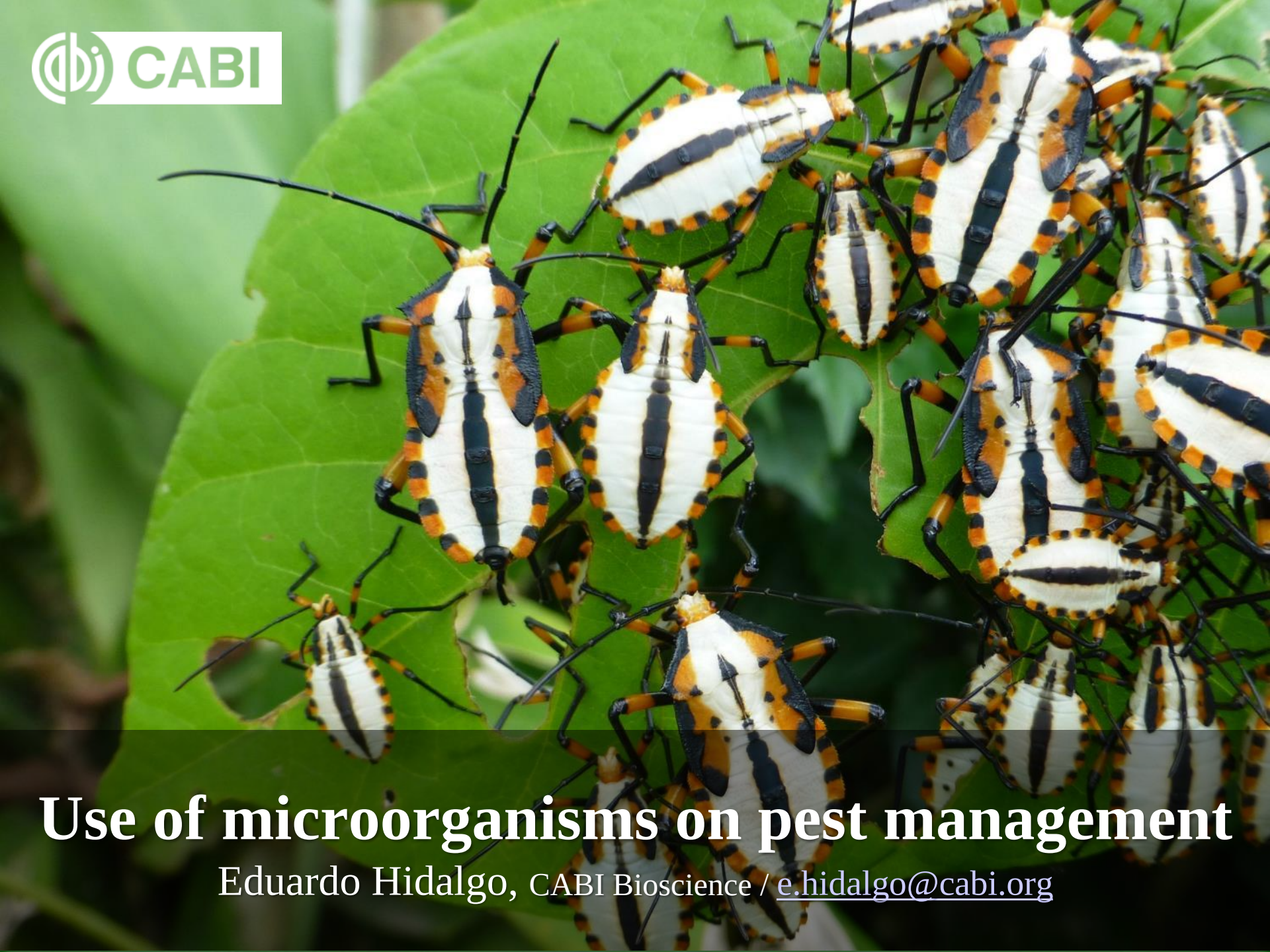
