

**WORKING GROUP 4 “Interactions with Field Biota”
ACTIVITY REPORT MARCH 2001-JULY 2002**

Outline of meeting/workshop activities for WG4 2001-2002

1. Ad hoc discussions about WG4 activities during 2nd Management Committee Meeting, Wageningen, The Netherlands, 30th Aug. 2001.
2. “Kick Off” for WG4 and Workshop, Royal Veterinary and Agricultural University, Copenhagen, Denmark, 30th Nov. – 1st Dec. 2001.
3. Ad hoc discussions about future workshop during 3rd Management Committee Meeting, Einsiedeln, Switzerland, 10th-12th Jan. 2002-08-23
4. **2nd WG4 meeting, “Nematode Ecology and Molecular Techniques” Instituto Canario de Investigaciones Agrarias, Tenerife, Spain, 14-15th June 2002.**

AUGUST 2001

Working Group 4, “Interactions with Field Biota”, met for the first ad hoc discussion during the 2nd Management Committee Meeting in August 2001, where it was decided to have a “kick off” meeting for the group in December 2001.

DECEMBER 2001

The “Kick Off” meeting was held in Copenhagen 30th November to 1st December 2001, with 14 participants from 10 member countries. The local organisers were Holger Phillipsen and Otto Nielsen and they did an excellent job. The 2-day workshop resulted in a report indicating selected activities for WG4 in COST 850. The report is currently an internal document for COST 850. The main WG4 activities selected are:

- “Safety of entomopathogenic nematodes”
- “Persistence and survival in soil (epns)”
- “Bringing epn (entomopathogenic nematodes) science to practise”
- “Multitrophic interactions”

JANUARY 2002

During the 3rd Management Committee Meeting and WG5 workshop in Einsiedeln, Switzerland, a list of current projects and desired projects was compiled under the topics selected at the Copenhagen meeting. The table below indicates current projects conducted by COST 850 members related to these topics in WG4.

Topic (Selected activity)	Current projects
SAFETY	<ul style="list-style-type: none"> • <i>Side effects of predatory beetles</i> • <i>Registration of epns against certain pests</i> • <i>Identify possible risks</i> • <i>Risk assessment of epn</i>
PERSISTENCE AND SURVIVAL (SOIL)	<ul style="list-style-type: none"> • <i>Fine scale epn distributions in soil over time</i>

<ul style="list-style-type: none"> • <i>Epn population dynamics in crop rotation systems</i> • <i>Survey of unknown areas</i> • <i>Looking for strains adapted to extreme conditions</i> • <i>Ecological data re epn/insects under cropping conditions</i> • <i>Transgenic approach of getting desiccation tolerant EPN/EPB complexes</i> • <i>To use epn's in organic farms</i> • <i>Biogeography (book chapter)</i> 	
<hr/> BRINGING EPN SCIENCE TO PRACTICE	<ul style="list-style-type: none"> • <i>Conservation Biological control in crop rotation systems/inoculative release</i> • <i>Foliar application (against caterpillars)</i> • <i>Control of BVW in Strawberry IPM system</i> • <i>Control of Hylobius abietis in conifers</i> • <i>IPM/use strategies epn's for hazelnuts, chestnuts, strawberries, mushrooms etc.</i> • <i>Formulation of epn's/micro-organisms for foliar application against the DBM</i> • <i>Biocontrol of Large Pine Weevil (Hylobius abietis)</i> • <i>Modeling – simulation foods in different crops</i> • <i>New epn strains for Mediterranean pest control and develop methods of application</i> • <i>New targets for epn's sawflies, moths, leaf beetles etc)</i> • <i>Biocontrol of Vine Weevil (mainly O.sulcatus)</i> • <i>Define soil and application conditions to make epn work.</i>
<hr/> MULTITROPHIC INTERACTIONS	<ul style="list-style-type: none"> • <i>Insect outbreaks and epn incidence</i> • <i>Interspecific epn competition in one habitat (site)</i> • <i>Correlation between epn and insect occurrence</i> • <i>Long-term effects of “ecotill” wheat on entomopathogens in soil.</i> • <i>Effect of natural populations of epn on insect hosts in urban parks and forests.</i> • <i>Interaction epn – insects in hazelnuts/chestnut plantations</i> • <i>Fly control in mushrooms with both epn + mites.</i>

JUNE 2002

In relation to monitoring and studying epns in soil, it was decided that a meeting should be held emphasizing the use of molecular techniques for identifying and/or quantifying organisms in soil. The meeting included invited speakers to address the molecular techniques topic, and in addition WG4 participants had the opportunity to present their latest work. Abstracts from the presentations are included in this report. The meeting gave an excellent insight into the molecular techniques that are available to us. The meeting was held in Tenerife and kindly hosted by Prof. Candido Santiago Alvarez and the “Instituto Canario de Investigaciones Agrarias”. Work by this institute was also presented during the meeting.

Abstracts from the Tenerife meeting: "Nematode Ecology and Molecular Techniques"
(chronological presentation)

INVITED SPEAKERS

1. Possibilities and limitations in the use of molecular techniques for identification and quantification of entomopathogenic nematodes in soil.

Alex Reid, CABI, UK.

