

# Safety of nematodes:

## Nematodes - Bacteria

- *Steinernema* spp.
- *Heterorhabditis* spp.



# Safety of entomopathogenic nematodes

- EPN used in plant protection since 1939 in USA and 1986 in Europe in ornamentals, tree nurseries, mushrooms, turf and home gardens without any known detrimental effects
- EPN pose much less threat to the environment (including personell in production and application) than chemical soil insecticides
- In most countries indigenous (exotic) species are exempted from registrations (macro-organisms)

# Use of exotic species with reported detrimental effects

- *S. scapterisci* in Florida and Georgia
- *S. glaseri* in NL and POR
- *S. riobrave* in many parts of the USA
- *S. carpocapsae* in Germany (1 natural record)
- *H. bacteriophora* in almost all parts in DE
- Commercial products from USA and Europe when used in Asia and LA

# OECD/COST Workshop Malente, Germany, 1995

## Possible risks to human health:

- No risk to general public, remote to production personnel for allergic reaction and toxicity when contaminants are present

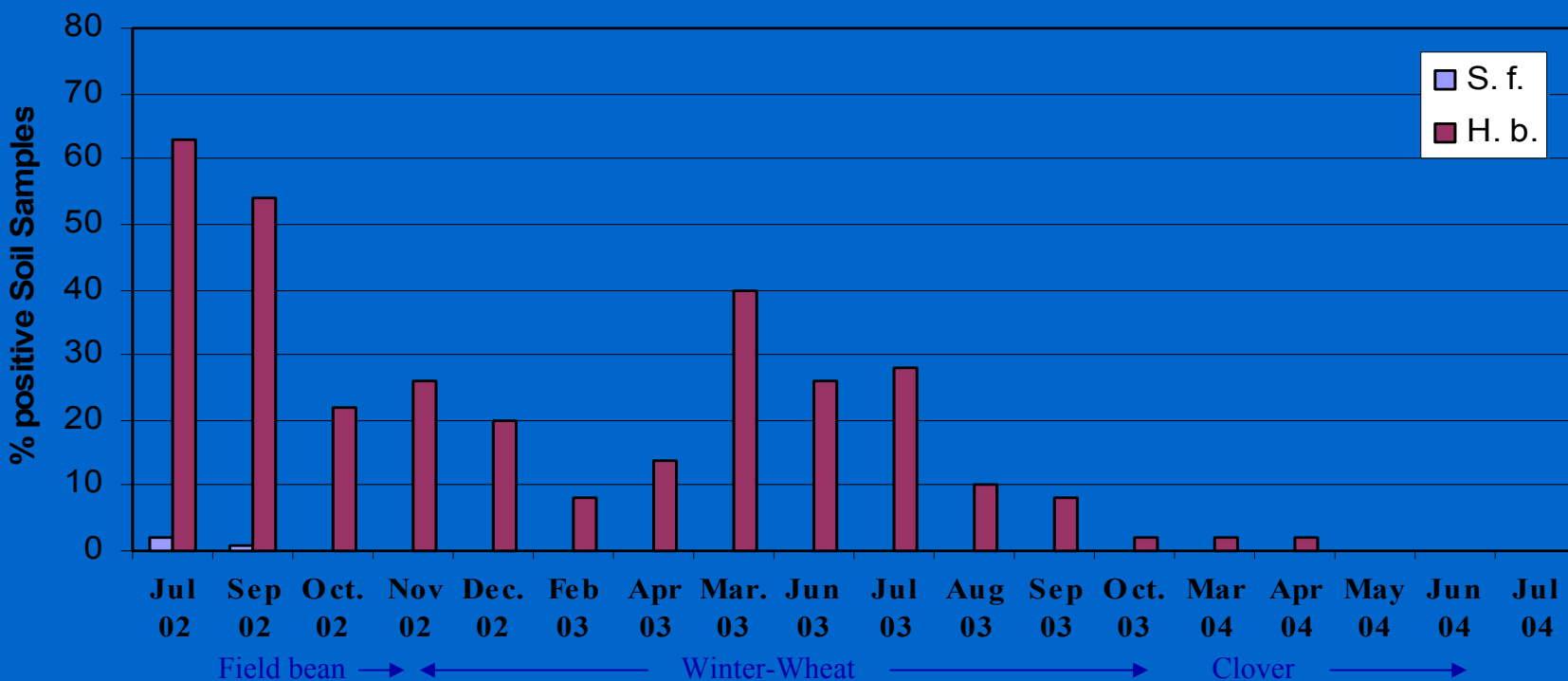
<ul style="list-style-type: none"> <li>• <b>RISK</b> ( 0 = no, 1 = remote, 2 = slight, 3 = moderate, 4 = high, 5 = very high risk)</li> </ul>	<ul style="list-style-type: none"> <li>• <b>RATING</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>NTOs in the soil</b></li> <li>• <b>NTOs in cryptic environments</b></li> <li>• <b>NTOs on foilage</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>3</b></li> <li>• <b>2</b></li> <li>• <b>1</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Vertebrates Warm blooded</b></li> <li>• <b>Vertebrates Cold blooded</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>0</b></li> <li>• <b>2</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Invertebr. Predators</b></li> <li>• <b>Invertebr. Parasitoids</b></li> <li>• <b>Invertebr. Pollinators</b></li> <li>• <b>rare or endangered species</b></li> <li>• <b>Earthworms</b></li> <li>• <b>Others</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>2</b></li> <li>• <b>2</b></li> <li>• <b>1</b></li> <li>• <b>1</b></li> <li>• <b>1</b></li> <li>• <b>0</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Plants</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>0</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Competitive displacement of native EPN in treated fields</b></li> <li>• <b>Temporary</b></li> <li>• <b>Permanent</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>2</b></li> <li>• <b>0</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Changes in ecosystem balance</b></li> <li>• <b>Local temporary suppression of NTOs</b></li> <li>• <b>Permanent suppression of NTOs</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>2</b></li> <li>• <b>0</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Contamination of ground water</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>1</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Gene transfer from exotic symbiont to other soil bacteria</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>2</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>Biological “pollution” with new EPN species</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>4</b></li> </ul>
<ul style="list-style-type: none"> <li>• <b>General biodiversity</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>1</b></li> </ul>