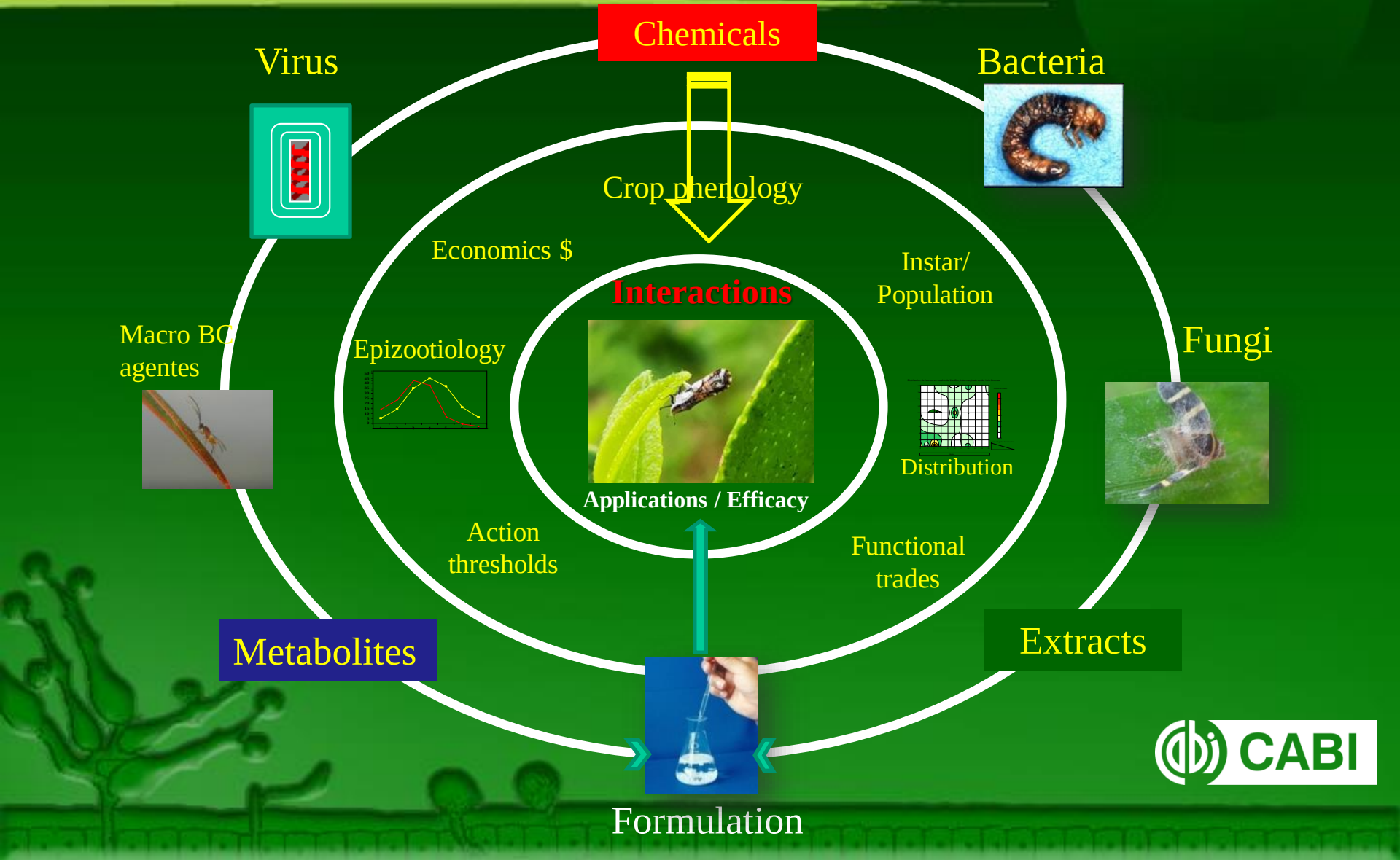


