Always thinking ahead BECKER UNDERWOOD

Quality assured mass production of entomopathogenic nematodes

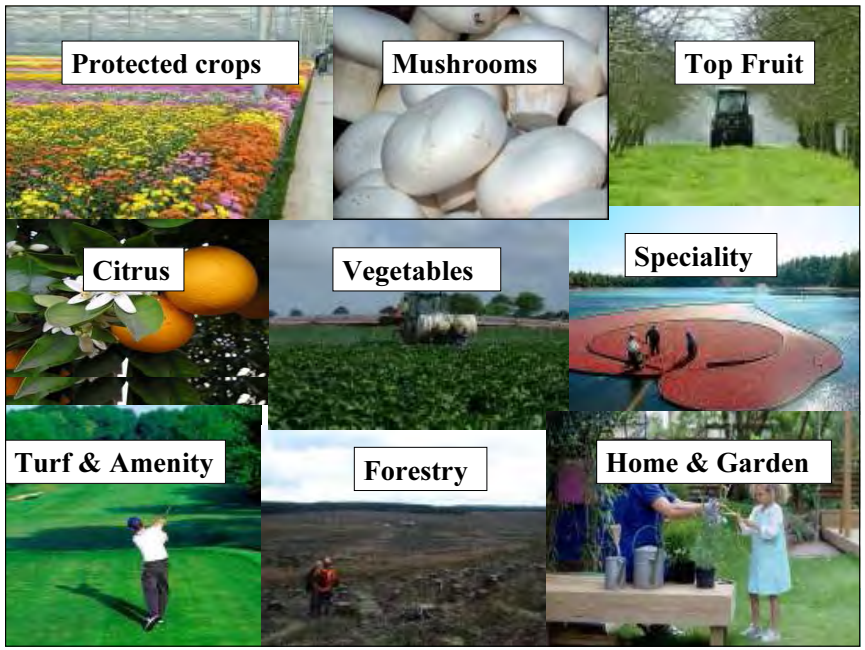
Brown A.P., Godliman J. and Pearce J.D.

Introduction

- Introduction to Becker Underwood.
- Overview of the range of beneficial nematode products available from Becker Underwood.
- History and evolution of mass production of entomopathogenic nematodes over the last 20 years.
- Problems specific to mass production of a multicelluar/unicelluar symbiotic complex.
- What the next 20 years holds for mass production of beneficial nematodes.

BioControl Products

Nematodes



Production facility

Becker Underwood is the worlds largest nematode manufacturer .

- Economies of scale:
  - Total capacity approx 90,000 litres
- Flexibility:
  - Ability to produce a range of nematodes at different volumes.
  - Range of fermenters from 10 litres to 25,000 litres
- Confidence in product quality:
  - ISO9001 Certified

History of nematode production

In vivo *production*

- Readily available insects e.g. *Galleria mellonella* infected with nematodes.
- Left on a White trap and emerging IJ collected.

In vitro *production - sponge*

- Nematodes grown in artificial media.
- Sponge cubes improves nematode survival over solid state media due to:
  - Improved air movement
  - More even media/nutrient distribution.

In vitro *production - sponge*

- Some companies still use this technique.
- Advantages:
  - Higher yields and cheaper than *in vivo.*
- Disadvantages:
  - Relative low yields and high costs.
  - Difficulty with application (sponge in bucket of water)

In vitro *production - liquid flasks*

- Simplest form of liquid production, some companies use this method.

- Advantages:
  - Higher yields and cheaper than sponge.

- Disadvantages:
  - Level of knowledge required.
  - More difficult to formulate than sponge.
  - Small scale production.

In vitro *production - liquid fermenters*

In vitro *production - liquid fermenters*

• Stainless steel fermenters can be used for large volume liquid production. Large companies use this.

• Advantages:
  • Higher yields and cheaper than liquid flasks.

• Disadvantages:
  • High setup cost.
  • Infrastructure required.
  • Level of knowledge required to design.
  • Highly skilled staff required to operate.

In vitro *production - large liquid fermenters*

In vitro *production - large liquid fermenters*

• Stainless steel fermenters can be used for large volume liquid production. Large companies use this.

• Advantages:
  • Economies of scale, larger fermenters produce cheaper units.

• Disadvantages:
  • Very high setup cost.
  • Infrastructure required.
  • Level of knowledge required to design.
  • Highly skilled staff required to operate.
  • Production of EPN at this large scale?

In vitro *production - large liquid fermenters*

Challenges with mass production of beneficial nematodes

Overview of other potential problems areas

As well as production there are other issues with mass production of nematodes and supplying the customer:

- Forecasting sales
- Product shelf life
- Formulations
- Quality control through storage
- Quality control of stock nematodes (inbreeding)

- Global shipping
- Customer support

Forecasting sales

Limited production capacity means ferementers have to be planned to fit in with different product sales periods:

- 15 Nemasys® branded products
- 54 different countries
- 5 different continents

Some products sell year round.
Some products have distinct seasons.
Unexpected sales in expanding markets needs flexibility.

