



Can entomopathogenic nematodes contribute
to the sustainable management of oil seed
pest insects?

Ralf-Udo Ehlers
E-nema GmbH, Schwentinal, Germany
ehlers@e-nema.de

e-nema GmbH



- **Founded in 1997**
- **Production of biological plant protection (bacteria, yeasts, fungi, nematodes)**
- **Development of production processes technology (liquid culture)**
- **More than 30 bioreactors from 5 - 60.000 ltr.**
- **Capacity currently increased to 700 cubicmeter**

Experience in production and downstreaming



e~nema produces fungi, e.g.:

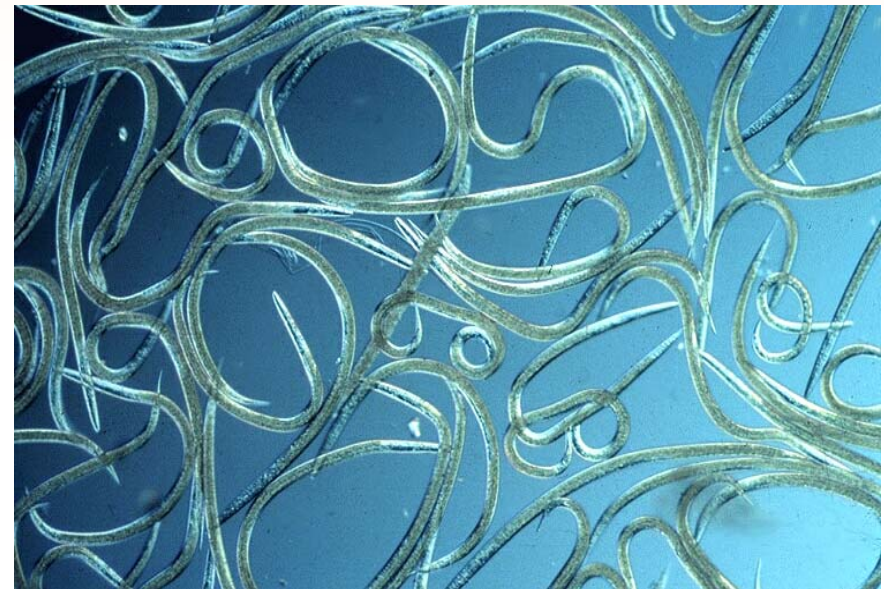
Gliocladium catenulatum,
Ampelomyces quisqualis

e~nema produces bacteria, e.g.:

Bacillus thuringiensis, B. subtilis, Bacillus spp.
Serratia plymuthica, S. entomophila,
Pseudomonas chlororaphis,
P. fluorescens

e~nema produces nematodes, e.g.:

Steinernema feltiae,
S. carpocapsae,
Heterorhabditis bacteriophora



What are entomopathogenic nematodes (EPN) ?