Use of microorganisms on pest management

Eduardo Hidalgo, CABI Bioscience / e.hidalgo@cabi.org

Alternatives and strategies



Biotic factors controlling *Diaphorina citri*

Name	Type	Stage of *D. citri* controlled
Tamarixia radiata	Parasitoids	Nymphs
Diaphorencyrtus aligarhensis		
Coccinellids (*Curinus coeruleus* Mulsant, *Exochomus childreni* Mulsant, *Harmonia axyridis* Pallas, *O. v-nigrum* Mulsant, *Cycloneda sanguinea* L., and *Coelophora inaequalis*),	Predators	Nymphs
chrysopids (*Ceraeochrysa* sp. and C*hrysoperla* sp.)		
Syrphid flyes		
histerid beetle: *Saprinus chalcites*		
Carabid, *Egapola crenulata*		
Cockroach *Blattella asahinai*,		
Spiders		Adults
Hirsutella citriformis	Entomopathogens	Adults (23 to 75% control)
Isaria fumosorosea (= *Paecilomyces fumosoroseus*)		Adults (up to 95% ctrl-Lab) (57-96% ctrl-field)
Lecanicillium lecaniil, L.longisporum		Adults (70% naural ctrl)
Beauveria bassiana		Adults (57-96% ctrl-field)
Cladosporium sp.		Adults

Qureshi, J. and Stansly , P. 2020. Asian Citrus Psyllid. CABInternational 2020

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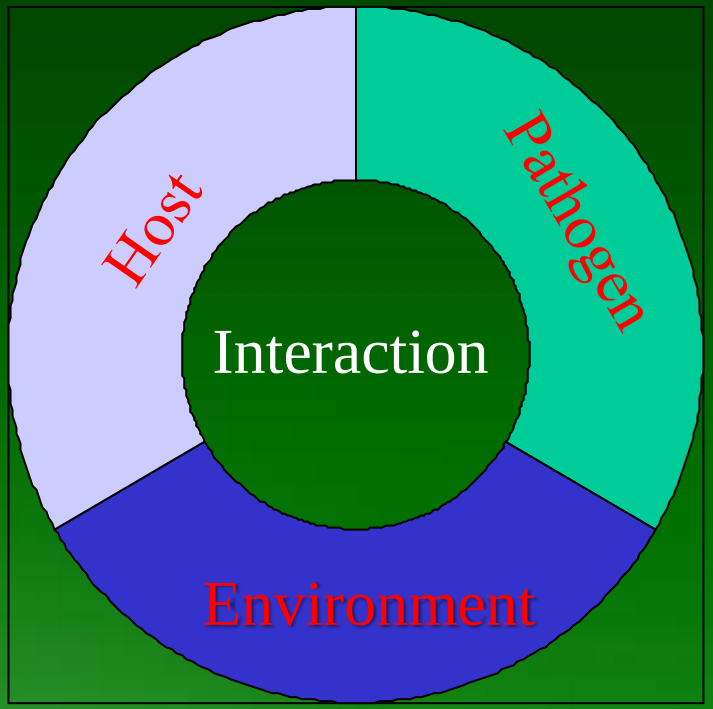
Disease (a dynamic process)

