

Advances in Entomopathogenic Nematode *In Vivo* Production and Application Methodology

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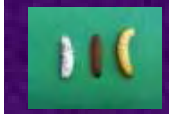

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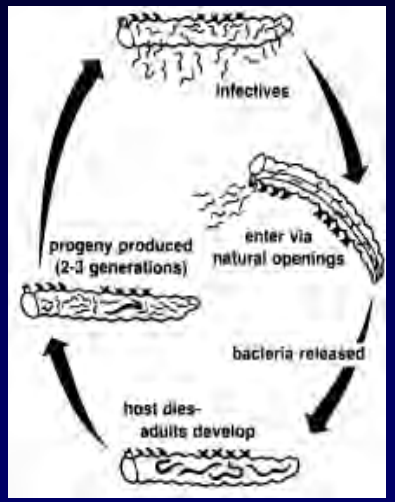
Outline:

- Introduction
- Production
  Standard approaches
  Improved *in vivo* production
  - insects, production system, nematodes
- Application
  Targets (N. America)
  Advancements in Application

Entomopathogenic Nematodes (EPNs) Steinernematidae & Heterorhabditidae



Commercialized Species

- *Heterorhabditis bacteriophora*
- *H. indica*
- *H. marelatus*
- *H. megidis*
- *H. zealandica*
- *Steinernema carpocapsae*
- *S. feltiae*
- *S. glaseri*
- *S. kushidai*
- *S. riobrave*
- *S. scapterisci*

> 13 commercial producers in US, Europe, Asia (Kaya et al. 2006)

Production: Approaches

- *In vivo* culture
- *In vitro*: solid culture
- *In vitro*: liquid culture

In Vivo Culture

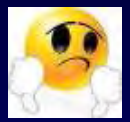
Basic Method

- Two dimensional process – a system of shelves & draws
- Often based on White trap
- Inoculation
- Incubation and Harvest
- Concentrate and Clean (automatic separation, antimicrobials)
- Storage

Factors Affecting Yield

- Insect: *Galleria mellonella, Tenebrio molitor*, others (bottom line = cost per IJ yield)
- Inoculation method
- Dose
- Host density
- Environment: RH, temperature
- Nematode sp or strain, e.g., 40,000 per *G. mellonella* in *S. glaseri* & >300,000 in *H. indica*

In Vivo Culture



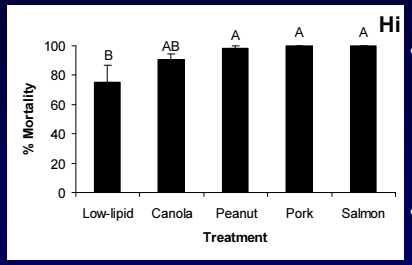
- Positive: Low tech, low capital input, easy to achieve quality (though problems exist)
- Negative: Scalability, Cost (labor & insect "media")
- Areas to Improve:
  - Media quality and efficiency of production
  - Production process/automation to reduce labor
  - Nematodes (quality of beneficial traits)

Our Focus: Improvement of *in vivo* culture using *Tenebrio molitor* as the host

Improved *In Vivo* Production: Improving the Media

- First Step: Grow your own insects!
- Next Step: Increase efficiency of insect host production (decreasing costs) & improve quality of nematodes
- For improving insect quality – see the talk of Morales-Ramos et al.! (also, Morales et al. 2010. J. Entomol. Sci.)
- For nematodes: targets for improvement = insect susceptibility, nematode yield, and virulence

Improved *In Vivo* Production: Improving the Media

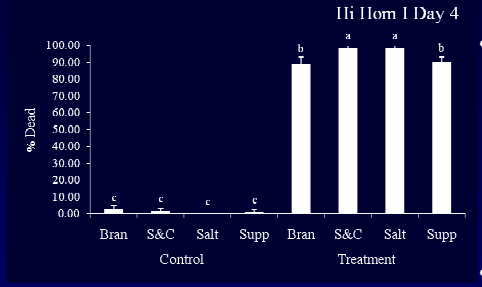


Effects of lipids in *T. molitor* diet on susceptibility to *H. indica*

- 1st Round: Discovered that lipid & protein content in host diets affects nematode virulence & fitness (can be positive or negative)
- E.g., lipids benefit *H. indica*