Nematode

Heterorhabditis

Steinernema

Bacterium

Photorhabdus

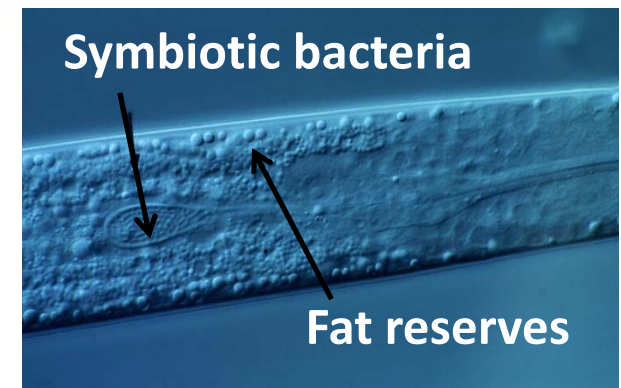
Xenorhabdus

Species: *H. bacteriophora*, *S. feltiae*, *S. carpocapsae*

The Dauer Juvenile



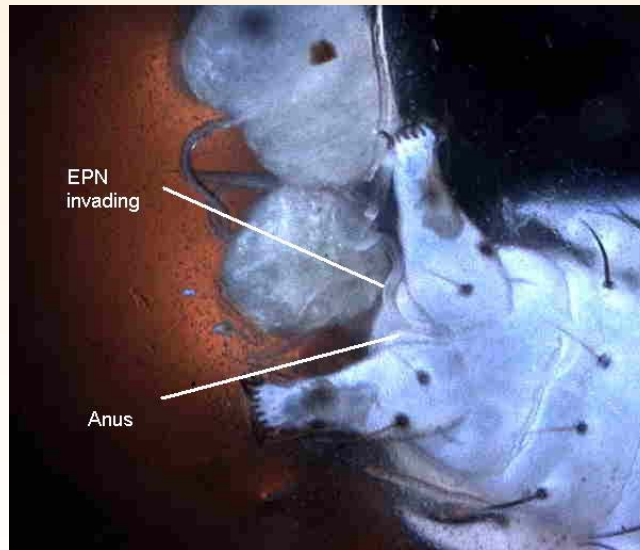
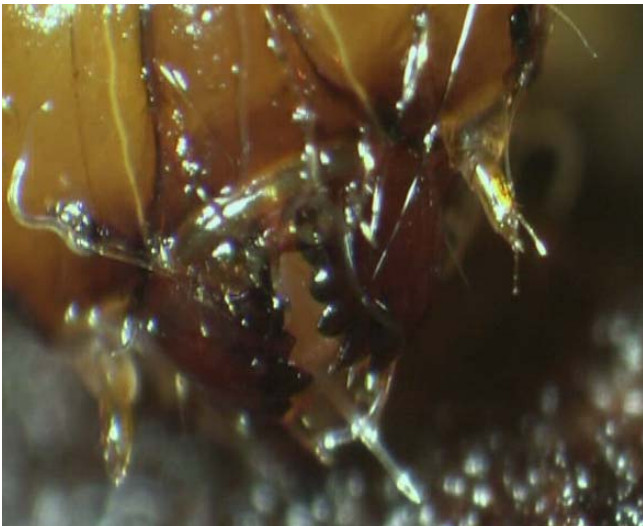
- Third juvenile worms of 0.5 - 0.9 mm
- Dauer juveniles (DJ) = enduring juvenile
- DJ adapted for long-term survival
- High in fat reserves
- Symbiotic bacteria in the intestine (arrow)
- Present in all soil environments
- Free-living in the soil
- Host recognition and host seeking
- Host invasion, enter in haemocoel
- Reproduction only in host insects