Epizootic: High mortality in short time

Enzootic: Low mortality all year round



Factors

Susceptibility

Disemination

Patogenicity

Virulence (DL and CL50) and TL50

Toxins

Latencia

Inoculum production

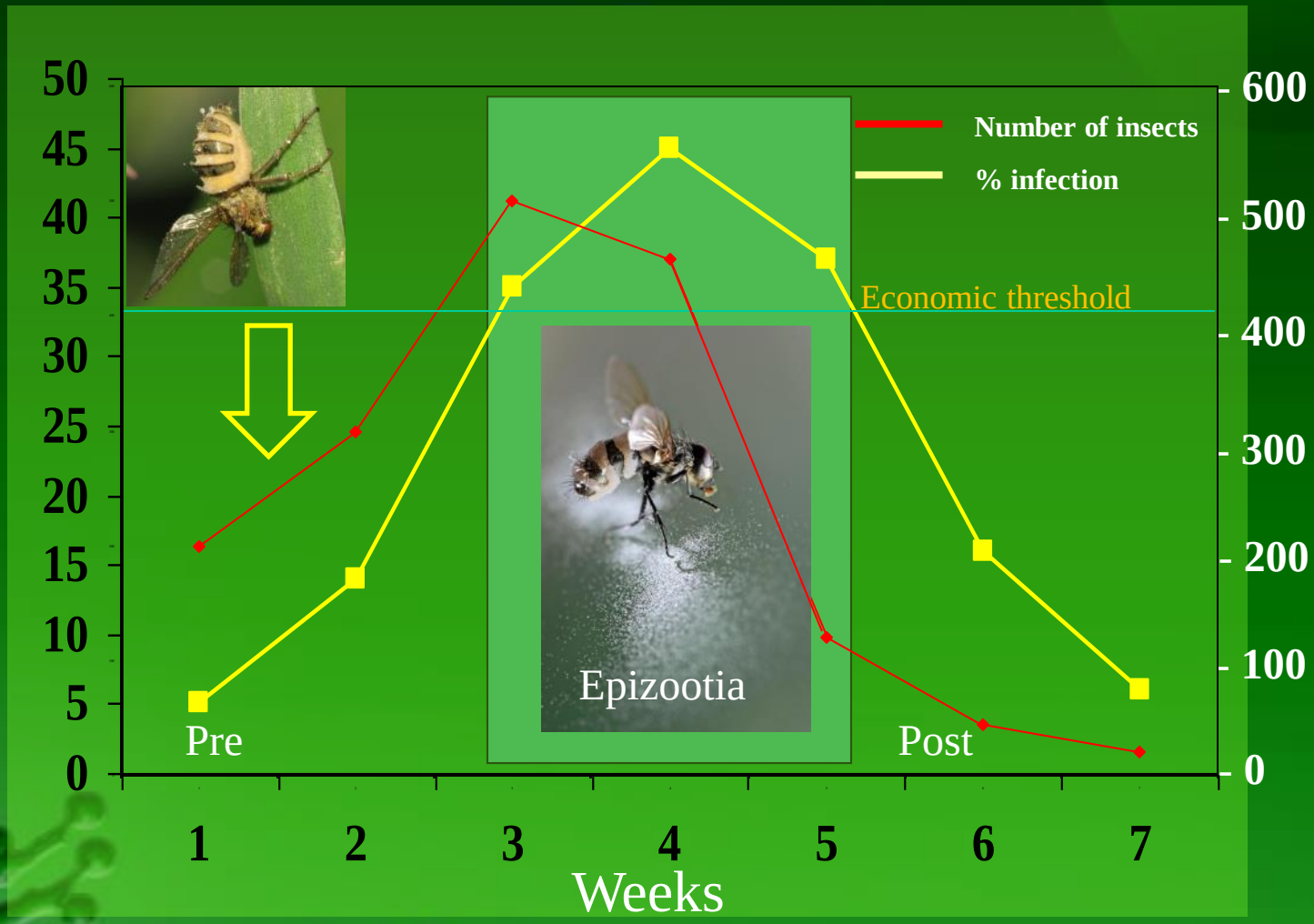
Temperature

Humidity

Radiation

Other (soil, plant, management practices)

