Biopesticides, a review of active agents

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Active agents

- Viruses
  - Baculoviruses – granuloviruses & nucleopolyhedroviruses
  - Cypoviruses
- Bacteria
  - *Bacillus thuringiensis, B. spaericus, Paenibacillus popillae*
- Fungi
  - *Metarhizium, Beauveria, Trichoderma, Verticillium……*
- Protozoa
  - *Nosema, Vairimorpha*
- Nematodes
  - *Steinernema, Heterorhabditis*
- Semiochemicals

Viruses infecting invertebrates

- DNA viruses
  - Double stranded DNA
    - Poxviridae
    - Iridoviridae
    - Baculoviridae *- NPV and GV*
    - Polydnaviridae
  - Single stranded DNA
    - Parvoviridae
- RNA viruses
  - Double stranded RNA
    - Rheoviridae – *cypovirus*
    - Birnaviridae
  - Single stranded RNA (-)
    - Rhabdoviridae
    - Bunyaviridae
  - Single stranded RNA (+)
    - Picornaviridae, Togaviridae, Tetraviridae, Flaviviridae, Nodaviridae

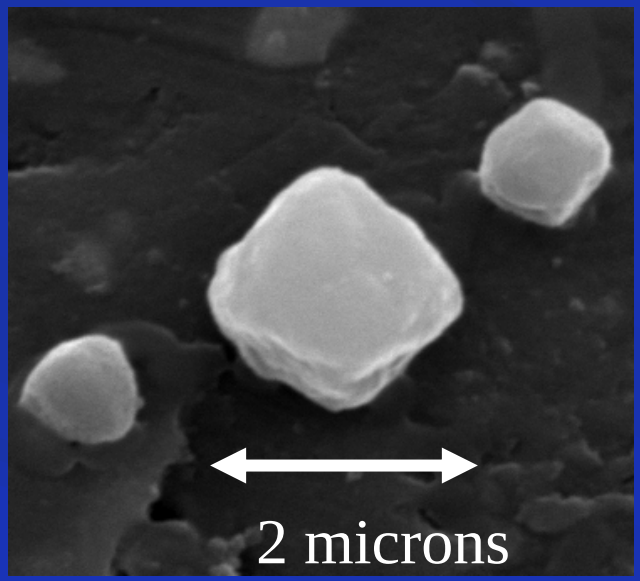
Entomopathogenic viruses

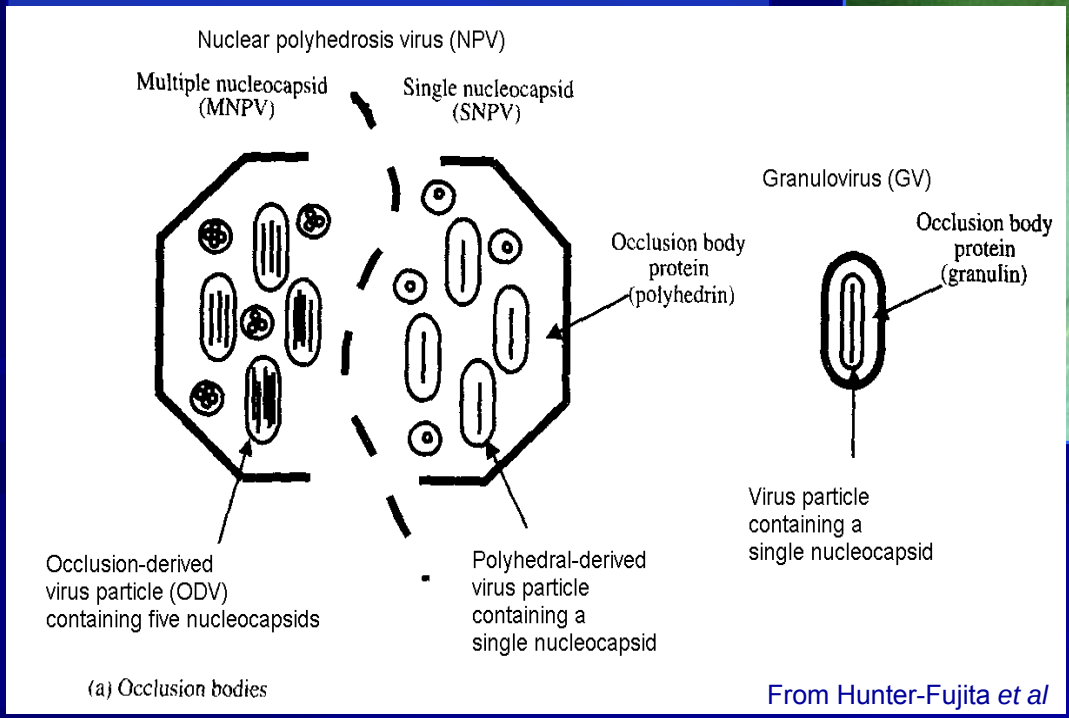
- Baculoviridae (ds DNA)
  - Nuclepolyhedrovirus
  - Granulovirus
- Reoviridae (ds RNA)
  - Cypovirus