(Shapiro-Ilan, Rojas, Morales-Ramos, Lewis, Tedders, 2008, J. Nematol)

Improved *In Vivo* Production: Improving the Media



Effects of salts & cholesterol in host diet on susceptibility to *H. indica*

- 2nd Round: Discovered that certain salt & cholesterol content in host diets improves host susceptibility & nematode virulence
- 3rd Round: Combined ingredients lipid + salt & cholesterol benefits to achieve optimum diet (data not shown)

Improving the Production Process Automation to Reduce Labor

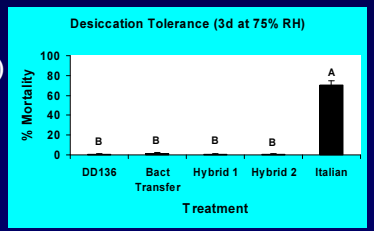
- Goal: Complete mechanization of the *in vivo* process, from rearing the insects to the nematode package
- Inoculation automated sprays, & optimization of inoculation density/rate
- Automated harvest deposited to final package

Improving the Nematodes

- Screen for the best species for primary target host(s)
- Discovery of new species/strains (e.g., Sr, Ss, GPS11)
- Hybridization or selection of desired traits, e.g., hybridization to increase environmental tolerance

(Shapiro-Ilan et al. 2005; Mukaka et al. 2010)

- Molecular/genetic Methods (Gaugler et al. 1997)



Shapiro-Ilan et al.

Strain Stability: During Nematode Culture
Once you have a good strain, how do you keep it?

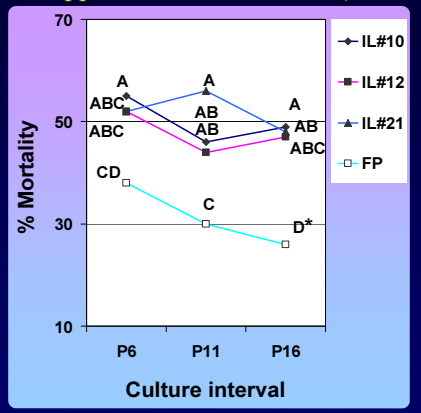
- When biocontrol agents including nematodes are cultured repeatedly, deterioration can occur in beneficial traits e.g., virulence, fecundity, environmental tolerance, etc. (Shapiro et al 1996; Wang & Grewal 2002)

Project initiated: Shapiro-Ilan, Gaugler, Adams, Hopper

- Both the nematode & bacteria partner contribute to trait deterioration during *in vivo* culture (Bilgrami, et al. 2006 Nematology)
- The cause of deterioration is (at least in part) genetically based (evidence indicates inbreeding depression) (Chaston et al. submitted)

Overcoming Trait Deterioration:
(Bai, Shapiro-Ilan, Gaugler & Hopper, 2005 Biol. Control)

- Inbred lines deter trait loss in Hb
- Problem can be deterred by selecting the "best" inbred lines
- Future = explore non-genetic factors, and molecular basis



Quality Issues for *In Vivo* Production

What can go wrong	Overcoming the Problem
• Poor nematodes (weak strain, deterioration)	• Strain improvement, inbred lines
• Poor inoculation	• Optimize inoculation rate, host density, environment (clean)
• Poor storage stability	• Optimize package density, medium & conditions
• Human error	• Improve training

Application Method for EPNs
Standard = Aqueous

Some Current Commercial Targets:

- Citrus root weevils, e.g., *Diaprepes abbreviatus*
- Black vine weevil *Otiorhynchus sulcatus*
- Fungus gnats (Sciaridae)
- Mole crickets, *Scapteriscus* spp.
- White grubs (Scarabaeidae)
- Also, billbugs, cutworms, cranberry girdler, fleas , thrips, etc.