Host Invasion via



Mouth

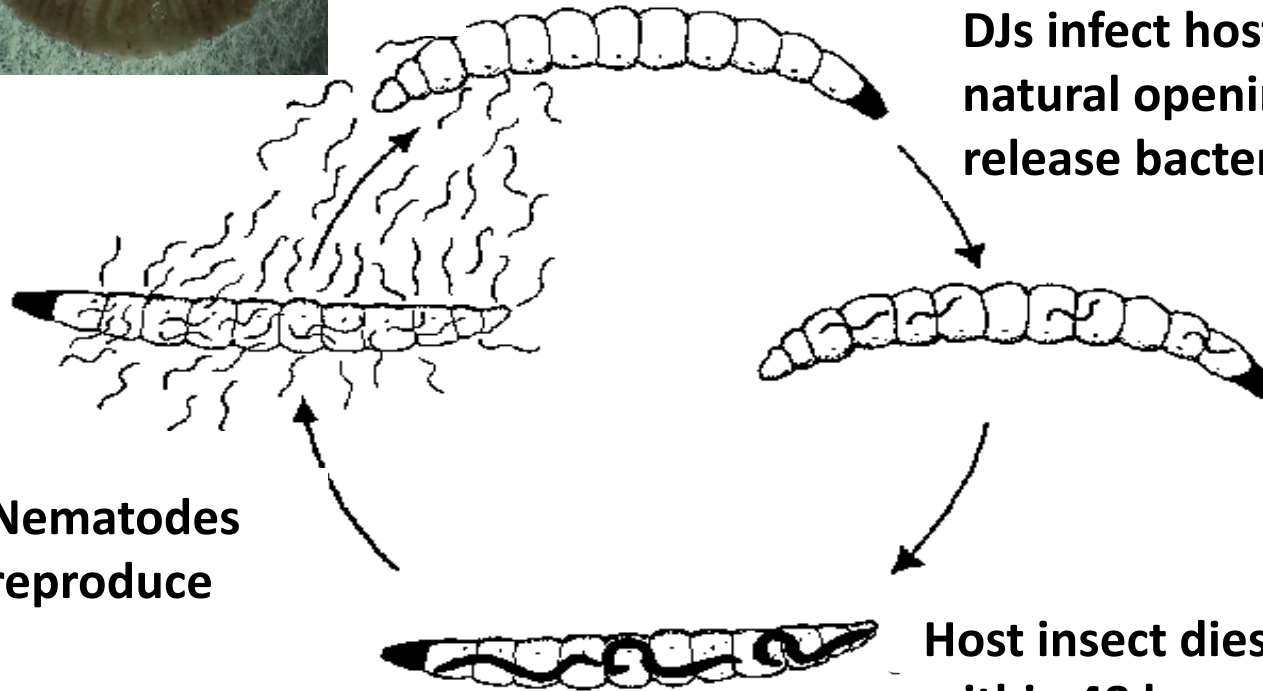


Anus

Spiracles



The Life Cycles



Red larva nematode-infected

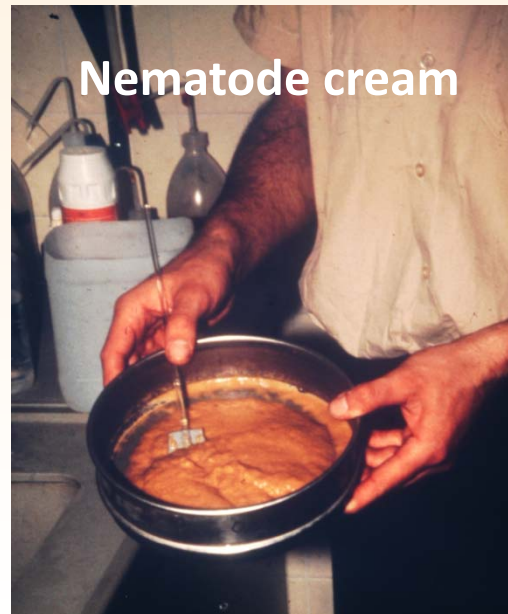


Nematodes recycle in host and infect surviving insect larvae

DJ excellent for product formulation



Dauer juveniles



Nematode cream



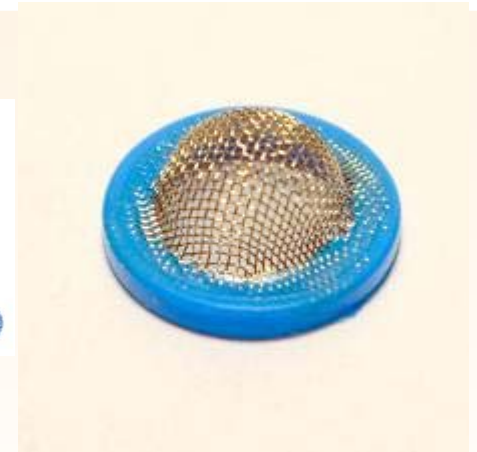
Inert clay carrier

Wash and concentrate DJs by centrifugation
Nematode cream mixed with inert carrier (clay)
Packed in plastic bags
Water content little below 100% relative humidity
Stored at 4°-12°C

Application Technology



- Pressure below 20 bar
- Remove filters
- Diameter of nozzles minimum 0,8 mm
- Use conventional nozzle systems
- Avoid nozzles with high shear forces
- Avoid heating of tank mix
- Use cool water