Product shelf life

All macro-biological manufacturers have this problem.

Nematodes are living organisms.

Have finite fat energy resource before they have to infect a host insect.

Shelf life can be improved by:
- Storage conditions
- Lower product contamination during production
- Formulation

Becker Underwood formulation specifically designed to cause desiccation so reducing IJ movement in storage.

Formulation

Formulations have a big effect on product storage, shipping, cost and final use.

Formulation is needed to give product structure.

However there are trade offs - increased formulation concentration results in bigger product.

- More difficult to store
- More expensive to ship
- More difficult to use
- Not acceptable in some markets – spray deposits

Of the products in the market, Becker Underwoods have the lowest percent carrier material = 10%.

Quality control through storage

Living organisms will vary in condition over time.

Have to monitor the condition of products in storage to ensure high quality viable products are sent to customers.

QC assessments are carried out during production, after production, after formulation and regularly through storage.

Quality control through fermentation

- Samples taken at intervals throughout fermentation.
- Assessed for nematode numbers and bacterial contamination.
- Data logging on fermentation parameters air-flow, dissolved oxygen, temperature, pressure.

Quality control of stock nematodes

Culture maintenance is important to ensure:

- Production batches are started with high quality populations.
- Prevention of inbreeding.
- Loss of traits due to *in vitro* production

Quality control of stock nematodes

Culture maintenance to ensure genetic diversity.
Academic work has demonstrated the risk of inbreeding and potential to loose viability.
Commercial companies have procedures in place to prevent this from occurring.

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Source of trait deterioration in entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* during *in vivo* culture

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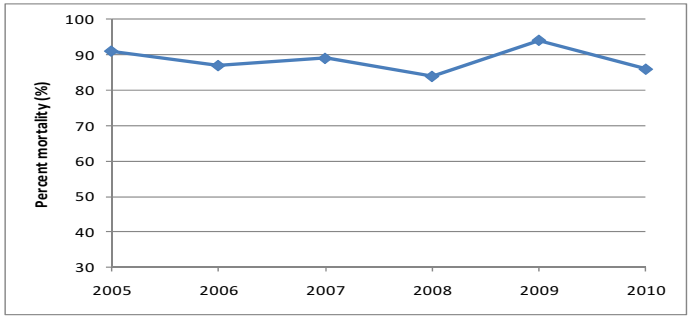
Quality control of stock nematodes

QC procedures of stock nematodes to ensure no loss of pathogenicity:

- *Tenebrio molitar* bioassay
- *Steinernema carpocapsae* pass mark = 70%



Quality control of stock nematodes

Independent work has demonstrated that mass production and Trans-Altlantic transport can be done (Caamano *et al* 2008) without compromising product quality.

Quality Assessment of Two Commercially Available Species of Entomopathogenic Nematodes: *Steinernema feltiae* and *Heterorhabditis indica*

Erick X. Caamano[1], Raymond A. Cloyd[2,5], Leellen F. Solter[3], and Declan J. Fallon[4]

ADDITIONAL INDEX WORDS. survival, biological control, pest management,

such as temperature and relative humidity (Choo et al., 1989; Hara et al., 1993; Lewis et al., 1992) as well as application technology (Gouge and Hague, 1995), because EPN are susceptible to desiccation (Ishibashi et al., 1987), temperature extremes, and ultraviolet radiation (Baur et al., 1995; Mason and Wright, 1997).

Despite logistical issues, the use of EPN has been successful in field and greenhouse environments to manage certain insect pests, including the black vine weevil [*Otiorhynchus*

Steinernema feltiae (NemaShield, Nemasys, Gnat Not, Horticultural Scanmask), and *Heterorhabditis indica* (GrubStake-Hi), based on eight shipments/samples of each EPN product received during a 5-month period (July to November). The estimated total number of EPN delivered per shipment (i.e., sample) was compared with the expected quantity listed on the label, and percent live EPN was determined for each shipment. One-half of the shipments of Gnat Not (four of eight) contained

billbugs [*Sphenophorus* spp. (Coleoptera: Curculionidae)], white grubs (Coleoptera: Scarabaeidae), fungus gnats [*Bradysia* spp. (Diptera: Sciaridae)] (Georgis et al., 2006; Hom,

"Entomopathogenic nematode survival (based on percent live IJs) was highest for Nemasys, with the most consistent percent survival (95% to 100%) across the eight evaluation periods"

The future of beneficial nematodes

The future of beneficial nematodes

This market will continue to grow.

Expansion in current markets – WFT in flowers (roses)

Growth into new markets – Slugs in potato

Growth into new territories – Those countries supplying EU supermarkets.

Genetic modification (e.g. stress tolerance) is unlikely to feature in commercial research as this may affect product perception by consumers.

The future of beneficial nematodes

Reasons for expecting growth:

Withdrawal of traditionally relied upon insecticides.

Desire for more environmentally sensitive growing.

Requirements for non-EU producers to fit strict European supermarket standards.

Economies of scale lowering end user price.

More expensive newer chemistries will be similar cost.

High and more reliable efficacy with greater understanding of products.

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