12th Wokshop of the Arthropd Mass Rearing and Quality Control working group at the IOBC

Industry Perspectives and Concerns

Mass production and nematode quality - contradicting targets ?

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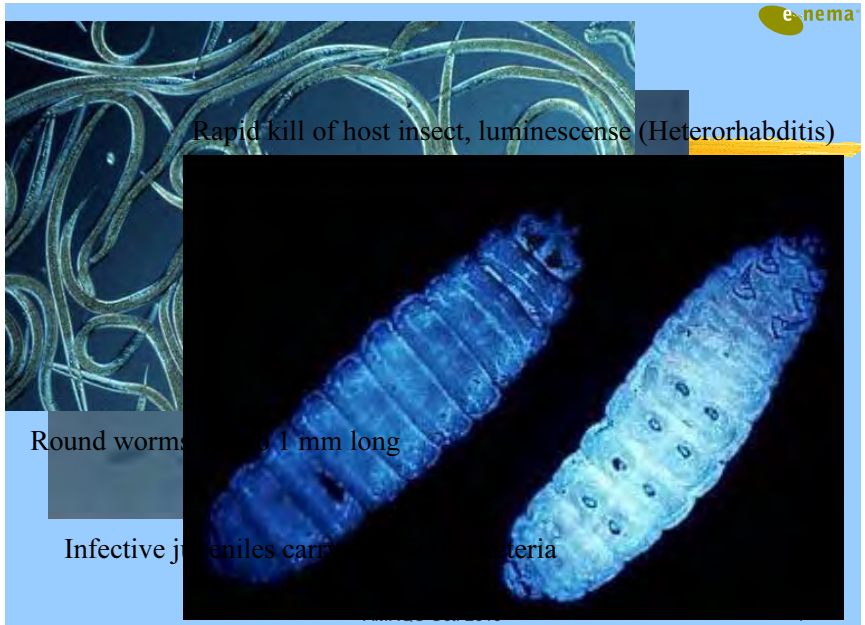
Mass Production

- Mass production is generally believed to result in poor quality
  - Food
  - Beverages
  - Cars
- Expensive products must be good –> mass production is done to reduce the costs -> the products must be less good
- Any statement on poor performance of mass produced beneficials will be a comfirmation of these customer prejudices

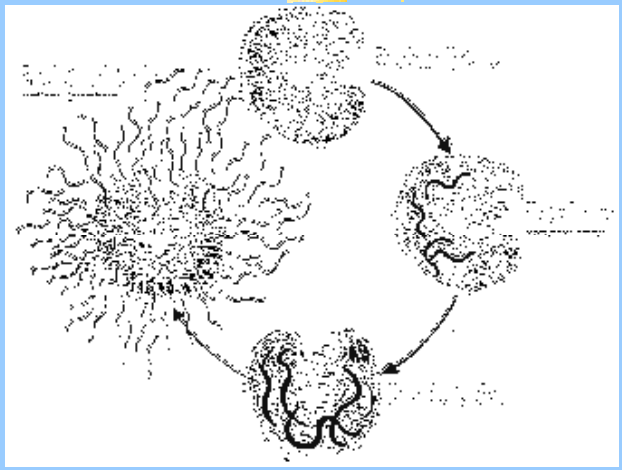
Are these prejudices justified ?

What determines nematode performance in the field ?