Pest, Common name	Pest, Scientific name	Nemas
Artichoke plume moth	*Platyptilia carduidactyla*	Sc
Banana moth	*Opogona sachari*	Hb, Sc
Banana root borer	*Cosmopolites sordidus*	Sc, Sf, Sg
Black cutworm	*Agrotis ipsilon*	Sc
Black vine weevil	*Otiorhynchus sulcatus*	Hb, Hm
Borers	*Synanthedon* spp.	Hb,Sc, Sf
Codling moth	*Cydia pomonella*	Sc
Corn earworm	*Helicoverpa zea*	Sr
Diamondback moth	*Plutella xylostella*	Sc
Fungus gnats	Diptera: Sciaridae	Sf, Hb
Japanese beetle	*Popillia japonica*	Hb, Sg
Leafminers	*Liriomyza* spp.	Sc
Mole crickets	*Scapteriscus* spp.	Sc, Sr, Ss
Plum curculio	*Conotrachelus nenuphar*	Sr

Shapiro-Ilan & Grewal (2008). Hb=*H. bacteriophora*, Hm= *H. marelatus*, Sc=*S. carpocapsae*, Sf=*S. feltiae*, Sg=*S. glaseri*, Sr=*S. riobrave*, Ss=*S. scapterisci*.

Expanding/Improving Targets

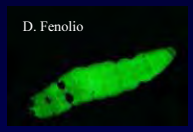
- Peachtree borer, *Synanthedon exitiosa*: 88-100% control (Sc) , curatively or preventatively, low costs (Shapiro-Ilan et al. 2009)
- Plum curculio, *Conotrachelus nenuphar*: > 95% control vs. larvae (Sr), need to integrate into management program (Shapiro-Ilan et al. 2008)
- Pecan weevil, *Curculio caryae*: Multiple pre-emergence applications results in <1% survival
- Small hive beetle, *Aethina tumida*: 78 to 94% control (*H. indica or S. riobrave*); (Ellis et al. 2010; Shapiro-Ilan et al. 2010)






Advances in EPN Application

- Cadaver approach



- Aboveground application

Improved Application Methods
Cadaver Application

- Approach = application of nematodes in infected hosts; pest suppression is achieved by newly released IJs; Reduces Labor!
- Superior efficacy e.g., in lab and greenhouse (Shapiro-Ilan et al. 2003)
- Facilitating application: Apply in hard-bodied insects or protective formulation, e.g., clay coating (Shapiro-Ilan et al. 2001) - or new automated tape coating (Morales-Ramos, Tedders, Dean, Shapiro-Ilan, Rojas);
  Efficacy demonstrated to small hive beetle & Diaprepes weevil (Shapiro-Ilan et al. 2010)

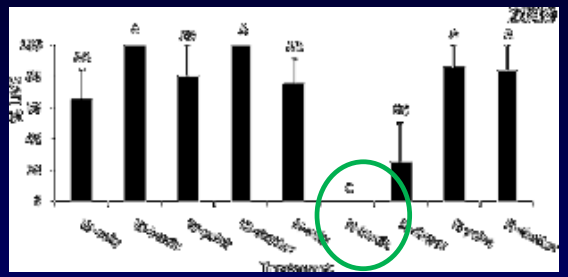


Improving Application Efficacy Aboveground:

Improved Formulations

- Adjuvants, e.g., antidesiccants;
  -surfactant-polymer combo (Schroer et al.), success versus *Plutella xylostella*
- Post-application "covers"
  - e.g., foam (Lacey et al.), sprayable gel……..


Sprayable "Fire" Gel
(Shapiro-Ilan et al. 2010. Biol. Control)



- Gel + nematodes (Barricade®) caused significant suppression of borer pests
- May have broad applications for use in other pests/crops



Summary/Conclusions

- *In vivo* EPN culture is a viable industry in North America; efficiency is hindered by labor and insect costs
- Production efficiency can enhanced via improved insect media (for nematode fitness), automation, superior nematodes & strain stability
- Improvements in application lead to superior efficacy, e.g., cadaver, spray parameters, improving soil environment, advanced formulations for aboveground use
- Future Research: Expanded automation, advances in strain improvement & formulation technology, basic studies (e.g., deterioration), quality control (tools)

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