# What makes EPN exceptionally safe antagonists?

- Limited host range in nature
- Natural epizootics are rare
- *Steinernema* a relatively balanced association with hosts
- *Heterorhabditis* epizootics more often
- Insects defence mechanisms cover EPN and bacteria, many unspecific cosmopolitan enemies
- Limited potential for dispersal
- Non-pathogenic for vertebrates

# Alper Susurluk: Limited potential to establish: *H. bacteriophora* in *Vicia faba*

Persistence of EPNs from June-2002 to July-2004

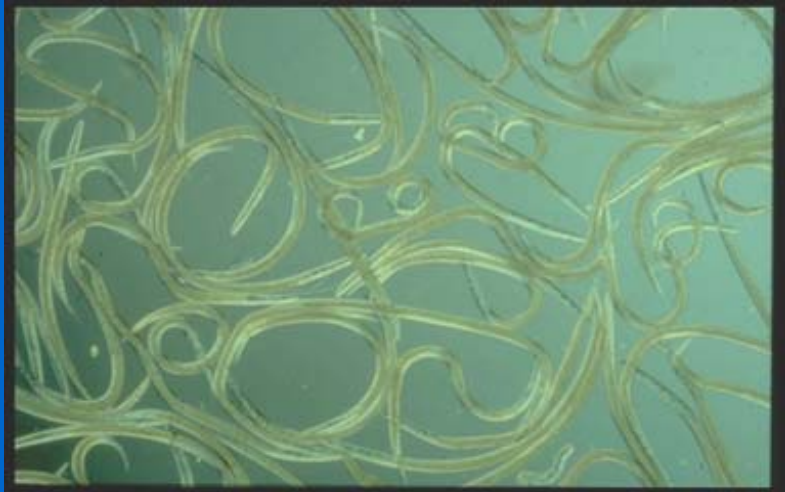


Tilling after field bean in October 2002, not after Winter Wheat

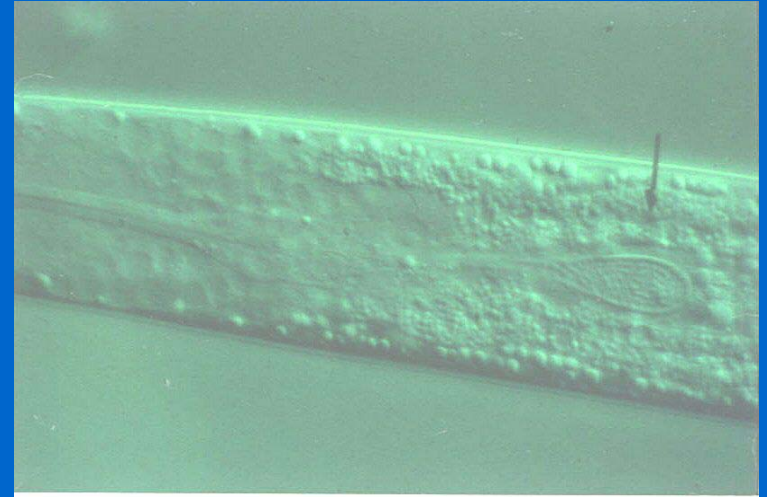
# Possible risks to humans

- No toxicity, no pathogenicity/infectivity reported
- Only detrimental effect reported is a remote allergenic reaction of production personell
- Biosys: 75 people worked with 5 species for over 12 years; 1 case of dermal reaction to nematode solutions
- CSIRO Australia worked with over 10 EPN species; one allergic reaction with *S. carpocapsae* in the eye, person continued working for the past 12 years
- At CAU 4 people exposed to EPN species from all over the world for 10 years without any allergenic response
- At Koppert + e-nema 3 species, BeckerUnderwood 8 species in production, no detrimental effects reported

# Significance of the associated bacteria



EPN dauer juveniles (0,8 mm)



Symbiotic bacteria (200-2000 cells) inside DL

**Users and consumers of nematode-based products hardly have a chance to get in contact with the symbiotic bacteria**

**In infected insects, gut bacteria take over after 1 week.**

**Symbiotic bacteria never isolated from soil samples.**

# Effect to Non-Target Organisms (NTO)

- Investigations could only identify **remote, temporary** effects on NTO, including predators, in soil and cryptic environments and on parasitoids of target organisms
- Reports on the effect on vertebrates (tadpoles) were not reproducible under natural environment conditions
- Competitive displacement of native EPN species possible (temporary)

# Effect on NTO

## Bathon *et al.*, 1996

- Release of *H. megidis* and *S. feltiae* on 100m<sup>2</sup> plots in beech forest, edge of pine forest, old orchard, and wheat field
- 3 year study, 1 Ph.D., 2 students
- 400.000 specimens were collected