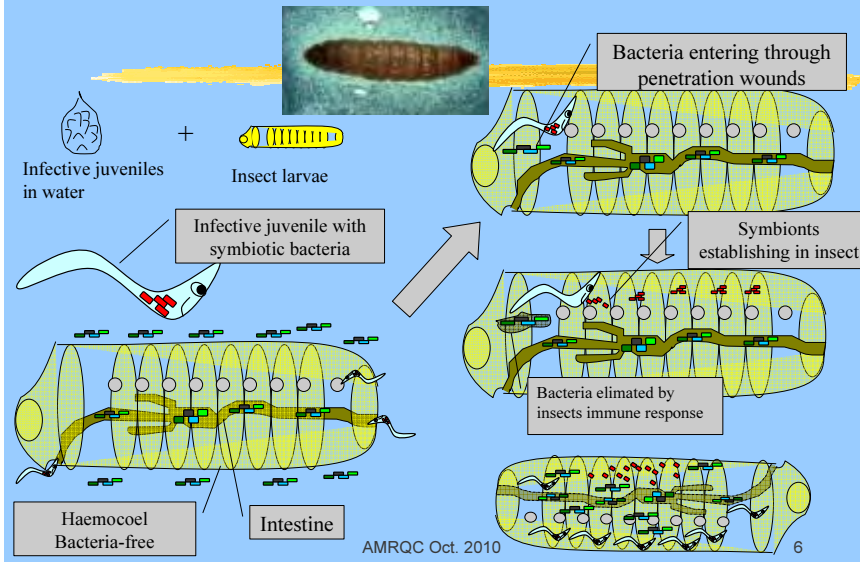
- Retention of symbiotic bacteria
- Movement
- Host finding
- persistence
- ...