Baculoviruses: Key reference

- OECD (2002) Consensus Document on Information used in the Assessment of Environmental Applications involving Baculovirus. Series on Harmonization of Regulatory Oversight in Biotechnology No. 20. OECD Environment, Health and Safety Publications. ENV/JM/MONO(2002)1.

Baculoviruses





From Hunter-Fujita *et al*

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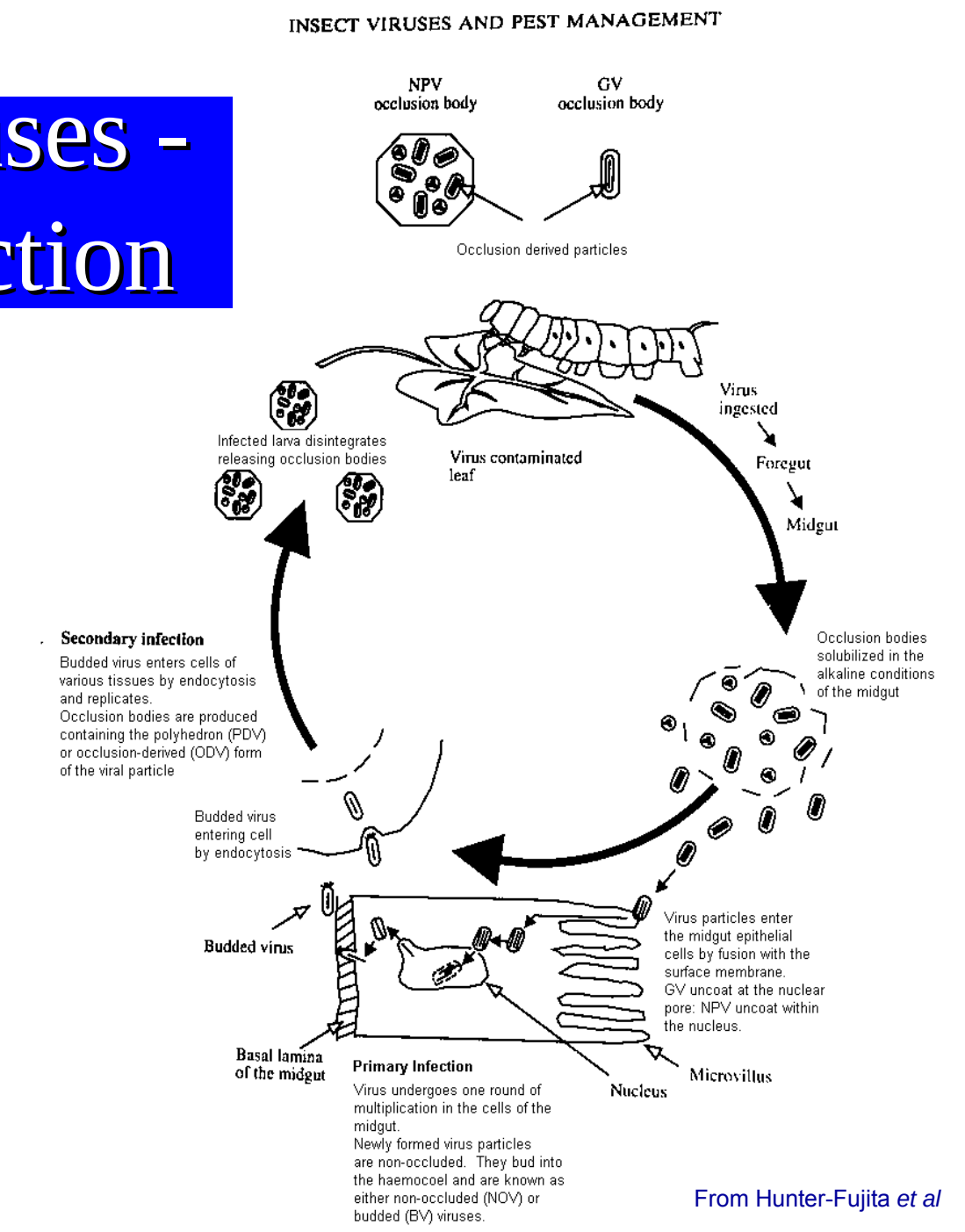
Baculoviruses - Mode of action