The revolution in molecular biology caused by the development of the polymerase chain reaction has opened many new avenues of research. The application of PCR based molecular techniques has impacted greatly on the study of entomopathogenic nematodes although the vast majority of work has focused on species diagnosis. The identification of EPN strains in laboratory culture has become commonplace and it is now time to use molecular techniques to answer more 'interesting' questions. These include rapid diagnosis directly from soil samples thus eliminating the need for *Galleria* baiting and all its inherent problems. The technology is available to achieve this goal now and by coupling real time PCR with species-specific primers in multiplex reactions gives the possibility of the definitive identification of the total EPN fauna from soil samples in the field. This would lead to a much more accurate assessment of the biogeography of EPN species than is currently possible. The next area for future research is the in depth study of the population biology of EPNs in the soil. Little is known about the population structure or the degree of gene flow between populations. Intraspecific genetic diversity studies have been conducted using fingerprinting techniques such as RAPD, AFLP and ISSR but none of these methods can easily be performed on individual nematodes and throw little light on levels of gene flow. However, the success of the ISSR technique shows that microsatellite type sequences are present in the genome of *S. feltiae* and that it would now be possible to isolate markers that could be used to assess gene flow on individual infective juveniles. The final area for future study is gene expression. Significant work has been undertaken by a handful of the EPN community with the construction of EST libraries and inroads have been made to isolate and characterise a number of EPN specific transcripts. However, much greater efforts need to be made to determine 'what makes these nematodes tick'.

2. Molecular approaches to differentiate and understand the pathogenicity of plant parasitic nematodes

**Vivian Blok, Plant Pathogen Interactions
Scottish Crop Research Institute.**

Molecular studies of the diversity and pathogenicity of plant parasitic nematodes and their interactions with their hosts use techniques that are applicable to a plethora of biological situations. Plant parasitic nematodes are of agricultural importance because of the damage they cause to crops but are also of fundamental interest because of the often complex and intimate associations they have with their hosts. These can be highly specific as in the case of *Globodera* spp., the potato cyst nematode and potato, or they can have very wide host ranges such as the root knot nematodes, *Meloidogyne* spp. making their management very difficult. Both of these groups of endoparasites manipulate the host's cells to produce multinucleate feeding sites, though the processes involved are different. The use of resistance to limit reproduction is the most environmentally friendly management tool however resistance is not always available for the crop or cultivars required. Variation in pathogenicity within a population can also pose difficulties. Various molecular approaches have been used to try to relate molecular and biological diversity in these nematodes. Random amplified polymorphic

DNA distinguished some groups within *G. pallida* and some within species structure was found in rDNA ITS sequence, however the latter was complicated by evidence for variation between tandem repeats and the suggestion of different molecular footprints of progenitors. Mitochondrial DNA studies of *G. pallida* were even more complex with the discovery of a multipartite mitochondrial genome with coding sequences distributed over various mini-circles and sequence redundancy between circles. The implications for the reproduction and functioning of this mtDNA genome are fascinating and merit further investigation.

To focus on variation in pathogenicity and differences in host responses, we have been using expressed gene approaches (i.e. cDNA AFLPs and suppressive subtractive hybridisation (SSH)) to identify differentially expressed sequences. These approaches have generated many sequences, which we are currently assessing for their time course and site of expression. Bioinformatics approaches are also being used to assimilate this information. Sequences have been assembled into contigs to eliminate redundancy and sequences of obvious poor quality were removed. Searches with these sequences were conducted against Solanum and nematode EST databases and further characterised for functional information by BLASTX searches. This information was then tabulated in relation to the various biological interactions examined. Surprisingly high numbers of parasite sequences were identified in some interactions and host response genes which are typically expressed following pathogen infection have been identified. Many sequences however cannot be identified as derived from host or parasite and many have no known function. Identifying candidates for further investigation which may be involved in pathogenicity or for manipulating the host response is in progress.

I would like to acknowledge the contributions my colleagues Miles Armstrong, Mark Phillips, Jane Wishart, John Jones, Bryony Banks, Paul Birch, Alison Paterson, Linda Cardle have made to this work and the funding received from the Scottish Executive, Environment and Rural Affairs Department and EU projects PL98-4235 (No Nematode), QLRT-1999-1462 (Dream) and QLK5-CT-1999-01501 (Nonema).

3. DNA/RNA amplification techniques and micro-array's in the study of soil micro-organisms

Arjen Speksnijder, Plant Research International, Wageningen, NL.

From studies it was derived that sometimes fewer than 1% of the total microbial community can be cultured and analyzed leaving a huge knowledge gap how microorganisms behave in natural environments. DNA-DNA re-association studies were performed in soil and indicated a possible diversity of 13.000 species. Shotgun-cloning of soil DNA was performed to look at genetic diversity of conservative genes. From these studies the ribosomal RNA gene became the most commonly used gene for studying microbial diversity. Carl Woese revolutionized the world with new phylogenetic insights using 16S rDNA data to reclassify the tree of life from the traditional five kingdoms of Animals plants fungi protista and monera (prokaryotes) into 3 primary domains, the Archaea the Bacteria and the Eukarya.

A whole database and web site are dedicated to this rRNA gene and in Germany bioinformatics have designed a program based on this data to design primers and probes. Suddenly we can target 99% of the community in stead of the 1% using these evolutionary conservative sequence regions, to pick out and amplify uncultivable bacteria. Primers are designed around the variable regions to generate amplicons by PCR, which reflect whole communities. Individual sequences from the mixed PCR product are cloned and randomly sequenced. These type of cloning analysis are very elaborate and maybe unnecessary in comparison studies. High diversity means also low coverage of the library. Sequence diversity of the PCR product can also be analyzed by fingerprinting. The most popular method at the moment is DGGE and TGGE. It has been demonstrated that sequences which differ only in

one base can be distinguished. A nice thing is that once these bands are separated they can be isolated and sequenced for identification by database comparison.