Life cycle of entomopathogenic nematodes



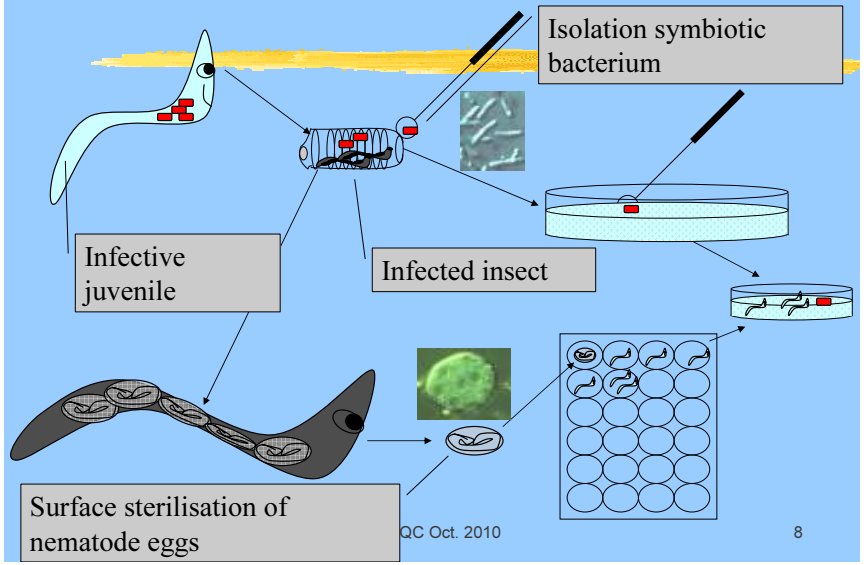
Production systems *in vivo*



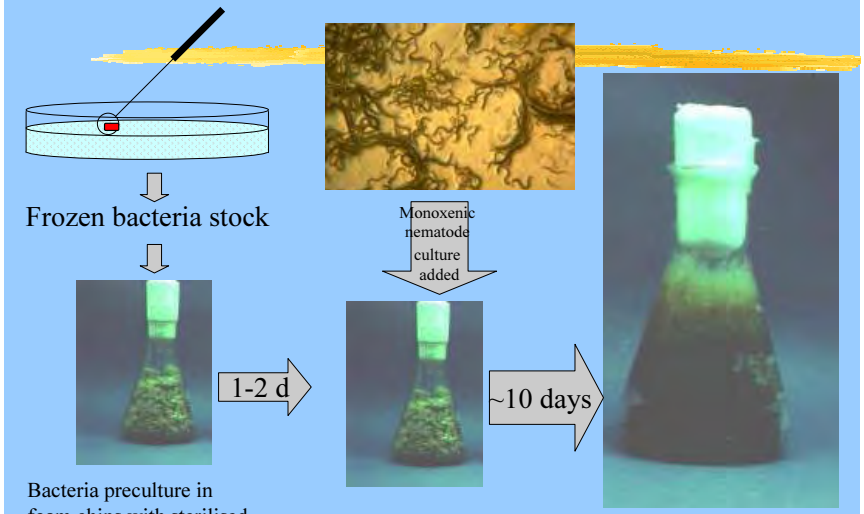
Production systems *in vitro*

- Monoxenic cultures must be established
- Onset of nematode development ("recovery") triggered by bacteria preculture
- Bacteria preculture from a frozen stock
- Contaminants excluded by sterile techniques, not by insect's immune system
- Monoxenic culture maintained until end of the process

How to obtain monoxenic cultures

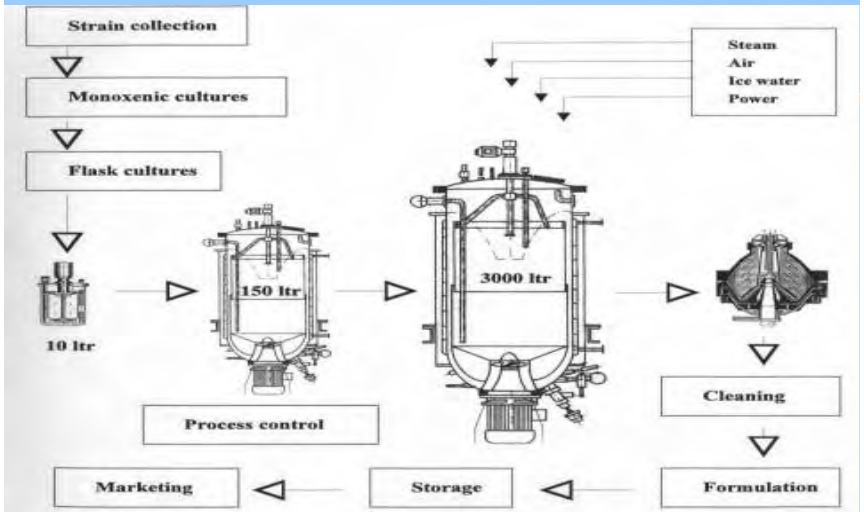


Production process *in vitro* solid state



Bacteria preculture in foam chips with sterilised growing medium

Liquid Culture Production



Mass Production in Bioreactores

E-Nema GmbH, Raisdorf

Quality assurance in different production systems

	In vivo	In vitro solid	In vitro liquid
Symbiotic bacteria	From nematode, variable	Frozen stock, uniform	Frozen stock, uniform
Contaminants	Only initially controlled by insect's immune system	Sterile technique	Sterile technique
Production units per 5 trillion (100 ha)	~ 10,000,000	~10,000	~ 1

Impact of contamination

- *S. carpocapsae* gives poor or no yield if grown on non-symbiotic bacteria (Ehlers & Stößel, 1989
- Likewise Heterorhabditis has low yield on non-symbiotic bacteria
- => Quality is self-assured in most *Steinernema* and *Heterorhabditis* species
  - Low reproduction if symbiotic bacteria is not present
  - Only symbotic bacteria are retained and transmitted
- Exceptions:
  - *Steinernema scapterisci*, *Steinernema glaseri*
  - *Heterorhabditis* species may grow on related symbionts while not retaining these bacteria (Han & Ehlers, 2001)
  - *Phasmarhabditis hermaphrodita* (slug nematode) with no fixed symbiotic association

Exception Phasmarhabditis hermaphrodita

- Grows on a range of different bacteria species
- Infectivity dependent on the bacteria-nematode combination
- Careful quality control during production required

Advantages of liquid culture for quality assurance

- Well defined stock cultures of symbiotic bacteria are used for every production process
- Assured sterility for complete process time
- Online measurement and control of temperature, O_2, pH, CO_2
- Deviations from standard will alert producer
- Quality checks are done from the complete uniform production unit