Filters



Soil-dwelling OSR Pest Insects



Order	Pest
Coleoptera	Stem Flea Beetle (<i>Psylliodes chrysocephalus</i>) Pollen Beetle (<i>Meligethes aeneus</i>) Rape Winter Stem Weevil (<i>Ceutorhynchus picitarsis</i>) Cabbage Gall Weevil (<i>Ceutorhynchus pleurostigma</i>) Rape Stem Weevil (<i>Ceutorhynchus napi</i>) Cabbage Stem Weevil (<i>Ceutorhynchus pallidactylus</i>) Seed Weevil (<i>Ceutorhynchus assimilis</i>)
Cecidomyiidae	Brassica Pod Midge (<i>Dasineura brassicae</i>)
Anthomyiidae	Cabbage Root Fly (<i>Delia radicum</i>)
Lepidoptera	Diamond Back Moth (<i>Plutella xylostella</i>)
Hymenoptera	Cabbage Leaf Sawfly (<i>Athalia rosae</i>)

In **red**, non-susceptible OSR pest species, in **green**, insect without no soil stage

Stem Flea Beetle Larvae
Psylliodes chrysocephalus
Coleoptera: Chrysomelidae



Hokkanen et al. 2006: S.f. 39% (F)

Other species, e.g., *Phyllotreta* spp.

**Li + Wang 1990: S.f. 86-100% (L),
77-94% (F)**

Reddy et al. 2014: S.c. 42 % (F)



Cabbage Root Fly, *Delia radicum*

Diptera, Anthomyiidae



Susurluk + Ehlers 2004: S.f. 85% (L)

Nielsen 2003: S.f. 77% (L), 88% (F)

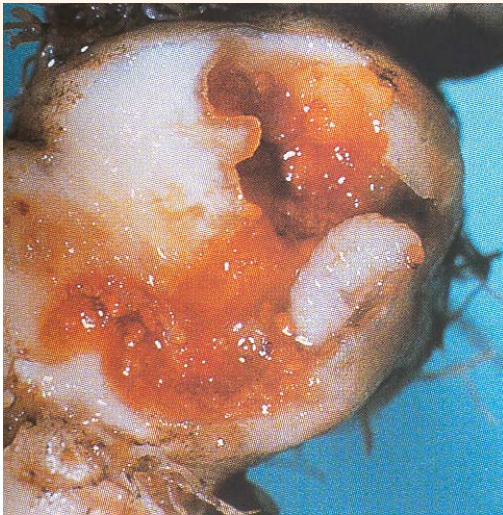


Rape Winter Stem Weevil
Ceutorhynchus picitarsis



No results available

Cabbage Gall Weevil – *Ceutorhynchus pleurostigma*



No results available

Diamondback Moth

Plutella xylostella



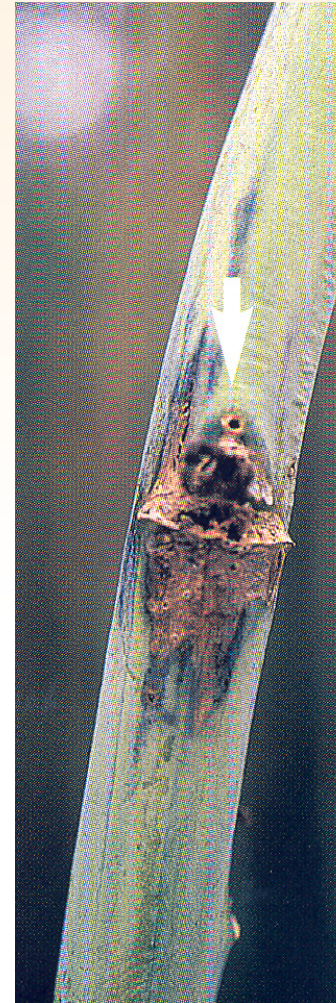
Baur et al. 1995: S.f. 67% (L), S.c. 95% (L)
Schroer & Ehlers 2005: S.c. 80% (L)
Schroer et al. 2005: S.c. 64% (F)
Somvanshi et al. 2006: S.ab. 46% (F)