From this data we can see what is present and different in community structures but we still do not know who and where the active members are. Probes must be designed to identify the species of interest. With a little trick RNA can be amplified by using RT. RNA level is supposed to be related to the activity of the cells. To look at active members simple RNA isolation of samples can be sufficient for screening with radioactive labeled probes. The detection limit is 10^{-4} cells ml. Fluorescent in situ hybridization is a very useful microscopy method to identify active members of interest but also for counting and sorting by flowcytometry for subsequent studies.

We still want to know what the function is of microorganisms. To humbly start with this enigma we can look what is known about other genes than the rRNA gene. It was shown that nif genes from within termite families were similar but clearly different between families. These genes show similarities with genes found in rice roots, and cautiously can be related to fixation activity.

But now with is this information flowing in there is more need for different type of analysis. Human DNA chips are evolving as well as potato chips. DNA chips or microarray's are small surfaces with spots containing part of genomes. We are designing a soil DNA chip with sequences from pathogenic organisms and important antibiotic genes. A microarray is more or less the same as a dot blot experiment but larger in numbers (400 3D to 400.000 2D). Probes are spotted and colored target DNA is added to the spotted probes. There are 2 way's of doing this, 2D and 3D. The 3D array spots probes in a matrix and has more surface available to the substrate.

The substrate is brought very close to the surface resulting in a fast high efficient hybridization (5 minutes) compared to conventional 2D glass surface array's (24 hours). The 3D array matches the detection limit of radioactive probes. DGGE is at the level of the 2d array's, 100 times less. Preliminary experiments are performed for the detection of Phytophthora, a pathogen widespread and well known in agricultural crops. De sequence information is available and probes are designed specific for each pathogenic strain. General primers can are applied to amplify the variable regions. Mixed PCR product with enriched pathogen sequences were analyzed on the 3D micro-array and being detected simultaneously. The microarray will be a powerful tool in the ongoing analysis of microbial communities and multiplex detection of specific bacteria and their functional genes.

WG4 PARTICIPANTS PRESENTATIONS

4. Effect of tree leaf beetles (Coleoptera: Chrysomelidae) on seasonal dynamics of natural *Steinernema feltiae* populations in the soil

Marek Tomalak, Department of Biological Control and Quarantine, Institute of Plant Protection, Miczurina, Poland.

Seasonal dynamics of natural populations of *Steinernema feltiae* was studied in the soil of urban forests in Poznan, Poland. The main objective was to elucidate relationships between this nematode and communities of tree leaf beetles (Coleoptera: Chrysomelidae) – the most abundant, potential host insects in the soil of this area.

Effect of 4 leaf beetle species – two pupating in May and early June (*Phytodecta rufipes*, *Phytodecta quinquepunctata*), and two pupating in late June, July, and August (*Phyllodecta laticollis*, *Altica quercetorum*), in 4 experimental combinations, was examined on 12

collection sites located within the area of some 12 ha. During March – May period isolation of nematodes gave positive results in less than 30% of collection sites. In late June the frequency of positive sampling started to grow on sites with *P. rufipes* and *P. quinquepunctata*, and from late July on, on sites with *P. laticollis* and *A. quercetorum* only. The presence of nematodes remained high until early winter.

Parallel laboratory experiments have shown that mature larvae, prepupae and pupae of all insect species under study were highly susceptible to nematode infection and the nematodes could reproduce in host cadavers.

The study conducted for 2 consecutive seasons revealed a positive correlation between changes in the abundance of nematodes (frequency of isolation) and presence of leaf beetles entering the soil for pupation. The regular growth of nematode population 3-4 weeks after leaf beetles descending to the soil suggests that these insects may play a significant role as natural hosts for *S. feltiae*.

5. Interaction between entomopathogenic nematodes (EPNs), different factors, and insects

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All types of ecosystems and most habitats on the Czech territory were inhabited by EPNs. However, nematodes were more abundant in tree habitats where they prevailed in poplar and lime-tree roadsides and oak forest. The EPNs were most common in light brown soils. Season and altitude had no significant impact on the EPNs incidence in soil samples. Nine steinernematids – *S. kraussei*, *S. feltiae*, *S. affine*, *S. carpocapsae*, *S. intermedium*, *S. bicornutum*, *S. areanarium*, *S. weiseri*, and *Steinernema* sp. B, and two heterorhabditids – *H. bacteriophora* and *H. megidis* were found in our survey for the Czech Republic. The most frequent were *S. kraussei* in forest habitats and *S. feltiae* in open habitats. *S. kraussei* prevailed in the heavy soil while and *S. feltiae* in the light soil.

Two laboratory temperatures of 15 and 20 °C are recommended for the *Galleria* baiting. Abundance of the EPNs in the soil sample can be assessed by the number of positive *Galleria* trap replicates and number infected of *Galleria* larvae.

A role of insect hosts seems to be essential for the EPNs incidence. The most suitable were localities with *Otiorhynchus sulcatus*, sciarids, bibionids, *Cephaleia abietis* and lepidopteran larvae where infection rate varied from 58 to 100%. Habitats with severe or moderate insect outbreaks were significantly more inhabited by the EPNs.

6. Competition and intraguild predation between entomopathogenic nematodes and *Bracon hylobii*, a parasitoid of pine weevil larvae.