  (whereas only subsamples can be taken from the multiple units in other systems)

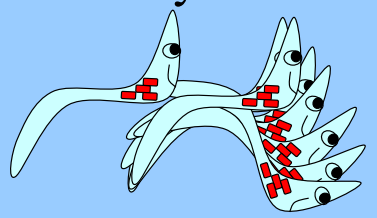
Disadvantage of liquid culture

- Limited to water soluble media ingredients (no saturated fatty acids)
- No sex for *Heterorhabditis bacteriophora*
- Still meiosis and crossing-over but multiple inbred lines in population
- Mitigation by regular reproduction in insects or on solid medium

Conclusion

- Comparing the currently employed production systems,
  liquid culture systems are suited best to assure a reproducible product quality

Thank you !



EPN products adapted to the climatic conditions

Control of Citrus Root Weevil (*Diaprepes abbreviatus*) with *S. riobrave* or *H. bacteriophora* or *H. indica*

Control of mole crickets (*Scapteriscus* spp.) with *Steinernema scapterisci* in Florida, USA

Why continue strain discovery ?

- Screening of EPN potential to control economically important pests
- Markets can only be conquered with effective EPN products, adapted to the climatic conditions
- Screening for potential best adapted species/strains
- One product is easier to produce and market than many niche market products
- Search for applications in which alternative control measures fail or need supplementation

Production

There is no alternative to liquid culture production

Producers Liquid Culture

- **Becker-Underwood, United Kingdom**
- **E-Nema GmbH, Germany**
- **Koppert, The Netherlands**
- **Capacity >20.000 ha**

Production Costs - Retail Price (100 Mio.)

In vivo	Liquid culture
1.000 G.m.	**350 - 900 ml**
10,00 Euro G.m.	0,50 Euro medium
80,00 Euro	30,00 Euro
Capacity?	Capacity EU $1,2x10^{14}$

Marginal differences in activity do not justify the higher costs

biosys: Did it fail because they were unable to compete with chemicals?

S. carpocapae **was not well adapted to relevant targets**

An ill approach: Marketing a bio-control product through chemical companies

Market potential overestimated - contract with ADM oversized

Future EPN company

- "Small-scaled cottage industry has emerged in US and EU" (Georgis, 2002)
- Companies producing in solid media struggle or have disappeared
- Companies producing in vivo can only serve the home gardening market
- Future for large scale providers which can furnish needs of local suppliers of BCAs
- EU providers capacity (now 50 m^3) will further increase
- The furtue EPN company owns its production plants, is flexible in capacity and strain, is near to market

Formulation

TASK: Increase shelf life, improve application and performance

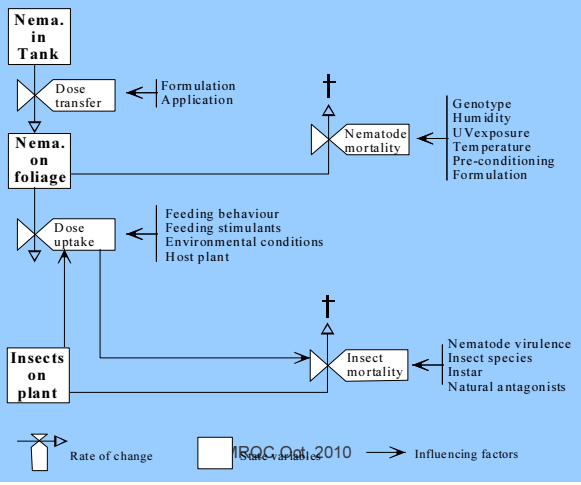
Product formulation with controlled environment to induce quiescence

Additives to prevent sedimentation in tanks, improve distribution in irrigation systems and sprayers (e.g. NEMATOP AD.)