Rape Stem Weevil

Ceutorhynchus napi



Hokkanen et al. 2006: S.f. 72% (F)



Pollen Beetle

Meligethes aeneus



Nielsen + Philipsen 2005: S. sp. 50-90% (L)

Menzler-Hokannen + Hokkanen 2005: S.f. 94% (F)

Hokkanen et al. 2006: S.f. 60% (F)



Cabbage Stem Weevil *Ceutorhynchus pallidactylus*



Nielsen + Philipsen 2004: S.f. 50% (L)
Hokkanen et al. 2006: S.f. 18% (F)



Seed Weevil *Ceutorhynchus assimilis* Coleoptera: Curculionidae



Philipsen + Nielsen 2003: S.sp. 55-93% (L)

Nielsen + Philipsen 2004: S.f. 22-75% (L)

Hokkanen et al. 2006: S.f. 48% (F)



Brassica Pod Midge, *Dasineura brassicae*

Diptera: Cecidomyiidae



Ehlers (unpublished): S.f. 10% (L)

Nielsen + Philipsen, 2004: S.f. 1% (L)

Hokkanen et al. 2006: S.f. 4 % (F)



Can EPN contribute?



Except for CRF, an effect in the year of application cannot be expected

Area-wide approach with effect on population dynamics over the years

Presence of other antagonists will support the effect

Can EPN contribute?