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The large pine weevil *Hylobius abietis* L. (Coleoptera: Curculionidae) is an important pest of coniferous reforestation in northern and central Europe. Amongst its natural enemies, the parasitoid *Bracon hylobii* Ratz. (Hymenoptera: Braconidae) and the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp. have potential as biological control agents. Both parasitoid and nematodes target the weevil larvae and hence there is potential for competition or intraguild predation. We examined the interaction of *B. hylobii* with *Steinernema carpocapsae* and *Heterorhabditis megidis* in laboratory experiments. Weevil

larvae that were still alive 1 day after exposure to nematodes were readily accepted for oviposition by *B. hylobii* females, while weevil larvae killed by nematodes were generally rejected by them. When nematodes were applied to weevil larvae on which *B. hylobii* had oviposited, few parasitoids developed to adult stage. Nematode-infected host weevils were less suitable for parasitoid development, and nematodes infected a proportion of those parasitoid larvae that did develop. The longer the time that had elapsed (1-7 days) between parasite oviposition and the application of nematodes, the less severe were the effects of nematodes on the parasitoids. Although parasitoid larvae were susceptible to nematodes, cocooned stages were not. Adult parasitoids were also susceptible in the laboratory but it is likely that under field conditions they will escape infection. Considered timing of nematode application is expected to minimise the impact of entomopathogenic nematodes on *B. hylobii* populations. Moreover, the ability of *B. hylobii* females to avoid nematode-infected hosts will lessen the impact of inundatively applied nematodes on *B. hylobii* populations and, by causing the parasitoid to concentrate its efforts on any remaining healthy weevil larvae, should complement the activity of applied nematodes in controlling weevil populations.

7. Application Pattern and Persistence of *Heterorhabditis bacteriophora*.

Mike Wilson, Department of Plant and Soil Science, University of Aberdeen, UK.

Scarab beetle larvae are important and widespread insect pests that can be controlled by the entomopathogenic nematode *Heterorhabditis bacteriophora*. The nematode is applied as an inundative biological control agent, with large numbers ($\approx 2.5 \times 10^9 \text{ ha}^{-1}$) being applied evenly to crops. In these systems nematode persistence is poor. Many theoretical mechanisms have been proposed whereby spatial structure can promote population persistence. We attempted to increase persistence of *H. bacteriophora* by applying it to 3 x 3 m plots of turf in three spatial patterns: uniform distribution; one central circular patch, diameter 1.12 m, or nine individual patches, diameter 0.38 m, situated in the center of each 1m² area within each 9 m² plot. Untreated control plots were also included. Nematode persistence (as estimated *Galleria mellonella* baiting) and spatial distribution (using SADIE analysis) were monitored over 1 year. The pattern of decline in nematode numbers was similar for all treatments. Over time the nematodes applied in patches moved from their initial application sites and became more evenly distributed, whereas the distribution of nematodes in plots with uniform application became more patchy as nematodes died. There were no significant differences in nematode numbers or spatial pattern from week 20 until the end of the experiment. Grub numbers were measured at the end of the experiment. Nematodes that had been applied evenly or in nine-patches significantly reduced grub numbers but the one-patch application did not.

8. Sex on the Beach – is it for everyone?

Rolston A.N., Downes, M.J. and Griffin, C. National University of Ireland

Entomopathogenic nematodes (EPN) of the genera *Steinernema* and *Heterorhabditis* are ubiquitous soil organisms with world-wide distributions. However, local populations are frequently patchy. The two genera have evolved two distinct reproductive strategies. The requirement for rapid reproduction in an infected host, typical of r-selected life history strategies, appears to be satisfied by self-fertility in *Heterorhabditis*. Yet *Steinernema* is neither self-fertile nor parthenogenic. So what selective advantages might *Heterorhabditis* spp. have over *Steinernema* spp. when intimately associated? Why does *Steinernema* need constant outbreeding when occurring intimately with *Heterorhabditis* which does not?

As part of a study exploring these questions, 80,000m² of a sand dune system located on Bull Island, Dublin Bay, Republic of Ireland was sampled for the distributions of the two genera. Soil cores (150mm x 10mm) were taken at 10m intervals in a grid covering 100m x 800m. The long axis of the grid paralleled the long axis of Bull Island with the short axis running perpendicular to this. Only two EPN species were found: *Steinernema feltiae* and *Heterorhabditis downsei*. Prevalence was low at 5.1% (45/880 cores). *H. downsei* occurred at a lower frequency than *S.feltiae*. *H.downsei* was more prevalent in the front 20-30m region of the dunes whereas *Steinernema* prevalence increased with distance into the dune system. These local distribution patterns may be due to the stability of the local environments, host distribution and the levels of parasite pressure acting on the two species of nematode.

9. The Use of the Glycosylase Mediated Polymorphism Detection for the Study of Gene Flow in Entomopathogenic Nematodes

Stephen BOYLE and Thomae KAKOULI-DUARTE. Department of Applied Biology and Chemistry, Institute of Technology Carlow, Ireland,

Entomopathogenic nematodes are traded for control of difficult pests. The greatest limitation on understanding how indigenous populations are affected by new strains or escaped genes is the problem of recognising the genetic structure of wild populations and the levels of gene flow between them. We are aiming at studying gene flow in Irish entomopathogenic nematode populations by detecting DNA polymorphisms in the intron regions of highly conserved nematode genes. We have used EPIC-PCR to amplify part of the major sperm protein gene from *Steinernema feltiae* and *Heterorhabditis megidis* containing a putative intron. DNA sequence analysis will follow to confirm the presence of the intron. Amplifications were achieved using degenerate primers designed after aligning the major sperm protein sequences from seven nematode species taken from GenBank. Our research is still ongoing for additional suitable genes. Genetic variation in the introns will be studied by the use of the glycosylase mediated polymorphism detection (GMPD). Introns will be reamplified using the four dNTPs including uracil and having adjusted the dTTP to dUTP ratio. Products will then be subjected to the action of uracil DNA glycosylase, which results in DNA cleavage at specific sites thus generating an allelic profile for each population studied.