Epizootic curve



Use of entomopathogens reported in the Caribbean

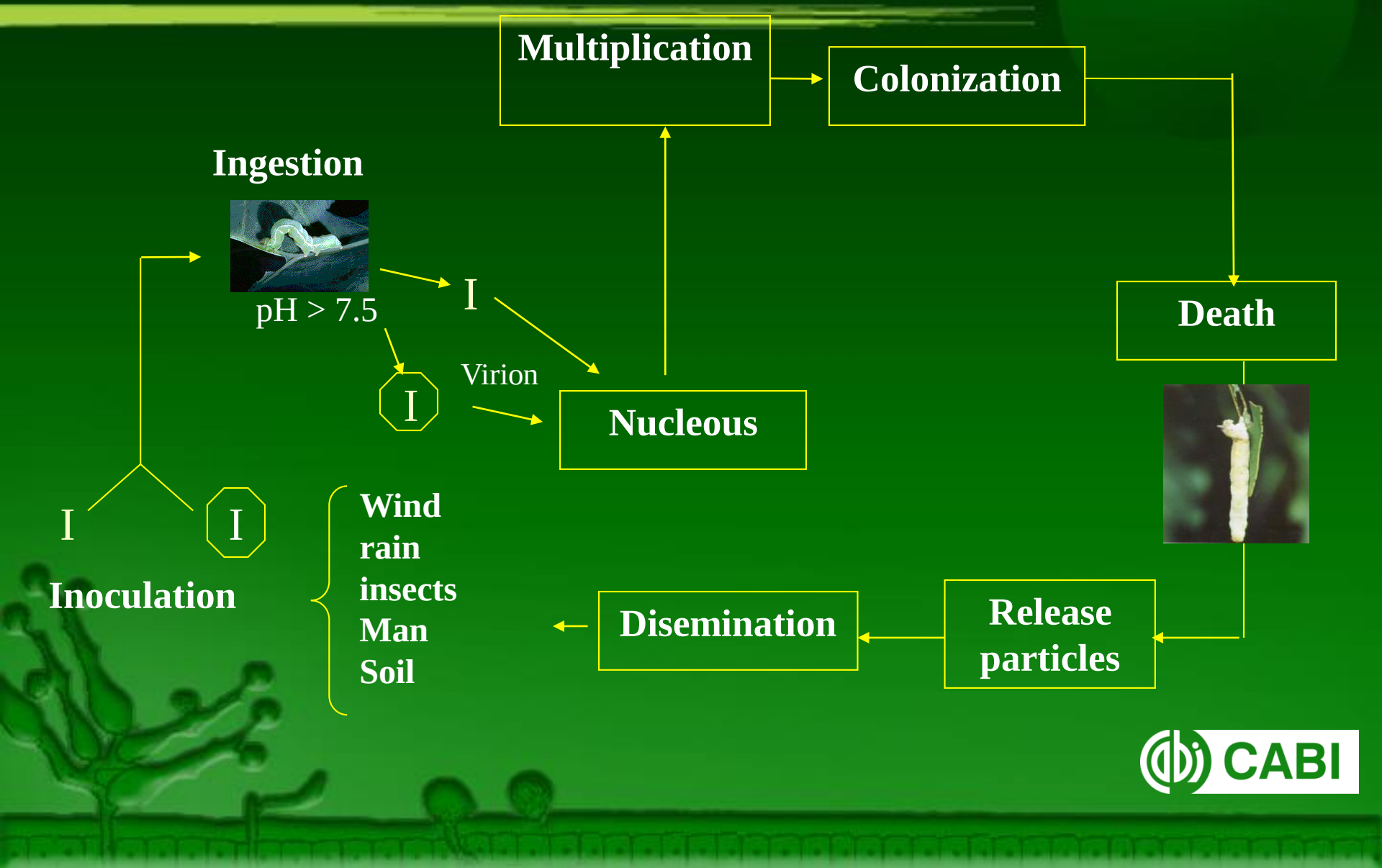
Natural enemy	Pest	Crop	Country
Metarhizium anisopliae	*Aeneolamia sp.*	Sugar cane	T&T[1]
	Aeneolamia flavilatera	Sugar cane	Guyana[2]
	Citrus weevils	Citrus	Jamaica[1]
Paecilomyces fumosoroseus	*Bemisia tabaci*	Tomato	T&T[1]
Neozygites parvispora	*Thrips tabaci*	-	Barbados[2]
Aschersonia aleyrodis	*Aleurodicus cocois* *Aleurothrixus floccosus*	-	Virgin[1] Islands
Beauveria globulifer	*Trhips*	Cacao	Grenada[1]
Beauveria bassiana	Citrus weevils Coffee berry borer *Orthezia praelonga*	Citrus Coffee	Jamaica[1]
Beauveria bassiana *Baculovirus* formulations	*Spodoptera exigua*	Onion, scallion, callaloo	Jamaica[1]
Trhichoderma ovalisporum	*Frosty pod rot*	Cacao	Jamaica[1]
Simplicillium sp.	*Red palm mite*	Coconut palm	Barbados[1]
Colletotricum musae (dead spores) *Gliocladium, Pythium, Trichoderma and Lecanicillium*	*Banana Crown rot (fungal complex)*	Banana	Various[1] countries

Qureshi & Stansly[1]. 2020, Hajek et al., 2016[2]