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Baculoviruses

Susceptibility of Alternative Hosts

- Found only in invertebrates
- No member of the family is known to infect plant or vertebrate
- Most have narrow host insect range, and infectivity is restricted to the original host genus or family

Baculoviruses

Toxicity studies - mammals

- Toxicity test results from 1970s/80s of 29 NPVs indicated no toxicity or pathogenicity. Doses were generally 10 – 100 x the “per acre” (1 acre = 0.45ha) field rate equated to a 70kg person.

- *Heliothis zea* NPV most extensively tested for toxicity in humans and led to registration of “Elcar” by Sandoz in USA.

Baculoviruses

Toxicity studies – mammals cont.

No effects of HzNPV found in:

- Acute toxicity-pathogenicity tests in mouse, rat, guinea pig, rabbit, monkey and man at $6x10^9$ – $3 x 10^{12}$ OB / kg.
- Skin irritation sensitivity tests in guinea pigs, rabbits and man at 10^6 and 10^7 OB / mm^2 skin.
- Eye irritation tests in rabbits with 10^5 and $2x10^6$ OB / eye
- Subacute toxicity-pathogenicity tests and subcutaneous injection into mice, rats, dogs and rhesus monkeys.
- Teratogenicity and carcenogenicity studies in rats and mice at 10^9 – $3.5x10^{12}$ OB / kg.

- Similar but less extensive results for many other NPVs from the 1970s/80s

Baculoviruses

Toxicity studies – wildlife

- Birds
  - Able to pass NPV through the alimentary tract unaffected
  - No deleterious effects
- Aquatic organisms
  - No adverse effects
- Beneficial insects
  - No direct effect on parasitoids, predators and pollinators
  - Indirect effects on parasitoids resulting from host death

Baculoviruses

Pathology studies

- Toxicity tests designed for testing effects of chemicals on vertebrates are insufficient
  - Results reported in Gröner (1986) indicate no virus induced antibody production in test mammals and chicken.
  - No cytogenetic effects of baculoviruses in mammalian cells either *in vivo* or *in vitro*.

Baculoviruses

Virus-cell interactions in vitro

- AcNPV inoculated into vertebrate cells can be taken-up and the degree of up-take depends on cell type, temperature, time and viral phenotype.
- BUT, none of the human and nonhuman vertebrate lines tested showed evidence of viral replication.
- NPVs unable to activate retroviruses in mammalian cell lines

Baculovirus registration

- The most comprehensive / developed registration guidelines come from the US EPA:

  http://www.epa.gov/pesticides/biopesticides/ai/viruses.htm

- European regulations well developed but complicated by the number of countries, despite the efforts at harmonising across the EC.