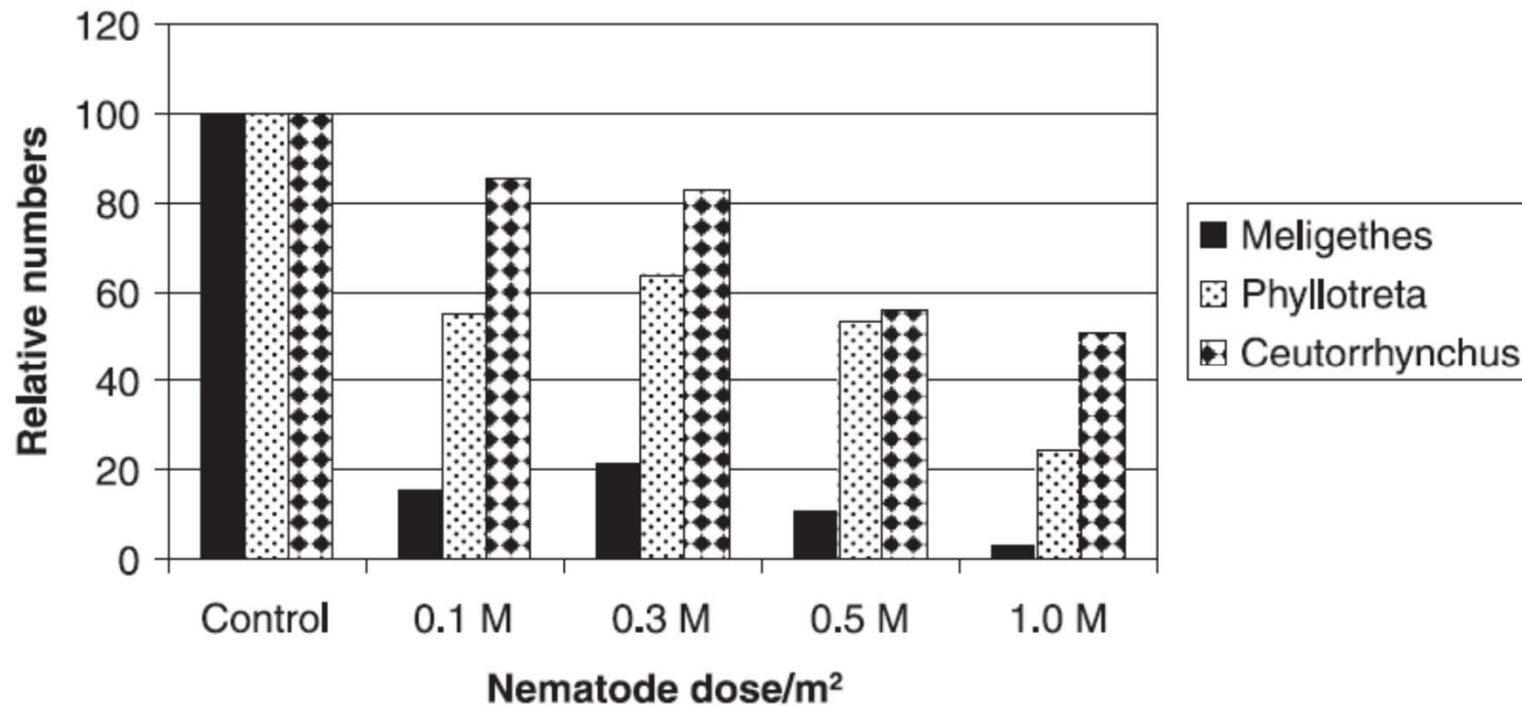
EPN not abundant in arable crops

Susurluk 2005: of 11.000 samples, 22 with S.f. = 0,2%

EPN need to be introduced

An effect on pest populations can only be expected if EPN can be successfully established

Hokkanen et al. 2006: Dose response *S. feltiae*



At 1 billion per ha the cost will be less than 100 €/ha

Successful establishment needs successful propagation in hosts



Nielsen 2000:

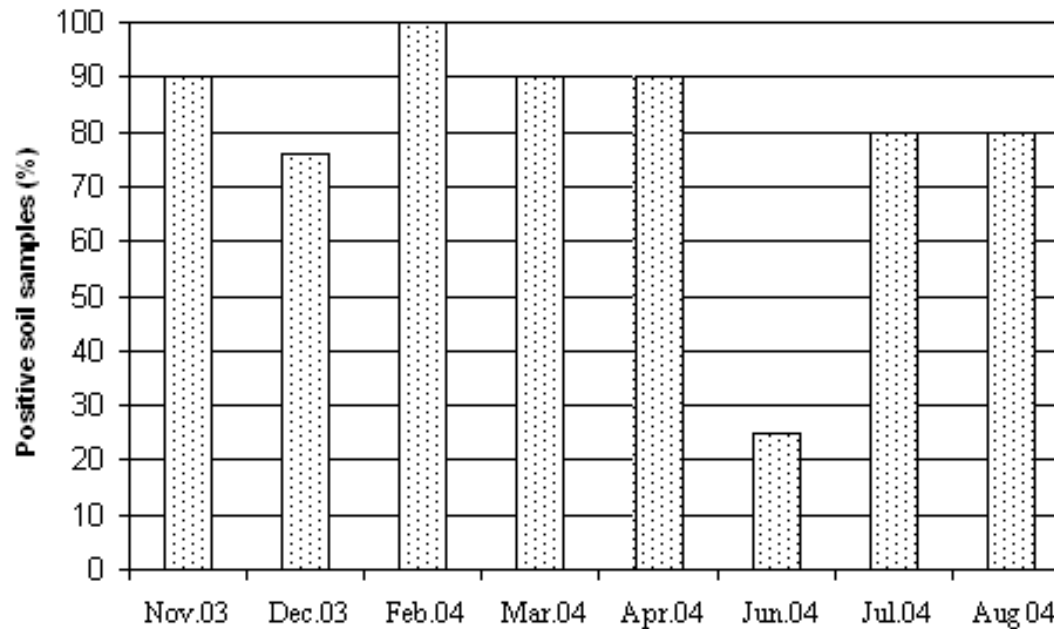
In lab assays, 25 S.f. per *Delia radicum* provided 80% control
Between 700 and 9.500 DJs harvested from infected *D. radicum*