10. Isolation of Entomopathogenic Nematodes in Santa Cruz de Tenerife province (Canary Islands).

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2.- Department of Animal Biology of the Universidad Autónoma de Barcelona. 08193 Bellaterra. Barcelona. Spain.

The objective of this survey was to obtain different native strains of entomopathogenic nematodes (EPN) for the control of the banana weevil *Cosmopolites sordidus*, an important pest in the Canary Islands

The survey for possible EPN was made in various important crop (banana, tomato, potato, grape) areas of the four islands of the province of Santa Cruz de Tenerife: Tenerife (175 samples), La Palma (60 samples), Gomera (25 samples) and El Hierro (30 samples).

Each sample consisted of approximately 1 kg of soil from 6 subsamples, and the nematode extraction was made using the *Galleria* baiting technique.

In this survey only two strains of EPN were isolated, both from samples taken in Tenerife island: a *Heterorhabditis*, from a banana plantation in northern Tenerife (80 m), and a *Steinernema* from south-facing mountain scrubland (2000 m).

The effectiveness of these two strains at different temperatures, and the susceptibility of the different stages of the banana weevil to these nematodes are currently being studied.

11. Entomopathogenic nematodes in Norwegian forest soils

Solveig Haukeland Salinas

The Norwegian Crop Research Institute, Plant Protection Centre, Norway.

A project on the use of entomopathogenic nematodes (epns) for control of the pine weevil (*Hylobius abietis*) has been initiated. In the first phase we are investigating the natural occurrence of epns in forest soils. Soil samples were collected from several clear cut forest sites in autumn 2001. Samples were taken around the roots of tree stumps at each site. Epns were isolated using the *Galleria* technique. Preliminary results indicate that several species in the genus *Steinernema* are present. So far *Steinernema krauseii* has been identified which is a new record for Norway. Identification of the nematodes isolated are being conducted using morphometric and molecular techniques.

12. Field Persistence of *Heterorhabditis bacteriophora*

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Persistence in strawberry fields

For control of *Otiiorhynchus sulcatus* in ornamentals and strawberries the company e-nema produces the product nematop[®] containing the nematode *H. bacteriophora*. Due to culture practice spraying of the product is not possible in strawberries. The soil is covered with plastic which prevents the migration of the nematodes into the soil. Attempts to deliver nematodes through the irrigation systems resulted in a loss > 90% due to holes in the pipes uneven distribution due to sedimentation of the nematodes in the tubes. Therefore EPN are applied plant by plant with a hose. This application method resulted in *O.sulcatus* control usually not surpassing 50%. Higher clay content of the soil prevented nematode migration. To deliver EPN at the site where the insects damage the plant dipping plants at the moment of plantation was tested. Planting of Frigo plants starts in May and lasts until August (for next years harvest). Egg laying of weevils is in May and the larvae are found earliest in June/July. Generally, EPN application against *O.sulcatus* is not done before the end of August against the larvae. Thus, if one wants to protect plants from damage caused by *O.sulcatus* using root application, the nematodes must persist at least until the first instars hatch from the eggs, which is in early July. Consequently, nematodes applied in May must persist for at least 1,5 months.

For a field trial a nematode suspension with 5,000 DJ/ml of *H. bacteriophora* was produced and the sticker CMC (Carboxymethylcellulose) was added at 0,5 %. The root system of one Frigo strawberry plant takes up approximately 2 g of this suspension. Each plant thus received between 10 - 20,000 DJ. Planting was on June 16, 2000. Soil samples taken at the day of planting were checked for the presence of *Heterorhabditis* spp. No EPN were detected. Whole plants with soil were sampled over a period of almost one year. The plants were transferred to pots with 40 *Tenebrio molitor* larvae. Insect mortality was assessed a week later.

The results indicate that *H. bacteriophora* persisted in the soil for over a year (Fig. 1). Insect mortality was between 30 and 95 % until 8 weeks after planting. The mean mortality dropped to 60 % after the winter had passed 345 days after planting. Two different CMC products were tested. When the dipping of the roots into the nematode-CMC suspension was compared

with the results obtained with plants which were sprayed, significant differences in insect mortality was observed only within the first two weeks of sampling, indicating that the soil allowed migration of the nematodes (Figure 2). On neighbouring untreated control plants *H. bacteriophora* was detected a month after planting of treated plants.

In this field trial larvae of *O. sulcatus* were not present. As we know from laboratory experiments (Lewis, 2002; Strong 2002) that *H. bacteriophora* cannot survive in the soil for very long time, we consider that alternative hosts must have been present, which supported nematode propagation. It can be concluded that dipping plant roots into nematode suspensions is a possible method of application of EPN. It will immediately provide protecting of the plants. The addition of the sticker will prevent sedimentation of the nematodes in the tank, where plants will be dipped. It also provides the plants and nematodes with enough moisture necessary to survive the time of planting.

Figure 1: Mortality of *Tenebrio molitor* larvae when added to strawberry plants in pots. The plants were taken from the field at different times after planting.