  (EC directive 91/414/EEC)

Baculoviruses

- a list of the baculoviruses regulated as pesticide active ingredients by the US EPA Office of Pesticide Programs as of May 2005

  - *Anagrapha falcifera* NPV
  - *Cydia pomonella* GV
  - Douglas fir tussock moth NPV
  - *Gypsy moth NPV*
  - *Helicoverpa zea* NPV
  - Indian meal moth GV
  - *Mamestra configurata* NPV (pending)
  - *Spodoptera exigua* NPV

Baculoviruses – US EPA fact sheet

III. ASSESSING RISKS TO HUMAN HEALTH

These viruses infect only the target insect larvae and closely related species. Toxicity tests show that the viruses pose no risk to the public. Workers wear protective clothing to prevent possible irritation from handling and applying the product.

IV. ASSESSING RISKS TO THE ENVIRONMENT

Tests show that the GV and NPVs that EPA has registered as pesticide active ingredients specifically infect only certain species of moth larvae. The viruses do not harm other organisms, including plants, beneficial insects, other wildlife, or the environment. These viruses occur naturally in their insect hosts.

Cypoviruses: Mode of action

- Polyhedra ingested and dissolved in larval midgut
- Virions released and attach to midgut columnar cells
- Viral core enters cell cytoplasm
- RNA transcription and replication
- RNA occluded in capsules
- Virus capsules occluded by virogenic stroma to form occlusion bodies

Cypoviruses (Rheoviridae)

- No CPV has been found infecting vertebrates or plants (Belloncik, 1989)

  - *Dendrolimus spectabilis* CPV registered in Japan in 1974. Safety test results generally negative.

    – Katagiri, K. (1981) Pest control by cytoplasmic polyhedrosos viruses. In: Microbial control of pests and plant diseases 1970-1980. (Ed Burges, H.D.) Academic Press.

Bacterial Entomopathogens

Spore-forming Bacteria

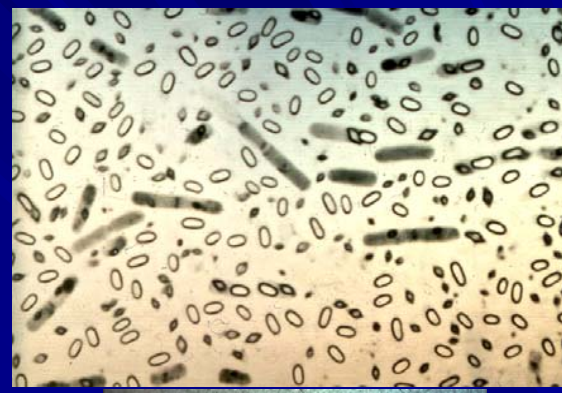
- *Paenibacillus (=Bacillus popillae)*
- *Bacillus sphaericus*
- *Bacillus thuringiensis*
  - Related mammalian pathogens:
    - *Bacillus cereus*
    - *Bacillus anthracis*

Non-spore forming spp.

- *Serratia entomophila*
- *Clostridium bifermentans*

Transgenics

- *Pseudomonas* spp.



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Bacillus popilliae

- Causes “milky disease” in scarab larvae
- Host-specific, apparently an obligate pathogen on scarabeid beetles
- Used for many years in control of scarab larvae
- Spores can last in the environment for several years.
- Ability to recycle

Mode of action

- Chronic – non-toxic mode of action
- Cause of death may be infection and physiological starvation
- Concentrations of bacteria in beetle haemolymph can be $3*10^9$ bacteria/ml

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B. popillae safety

- Highly specific, non-toxic mode of action - no toxicity known to mammals, birds, or fish.
- Does not grow above 35.5 ^{o}C so can not persist in vertebrates
- Non-target effects: None known