C. assimilis: 1.250-1.400 DJs of S.f. per larva

C. pallidactylus: > 100 DJs S.f. per larva

Meligethes sp.: 850 – 1.260 DJs S.f. per larva

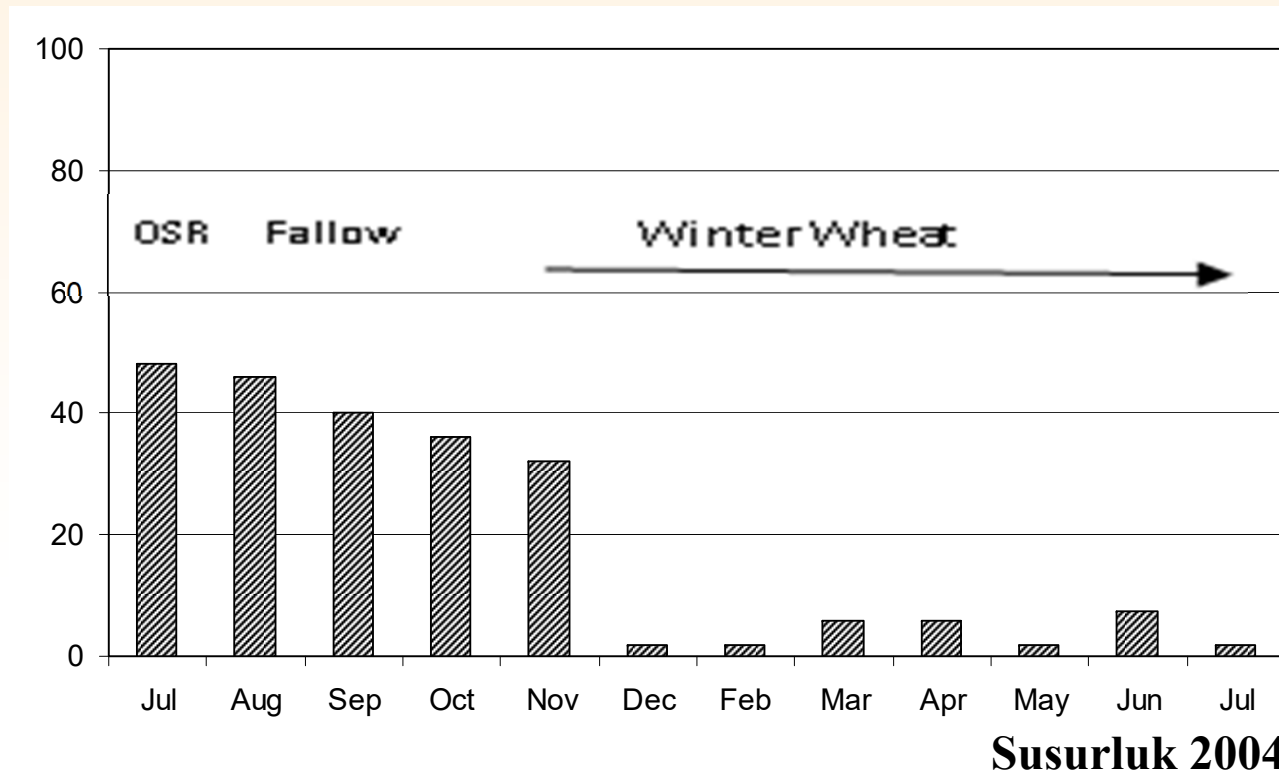
Establishment and persistence *S. feltiae* in OSR



Susurluk 2004

Steinernema feltiae sprayed on November 11, 2003. Percentage positive soil samples (n=50 per 250 m² on each date) from oil seed rape field infected with 3rd instar larvae of the cabbage root fly *Delia radicum* (mean of n=50 plants of 2,26 ± 0,8 larvae per plant). Sampling not continued after harvest. In May spray against *Meligethes aeneus*.