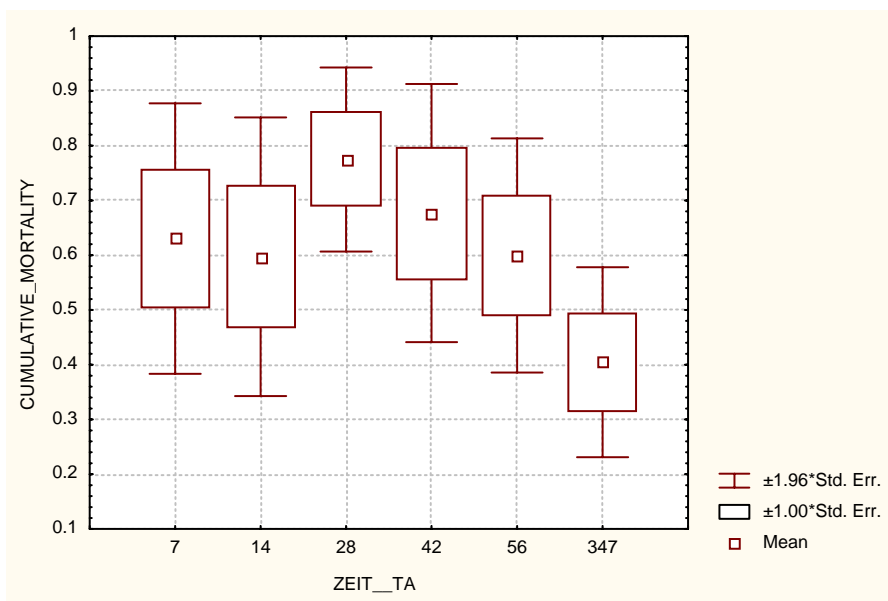
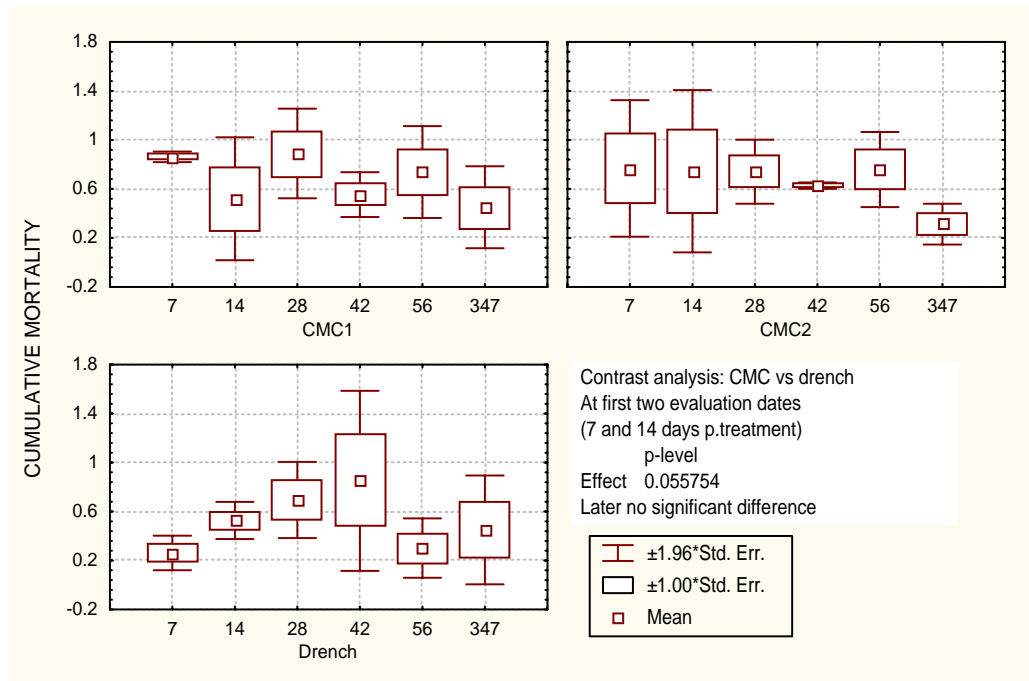


Figure 2: Nematode persistence measured as mortality of *Tenebrio molitor* larvae added to plants and soil taken from the field at different time after plantation of the plants. Two different Carboxymethylcellulose products (CMC) used for dipping plants are compared with a drench. Approximately 10,000 nematodes/plant were applied on July 15, 2000.



Persistence in turf

For control of grubs the company e-nema GmbH sells the product nema-green[®]. It also contains *H. bacteriophora* which can provide excellent results of up to 90% control against the garden chafer *Phyllopertha horticola* (Sulistyanto and Ehlers, 1996; Ehlers & Peters, 1998) and also against other grubs like *Aphodius contaminatus* or *Hoplia philanthis*. The product is sold in Germany since 1997. In 1999 several of the golf courses which had used the product a year or two before were surveyed for the presence of *H. bacteriophora*. Three to five samples of 500 g each were taken from fairways which had been treated with nema-green[®]. Baiting with last instar *Galleria mellonella* detected *H. bacteriophora* in 25% of the samples. Of the 14 golf courses all were positive in at least one sample.

The results indicate that *H. bacteriophora* can persist for a very long time. The nematodes can be successfully established by spraying but also by dipping plant roots into nematode suspensions. Long term persistence provides sustainable control effects and is another advantage over traditional insecticide spraying. Dipping plant roots into nematode suspensions is used for protection against *O. sulcatus* in strawberries and ornamentals. The addition of the sticker improves nematode distribution and provides moisture until the plants are planted. Dipping might be superior to spraying when the soil has a high clay content.