Entomopathogenic viruses

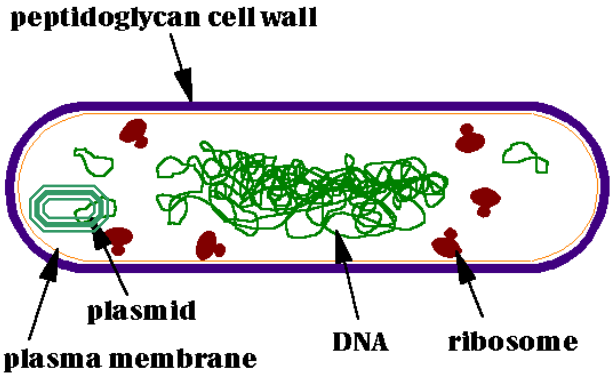
- DNA/RNA chains + protein
- Obliged pathogens
- Frequent in Lepidopteran larvae
- Cause disease and death in few days
- Can cause epizootics
- Various families reported but Baculoviridae the one commonly used

How to use virus for pest control



Entomopathogenic bacteria

- **General information**
  - No membrane in nucleoplasm
  - Reproduction by fission
  - Saprophytic, symbiotic, pathogenic



- Direct or potential pathogens
- Spore forming / non-spore forming
- Bacillus, Serratia, Pseudomonas

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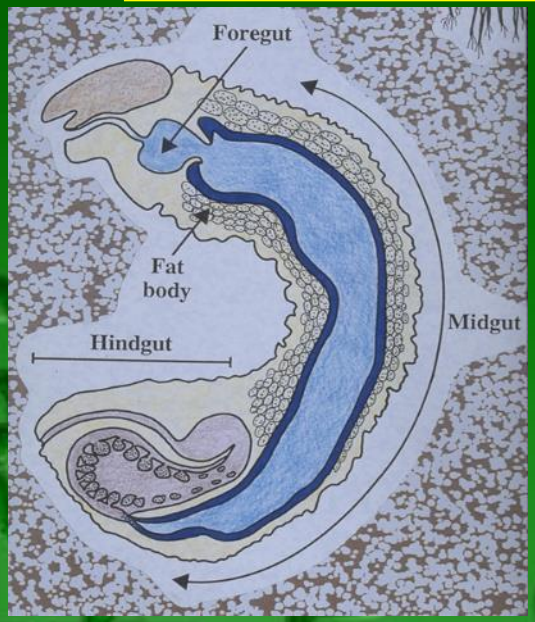
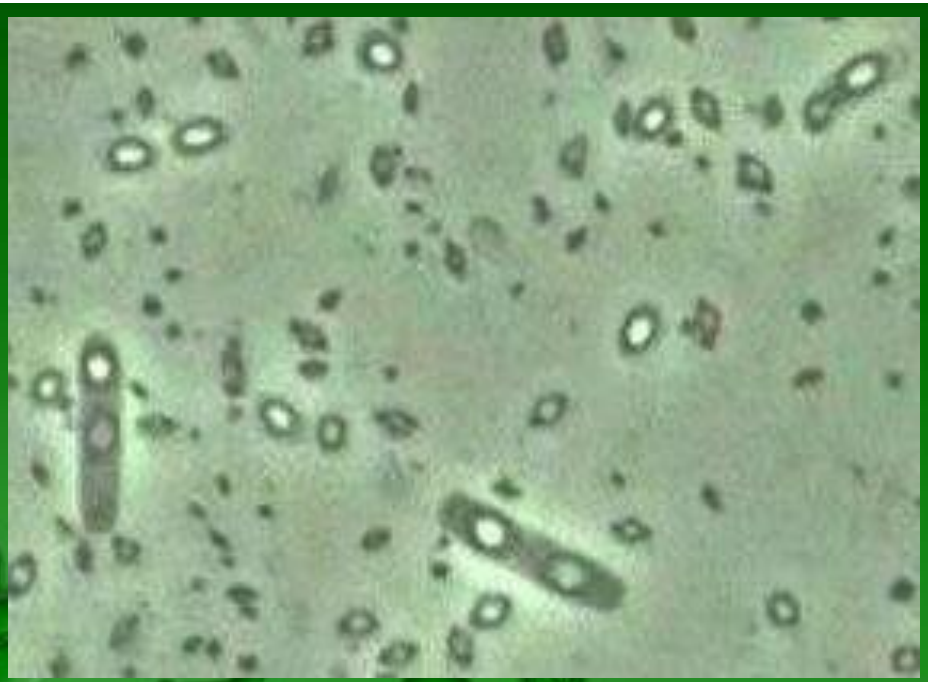
Entomopathogenic bacteria

