora*. Unidentified factors must therefore be responsible for the lack of results in Denmark. The general opinion is that *H. bacteriophora* is too sensitive to variations in factors like application technique, application timing, irrigation, weather conditions and soil – and turf conditions to give a successful result.

Guiding commercial introduction of *Heterorhabditis bacteriophora* in the Netherlands

Henk Vlug

Insect Consultancy, Scherpenzeel, The Netherlands

In 2000 the commercial introduction of *H. bacteriophora* was accomplished by close cooperation of E-nema, ProGrasS and Insect Consultancy. The firm Insect Consultancy was especially erected for this purpose. Applications in the field have been closely guided, taking samples of the sprayed nematodes and at the spot estimation of the quality and quantity of the applied nematodes. After three years of nematode applications and judgment of the results it becomes clear that the final solution has not yet been completely reached. In the Netherlands six species of grubs were found in turf. None of them has the same susceptibility for parasitic nematodes. Mixed populations of grub species enhance the problem.

Control of *Melolontha melolontha* white grub with entomopathogenic nematodes

Silvia Hellingman

Business Consulting & Vertalingen, Hercules Segherslaan 20, 3723 GT Bilthoven,
The Netherlands

Melolontha melolontha large white grubs are responsible for a lot of damage in trees, ornamentals and turf in the Netherlands. The eastern part of the country has the highest concentration of those pests. Henk Vlug will give you an explanation about the demographic spread of the *M. melolontha* grubs. My contribution to this meeting will consist in my explanation about the efforts. I'm doing for 3 years trying to control the white grubs by using nematodes and the results I have got so far. I also will tell about an official project we are running to control large white grubs.

**COST 850 workshop, 3 + 4 Working Group Meeting, Merelbeke 2-3rd
May 2004:**

**Use of Entomopathogenic Nematodes to Control White Grubs in
Turf**

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