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13. Signals in Soil

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Host-finding of plant parasitic (PPN) and entomopathogenic nematodes (EPN) comprises a chain of processes: activation or hatching, orientation (attraction/repellence), migration (motility and mobility), rhizosphere exploration and penetration. Amphidia and phasmidia are considered the most important sensors for host-finding. Nematodes respond to both water-soluble and volatile compounds emanating from their host or from the host environment. In soil even short gradients make sense due to limited connectivity of soil pores. Combined signals increase sensitivity and specificity of gradient perception by nematodes.

We developed various methods to study host-finding behaviour of PPN and EPN, ranging from soil columns and boxes with a scale of decimeters down to in vitro tracking and activity tests on a millimeter scale. The distance and rate of host-finding reaction varied with nematode species. *Paratrichodorus teres* reacted from more than 7 cm away from a host plant when the plant signals were transported by infiltration water (5mm/d). Without a plant signal this nematode remained quiescent. Plant signals involved in host finding could be neutralised by organic amendments. Another interesting phenomenon is the attraction of EPN towards plant roots, apparently because there they are likely to meet hosts. However, EPN are not complete opportunists; *Thuja occidentalis* roots that had been damaged by the host insect *Otiorynchus sulcatus* were more attractive to *Heterorhabditis megidis* than mechanically damaged or undamaged roots. This suggests that host induced cues were present.

It is concluded that plant signals are of major importance to PPN and EPN. The methods to study host finding can be used for both, if properly adjusted with respect to distance and time. The challenge now is to identify specific semiochemicals involved in host-finding of either PPN or EPN in order to develop specific strategies to lure and kill or reduce host-finding of PPN, and increase host-finding efficiency of EPN without negative side effects. Study of the chemical identity and of effects of compounds in their natural setting will be of prime importance.

14. Use of EPN to control soil dwelling stages of Western Flower Thrips

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Western Flower Thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is worldwide one of the most important pests on vegetables and ornamental crops under greenhouse and field conditions. Conventional chemical and biological control tactics, targeting only at the foliar-feeding stages, usually provide no satisfactory control levels. Thus we investigated the potential of entomopathogenic nematodes (EPNs) and soil inhabiting predacious mites (*Hypoaspis* spp.) against soil-dwelling life stages of WFT. Our results indicate that all soil-dwelling life stages of WFT are susceptible to the tested EPN strains/species. Virulent strains, applied at a dose rate of 400 infective juveniles cm⁻² resulted in 80 and 40-60% WFT mortality under laboratory and microcosm conditions, respectively. Releases of *H. aculeifer* (Canestrini) at 2,800 mites m⁻² reduced WFT population by 78%. Combined applications of EPNs and *H. aculeifer* significantly lowered the number of emerging WFT adults compared to the

untreated control as well as to individual releases of EPNs and predacious mites. These findings may open up a new venue for biological control of WFT.

15. Influence of EPN on leaf feeding insects

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16. Fatty acid profile and thermoadaptation in *Steinernema* species

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The fatty acid composition of phospholipids as well as the fluidity of phospholipid vesicles obtained from *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, *S. arenarium* and *S. sp.* Morocco were studied. The nematodes were grown in last instar larvae of wax moth, *Galleria mellonella* at 18C and 25C. Phospholipid methyl esters were separated by gas chromatography and the membrane fluidity was measured by infrared spectroscopy. Phospholipids were characterized with high proportion of eicosapentaenoic(20:5n-3), linoleic(18:2n-6), oleic(18:1n-9) and stearic(18:0) acid in the *S. carpocapsae* and *S. feltiae* group. In the *S. glaseri*, *S. arenarium* and *S. sp.* Morocco group oleic(18:1n-9), stearic(18:0) and palmitic(16:0) acid were the major phospholipid components. The saturated unsaturated ratio in all the long nematode strains was higher than the *S. carpocapsae* and *S. feltiae* group. The *Polish* and *NC513* strains showed lower ratio than those long nematodes adapted to warmer conditions- *Glaseri*, *Az26*, *Azores*, *Italy*, *Morocco*. In the *S. carpocapsae* and *S. feltiae* strains S/U was lower and appears to be independent of their respective ecotype. *S. carpocapsae* and *S. feltiae* strains from colder areas contain a relatively lower proportion eicosapentaenoic acid(20:5n-3) EPA than those found in warmer climate. This did not influence their ability to regulate the membrane fluidity. Moreover, the strain *VIJE* can keep its membrane more fluid, despite its unexpectedly low amount of EPA even at lower temperature, by lowering the amount of cholesterol built in its membrane. Our results suggest that the ability of thermoadaptation effects the geographical distribution of these nematodes. Strains of *S. carpocapsae* and *S. feltiae* are well represented at different climatic areas. Strains of *S. glaseri* and *S. arenarium* appears to be less adaptive based on their fatty acid composition. Further experiments are needed to investigate their behaviour at extreme temperatures.

17. Preliminary field trial results on the biological control of *Saperda carcharias* larvae on poplar groves

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The Cerambycidae (Coleoptera) *Saperda carcharias* is a serious pest to poplar, especially in young plantation. In Italy, it completes one generation in two years. The adults appear from July and they lay isolated eggs in the thickness of the bark during all summer and autumn. Part of the eggs overwinter and hatch the following spring. Neonate larvae remain, for approximately a month, in the thickness of the bark and penetrate in the wood where they dig ascending galleries. Such galleries communicate with the outside with a hole, from which

abundant frass, mixed with the lymph of the tree, comes out. The larvae overwinter in the trunk and they resume their activity in the spring of the successive year and pupate and the adults emerge in July-August.