Bacillus sphaericus

- Activity restricted to certain Diptera
- Effective against mosquitoes
- Ability to recycle to produce new infections
- Spore and multiple toxin crystals
- Lasts up to nine months in aquatic environments
- Better than *Bt israelensis* in polluted waters
- No measurable health effects or environmental risks

Bacillus thuringiensis

Number one biopesticide world-wide

Uses:

- field crops
- forest pests
- vector control in aquatic ecosystems

- Foliar applications have a typical persistence of 4-5 days.
- Spores are viable for years in the soil (not exposed)
- Rarely recycles in nature

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Bt varieties

- *kurstaki* – standard lepidopteran strain
- *aizawai* – lepidopteran strain
- *israelensis* – diptera (mosquito, blackflies)
- *tenebrionis* – some beetles (e.g.Colorado potato beetle)

Bt Mode of Action

- Kills by direct toxicity, not infection
- Parasporal bodies = occlusion bodies = crystals containing toxins.
- Crystal toxins = δ (delta)-endotoxins
- Other toxins:
  - Haemolysins
  - β-exotoxins
  - enterotoxins

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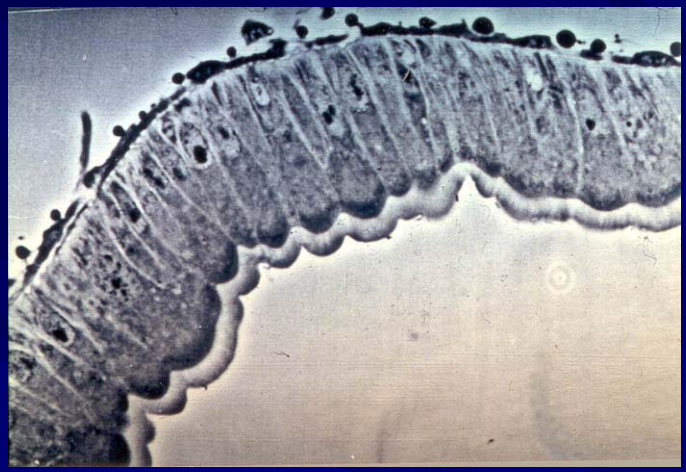
Delta Endotoxins

- Activation of endotoxins
  - monomeric protoxin to active δ-endotoxins
- Mode of Action
  - active δ-endotoxins bind with gut receptors
  - creates pores interfering with ion transport
  - results in gut paralysis
  - high doses lead to gut lysis and rapid death

2h after distilled water



3h after Bt endotoxin

Effect of Bt endotoxin on insect gut tissue

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Beta Exotoxins

- Proteinaceous, sometimes excreted in the growth media.
- Detected in several strains from several sub-spp.
- Inhibits biosynthesis of RNA
- Broadly toxic to vertebrates
- Not associated with the crystal proteins.
- Are genetically associated with crystal proteins (on same plasmid)

Enterotoxins

- Known from the closely related *Bacillus cereus*. Some varieties of *B. cereus* cause gastroenteritis (food poisoning).
- Some commercially used strains of Bt can produce enterotoxins. No conclusive case of Bt-induced gastroenteritis
- Bt enterotoxin ten times less than that produced by *Bacillus cereus*.

Safety of Bt

- Humans – After 60 years of use, clinical infections sufficiently rare that they are printed in medical journals.
- Human testing showed no undesirable effects
  - ingestion (3-10 * 10^9 spores/day for 3-5 days)
  - inhalation (100mg for five days)

Mammals

Oral

- No undesirable effects ever reported
- Bt remains confined to the gastrointestinal tract (McClintok, 1995)

Acute Pulmonary

- $2.6*10^7$ spores/kg.
- Clearance from lung with no adverse effects.