Persistence of *H. bacteriophora* applied in OSR



Winter wheat a poor provider of EPN hosts, but better than pasture or potato

Can EPN contribute?



Presence of host insects and good agricultural practice essential:

- no insecticide spraying
- conservation tillage after OSR
- rotation with „catch crops“
- improved agroecosystem management (e.g. OSR neighbouring OSR in rotation)

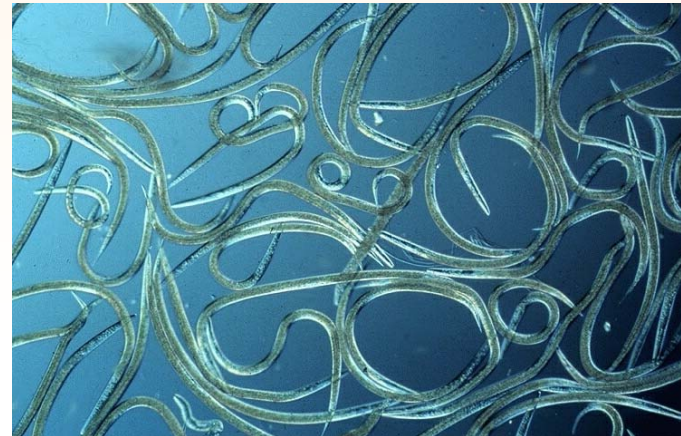
Questions:

- can better adapted EPN strains be identified (virulence, persistence, etc) ?
- can necessary traits be improved by genetic selection?
- when to apply, how many, against which pest, density depended application?
- how much water is needed, effect on OSR development?
- can cover crops in following crops enhance persistence (clover) in rotation?
- can the effect be measured on neighbouring OSR fields in following year?
- can farmers be organised for a area-wide approach?
- will this approach be cost-effective?



Thank's!

Ralf-Udo Ehlers
ehlers@e-nema.de
www.e-nema.de



Application of Nematodes in OSR



Appl. Dates and times (hour)	Nematodes species	Plants	Plant heights (cm)	Soil/air temp.(°C)	IJs/cm ²	IJs/cm ² in control (without plant)
23.10.01-5:30	<i>S. feltiae</i>	Clover (<i>Trifolium pratense</i>)	19-25	6,5 / 8	29,8±12,0	48,3±16,0
23.10.01-5:30	<i>H. bacteriophora</i>	Oil-seed rape (<i>Brassica napus</i>)	9-14	6,5 / 8	38,5±7,5	49,5±12,0
11.06.02-14:00	<i>H. bacteriophora</i>	Bean (<i>Vicia faba</i>)	58-70	15,5 / 22	45,7±6,8	52,1±18,0
19.06.03-16:30	<i>H. bacteriophora</i>	Wheat (<i>Triticum durum</i>)	78-90	15,5 / 20	26,2±9,0	53,8±13,5
19.06.03-16:44	<i>H. bacteriophora</i>	Pasture	5-7	15,5 / 20	41,8±11,2	54±11,9
19.06.03-17:12	<i>H. bacteriophora</i>	Oil-seed rape (<i>Brassica napus</i>)	97-110	15,5 / 20	2,3±1,9	49,7±13,9
08.07.03-15:00	<i>H. bacteriophora</i>	Lupine (<i>Lupinus angustifolius</i>)	90-110	17,5 / 25	3,0±1,8	48,5±9,7
08.07.03-15:17	<i>H. bacteriophora</i>	Potato (<i>Solanum tuberosum</i>)	65-70	17,5 / 25	42,6±5,3	54,8±18,9
08.07.03-15:42	<i>H. bacteriophora</i>	Pea (<i>Pisum sativum</i>)	77-91	17,5 / 25	23,6±6,7	53,3±8,0
11.11.03-15:00	<i>S. feltiae</i>	Oil-seed rape (<i>Brassica napus</i>)	14-17	5 / 7	41,1±13,6	52,9±15,7