The larval activity causes a depreciation of the wood quality in the case of low population density or the death of the plants in the case of extreme infestations.

Objective of this work was to verify the effectiveness of products based on entomopathogenic nematodes, one of these active at low temperatures in comparison with a new chemical insecticide proposed by the standard procedure of integrated production.

Materials and Methods

The trial was carried out in northern Italy, in a poplar grove in the province of Reggio-Emilia. It went on from the second part of the winter until the first part of the summer. Late winter and early spring were the periods to better evaluate the potentiality of the cold active nematode. The trees (Canadian poplar) were 8 years old. For each treatment, 16 galleries in 5 or more trees, were chosen. The treatments were disposed in randomised blocks. The cold active nematode was applied, with its control, in winter toward over-wintering larvae and assessed before the temperature rose. All other treatments were targeted toward newly hatched larvae. Products, doses, dates of application and dates of assessment are summarized in table 1.

Table 1.

Product	Active Ingredient	Dose	Date of treatment	Date of assessment
Ildenal	Propoxur + Ciflutrin	2,20 ml/l	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Daskor	Clorpyrifos- methyl + Cipermethryn	2,20 ml/l	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Nema-Bit	<i>H. bacteriophora</i>	13,53 x 10 ⁶ IJ/l	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Larvanem	<i>H. megidis</i>	13,53 x 10 ⁶ IJ/l	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Cold active	<i>S. kraussei</i>	13,53 x 10 ⁶ IJ/l	Feb. 10th	May 11th; Jun. 6th; Jul. 3rd
Naturalis	<i>Beauveria bassiana</i>	800 ml/l	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Naturalis	<i>Beauveria bassiana</i>	400 ml/l	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Water control	Water	10 ml/hole	Feb. 10th	May 11th; Jun. 6th; Jul. 3rd
	Water	3 ml/hole	Jun. 6th	Jul. 3rd, 21st Aug. 11th
Control				Jul. 3rd, 21st Aug. 11th

Results

Results are summarized in Figure 1. for the treatments with the “cold active” nematode and in Figure 2. for all other treatments. In Figure 1 insect mortality was defined according to Abbott’s correction, while in Figur 2 all the treatments were different from the control, but each other effectiveness was not different according statistical χ -squared analysis.

Figure 1.

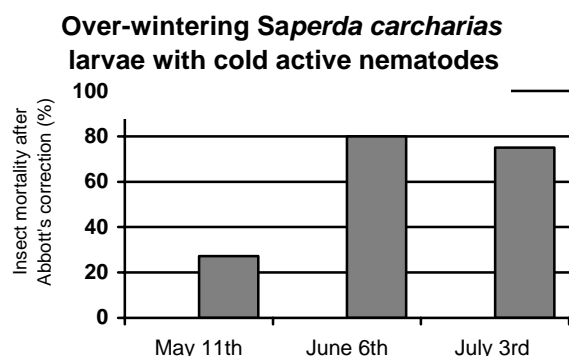
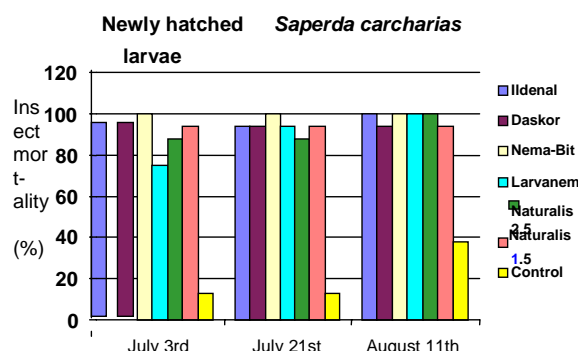


Figure 2.



Discussion

Entomopathogenic nematodes are very effective against *Saperda carcharias* larvae and comparable with chemicals. Particularly, “cold active” *S. kraussei* strain controlled over-wintering larvae, but its effect was well defined in late spring. The newly hatched larvae were 100% killed by Nema-Bit a month after the treatment, while chemicals and *B. bassiana* controlled about 94% of *S. carcharias* larvae in the same period and reached 100% of mortality 65 days after the treatment. Larvanem was not significantly less effective than the others a month after the treatment (75% larval mortality) , but 100% of insect mortality was reached at the trial end. Such a mortality rate (from 12% to 37%) was pointed out in the untreated control (water).

Spray applications are very simple to carry out, and commonly employed also for the chemicals, therefore the biological control by entomopathogenic nematodes is going to be put in poplar guidelines .

18. SUMMARIZING THE CURRENT BASIC TECHNIQUES FOR ISOLATING EPN FROM SOIL – OUTCOMES AND LIMITATIONS

Presented by Bill Hominick, CABI, UK

	Flotation	Baermann funnel	Bioassay
Nematode activity	Passive	Active	Infective
Efficiency	High	Lower	Lowest
Nematode populations recovered	Mixed	Mixed	One
Labour needed	High	High	Lower
Lab cultures established	No	Possible	Yes
Stages recovered	Dauers	Dauers	All
Taxonomic expertise needed	High	High	Moderate
Taxonomic work possible	No	No	Yes
Quantative	Yes	Yes	Yes