Mammals

Intravenous

- *Bt israelensis* declines steadily in the bloodstream, lungs, kidneys, brain.
- Indicative of inability to replicate and/or persist

Intraperitoneal

- No effects in mice at doses of 10^6 or 10^7 spores
- At 10^8 spores, substantial mortality appears (as also happens with “harmless” bacteria)

Mammals

Dermal/Ocular

- Slight to moderate skin irritation has been documented.
- Reversible in 7 days
- Cornea undamaged.
- Formulation, not Bt. was assumed to be the cause.

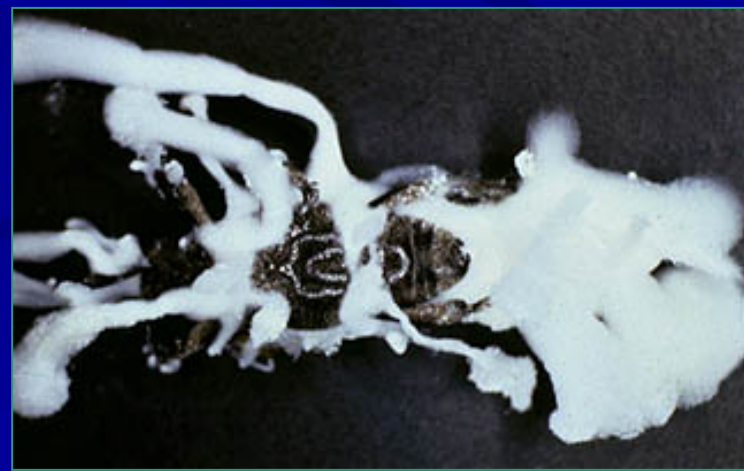
Birds

- No undesirable effects in birds from feeding wild birds or chickens with spores or with infected insects

Fungi as biocontrol agents for plants, plant diseases and arthropods

- Potential safety issues:
  - Competitive displacement of non-target organisms
  - Allergenicity
  - Toxigenicity to NTOs
  - Pathogenicity to NTOs

Callosobruchus infected with B. bassiana. Photo by Georg Goergen, IITA, 2002



Competitive displacement:

- Competitive occupation of niches
  - Intended / unintended

Allergenicity

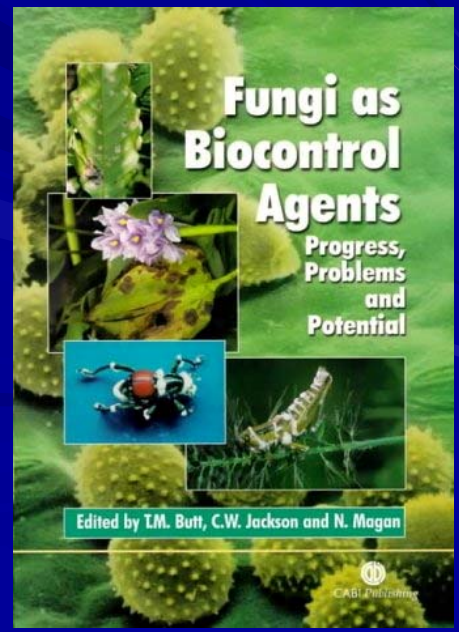
- Spores can cause allergic reactions
- Reports of allergic reactions among mass production workers
- Fungal bca are not among the species responsible for production of common allergies

Toxicity

- Fungi secrete wide array of compounds with biological activity against other organisms
- Function differs depending on the ecological niche of the fungus.
- May play a role in pathogenesis-antagonism in bca
  - *Metarhizium anisopliae* – destruxins and cytochalasins
  - *Beauveria bassiana* – bassianin, beauvericin, beauveriolide, bassianolide
  - *Beauveria brogniatii* - oosporein
  - *Hirsutella thompsonii* – hirsutellin
  - *Trichoderma harzianum* – peptaibols
  - *Fusarium* spp. – trichothecenes, beauvericin
  - *Colletotrichum* - colletotrichin
- Toxin production varies according genus and isolate

Toxins – a reason for exclusion?