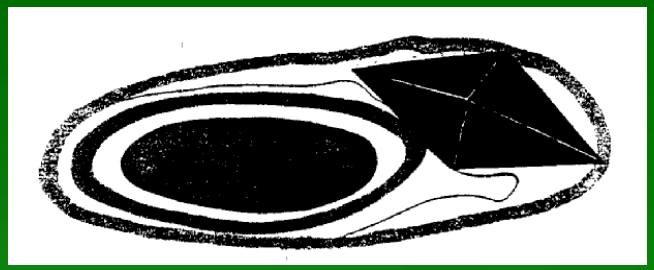
- **Midgut bacteria**
  - ***Serratia entomophila***
  - ***S. Proteomaculans***
  - **-Slow action**
  - **-Larva stops feeding**



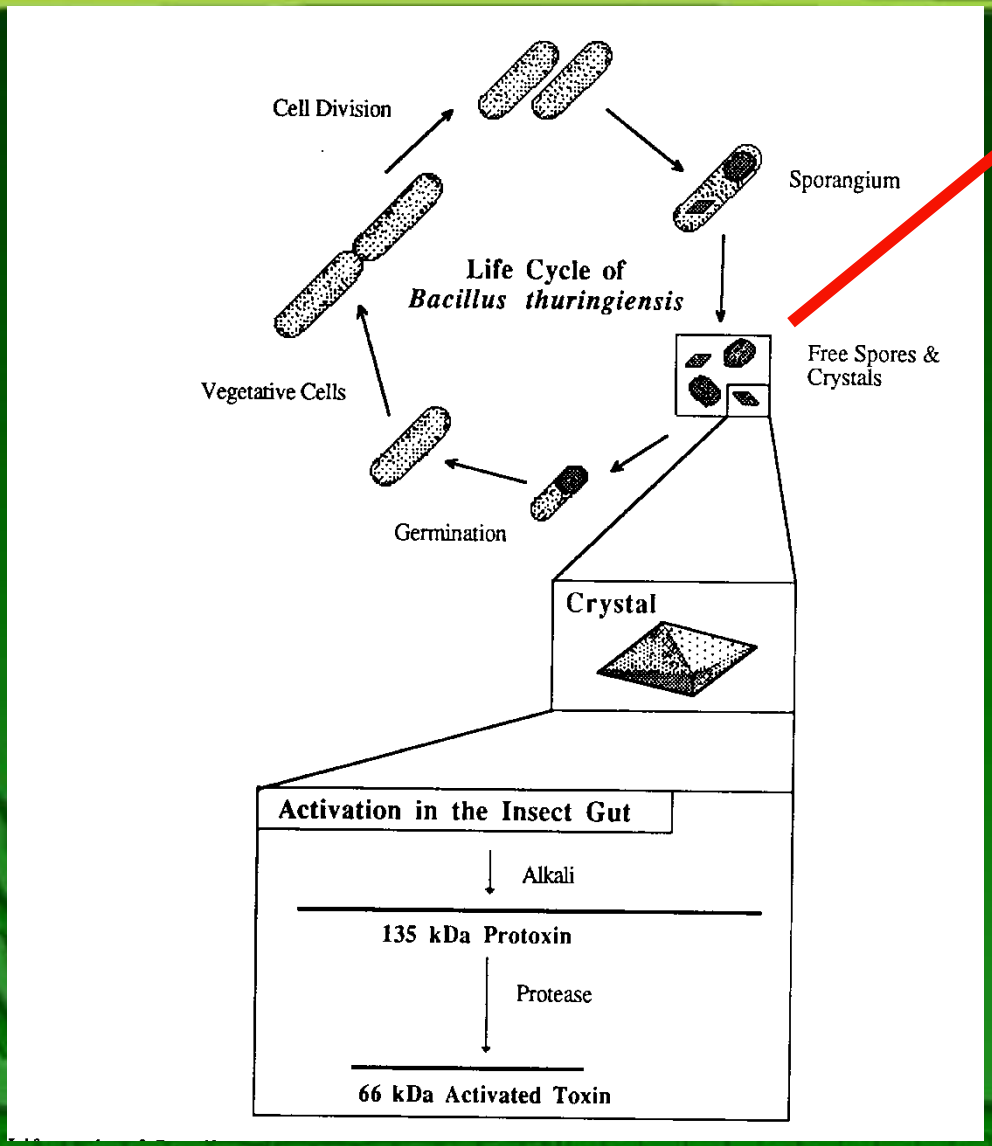
Bacillus thuringiesis

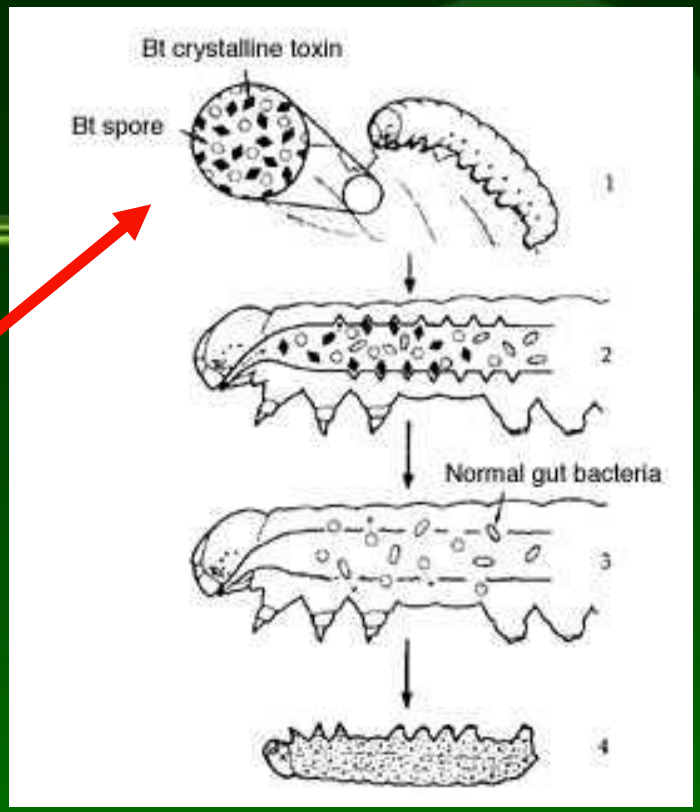
- **Mainly for Lepidoptera but there are strains for coleopteran and mosquitos**
- **Can be isolated from soil and infected larvae**