Additives to prolong survival on foliage through biopolymers (e.g. NEMASYS T)

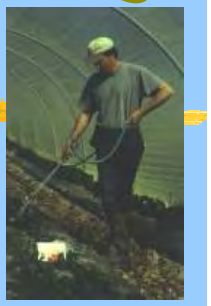
There is a market for EPN foliar application

Modelling foliage application



Application or Establishment

- Spraying
- Plant to plant application with hose and spear
- dipping roots into EPN suspensions with stickers



Characterize ecological potential

What makes EPN succeed?

Behaviour in soil or on foliage

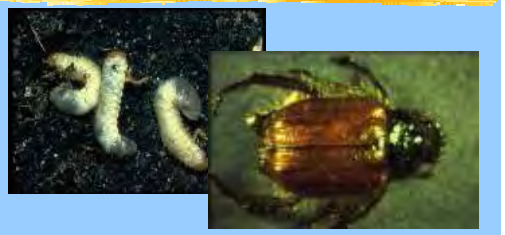
Pest population density + EPN efficacy

Sustainable effects

Alternative hosts

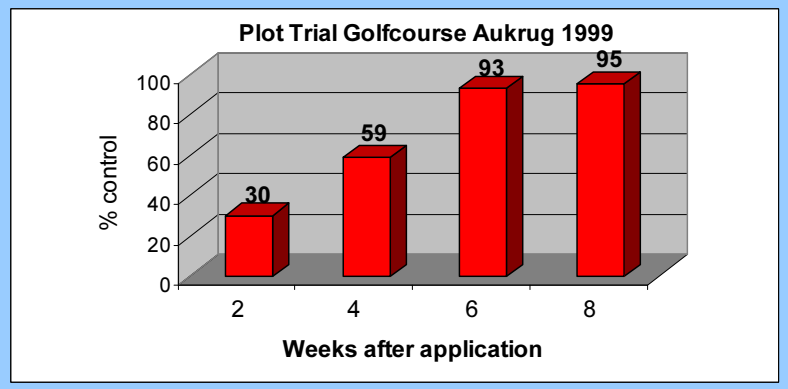
Recycling

nema-green®



- *Heterorhabditis bacteriophora*, 0.5 Mio / m^2
- For control of Garden Chafer (*Phyllopertha horticola*) and related grub species (*Hoplia philanthus, Aphodius* sp.*)*.
- In turf (sport fields, golf courses and home gardens).

Test results



Other fields for EPN use

Soil: Grubs like June Beetle (*Amphimallon solstitialis),* Cockchafer *(Melolontha melolontha*), *Diabrotica* sp.

Urban Pest Control: Cockroaches (*Blattela germanica*)

Foliage application: Thrips in soil and on foliage, applications in which insecticides fail (e.g.: *Plutella xylostella*)

Sustainable agriculture: Reduce populations of fruit flies (Med fly, Cherry Fly) or curculionidae (sugarbeet weevil)

Organic farming: EPNs not yet used

Producers willing to give support

Sciarids

Foto di Dr. D.H. Gouge e G.O. Poinar

- **What makes them succeed in mushrooms?**
- **What can we do to increase impact on phorids?**

Registration

- OECD/EPPO working groups want to implement registration for macro-organisms (incl. EPN)
- Major advantage of nematodes: Products have immediate access to market
- There is no need for further burocratic hurdles which only increase costs, but cannot provide more safety

Marketing

- Biocontrol is a 200 Mio. Euro market in EU
- BCAs need adapted marketing strategies
- EPN suffer from market structure - no targets in greenhouse
- EPN at the step into outdoor markets
- New products will be developed and product costs will be adapted
- EPN will benefit from increasing number of highly effective BCAs

Conclusion

- EPN sales increase every year
- New growers generation demands high quality + cost effective EPN products
- They are open for a change to sustainable control systems in agri- + horticulture
- Without input from R&D and support from extension services progress will be slow

Symbiotic association with insect-pathogenic bacteria