- NTO effect was temporary, spatially restricted and effected only part of the affected populations

# References

- **OECD-COST Workshop Malente, Germany (1995) published in Biocontrol Science and Technology 6, 1996 (190 pp.)**
- **Barbercheck + Millar (2000): Environmental impacts of EPN used fro biological control in soil. In: Follett and Duan: Nontarget Effects of Biocogical Control. Kluwer Acedemic Publ.**
- **Akhurst + Smith (2002) Regulation + Safety of EPN. In. Gaugler: Entomopathogenic Nematology, CABI Bioscience**
- **Ehlers, R.-U. (2003): Biocontrol Nematodes. In: Hokkanen + Hajek: Environmental Impacts of Microbial Insecticides. Kluwer Acedemic Publ.**

# Reports of *Photorhabdus luminescens* infecting humans

- Between 1977 and 1989 six isolates in USA
- Until 1999 four isolations from Australia
- Infections associated with skin lesions
- Some patients reported spider bites
- Infections often in association with other human pathogens (e.g. *Staphylococcus aureus*)
- Bactericemia reported from 4 of the 10 cases
- All isolates responded to treatments with antibiotics
- No case of infection with symbiotic *Photorhabdus* or *Xenorhabdus* reported

# Reports of *Photorhabdus luminescens* infecting humans

- US strains recently described as *P. asymbiotica*
- Australian strains are more closely related to *P. asymbiotica* than to the nematode-symbiotic strains
- 16S rDNA sequencing suggest that Australian strains should be classified as new species (Akhurst, pers. comm.)
- Molecular phylogeny indicates that symbiotic and asymbiotic species are much less related than *Bacillus thuringiensis* with the clinical *B. anthracis*

# Risk assessment for *Photorhabdus luminescens* necessary?

- The risk for infection with symbiotic bacteria can be considered to be low considering the experience from production plants (3 in USA, 1 AUS, 8 EU)
- An analysis of *P.I.* genes has shown that many pathogenicity genes described from human pathogens are also found in *P.I.* (Ffrench-Constant *et al.* 2000)
- Growth under continuous culture conditions has shown that *P.I.* (*H. indica*) can adapt to 39°C and only at 40°C growth was inhibited (Ehlers *et al.* 2000)
- *P.I.* from *H. indica* used in transgenic maize (Dow, US)
- Safety tests should be conducted before a new species is used commercially in order to protect personnel