- Presence of toxins should not necessarily preclude use of BCA in biocontrol
- Considerations:
  - Presence of toxin in formulated product?
  - Fate of toxin after application?
  - Accumulation of toxin in host / environment?
- No documented accounts of toxin levels rising after epizootic/epidemics
- No reports of bca metabolites entering the food chain
- Risks are very low



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RAFBCA

(**R**isk **A**ssessment of **F**ungal **B**iological **C**ontrol **A**gents)

http://www.rafbca.com

EU project that has generated:

1. Data on fungal metabolites
2. Tools for risk assessment

in order to accelerate registration of fungal bca in Europe

Major conclusion of RAFBCA is that metabolites of selected fungal BCA do not enter the food chain, and therefore do not pose a risk to consumer and animal health.

Risk assessment of each metabolite produced by a microbe would be very expensive and certainly deter most small and medium sized enterprises

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Pathogenicity


Photo: G. Goergen

- Pathogenicity towards host is desired effect
- Ability to infect is usually constrained within a host group

- But, pathogenicity towards NTOs, including vertebrates, is reported:

  - Reports include:
    - *B. bassiana* infecting alligators, giant tortoises, inland silverside fish embryos, grass shrimps
    - *M. anisopliae* infecting inland silverside fish embryos, immunocompentent and immunoincompetent humans
    - *Paecilomyces lilacinus* in immunocompentent and immunoincompetent humans
    - *Conidiobolus coronatus* associated with lesions in horses and humans

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Entomopathogenic microsporidia

- Microsporidia are genetically aligned with fungi
- Principal genus = *Nosema*.
- *Nosema locustae* is the only sp. Registered by US EPA as biopesticide.
  - Used in locust control for > 20 yrs.
  - Conventional and maximum challenge testing gave no illness or infection.
- Weight of evidence is against infection in mammals.
  - Reproduction in some mammalian cell lines at <37°C.
- No effect on physical environment
- Safety concerns relate only to effect on NTOs

Non-target effects of microsporidia

- Same as for fungi –
  - Competitive displacement
  - Allergenicity
  - Pathogenicity
  - Toxicity of metabolites and antibiotics

Entomopathogenic nematodes

- Genera = *Steinernema* and *Heterorhabditis*
  - Symbiotic relationship with specific bacteria spp in *Xenorhabdus* and *Photorhabdus* respectively
  - Wide host range
  - Highly effective
  - Persistent and can recycle
  - Mobile
  - Large scale mass production possible

EPN effects on non-target hosts

- No ecological problems ever recorded
- Considered safe for user and environment

Vertebrates:

- No detrimental effects for mammals or plants
  - No evidence of infection in humans of nematodes or associated bacteria
  - Slight risk of allergic effects from nematode-bacterium material
  - Remote risk of toxicity from contaminating bacteria

EPN non-target effects

Arthropods:

- Risk for NTOs with soil-dwelling stage is greatest
- Soil arthropods can be infected if exposed to high concentrations but effects not long lasting.
- May affect immature parasitoids directly / indirectly
- Some evidence for infection of predators feeding on infected hosts (mostly in laboratory)
- Honey bees susceptible if directly sprayed but colony not adversely affected

EPN non-target effects

- OECD-COST expert group identified risk for invertebrate NTO to be slight in treated fields. Data gathered to date indicate nematodes currently used in biocontrol pose few problems for natural populations of invertebrate NTOs.

Recommendations (from OECD-COST expert group)

- Indigenous EPN should not be regulated
- Release of exotic EPN should be regulated as macro-organisms
- Regulation should be at species level
- Future regulations may require tailoring with regard to EPN

Semiochemicals

- Allelochemicals
  - Allomones
  - Kairomones
  - Synomones
- Pheromones

Semiochemicals

- Non-toxic – modify behaviour
- Highly specific
- Natural occurrence
- Effective at very low rates
  - Comparable to natural emissions
- Humans and NTO have very low direct exposure
  - Dissipate rapidly
  - Rapidly degraded
  - Crop residues extremely low

- Risk of adverse effects minimised
  - No reports of adverse effects

Thanks for your attention

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