- **Forms a spore and protein crystal**
- **Can be mass produce by fermentation**





Bacillus thuringiensis





Bacillus popilliae

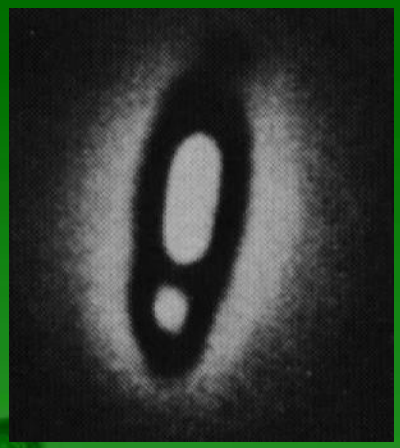
Bacillus popilliae

(*Paenebacillus popilliae*)

– Specific for Scarabaeidae

– Produces a spore and parasporal bodies

– Milky disease

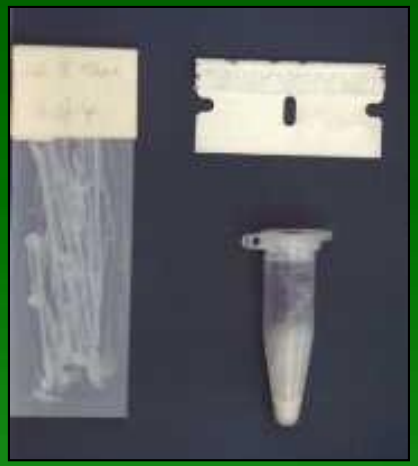


in vivo production of *Bacillus popilliae*

Inoculation

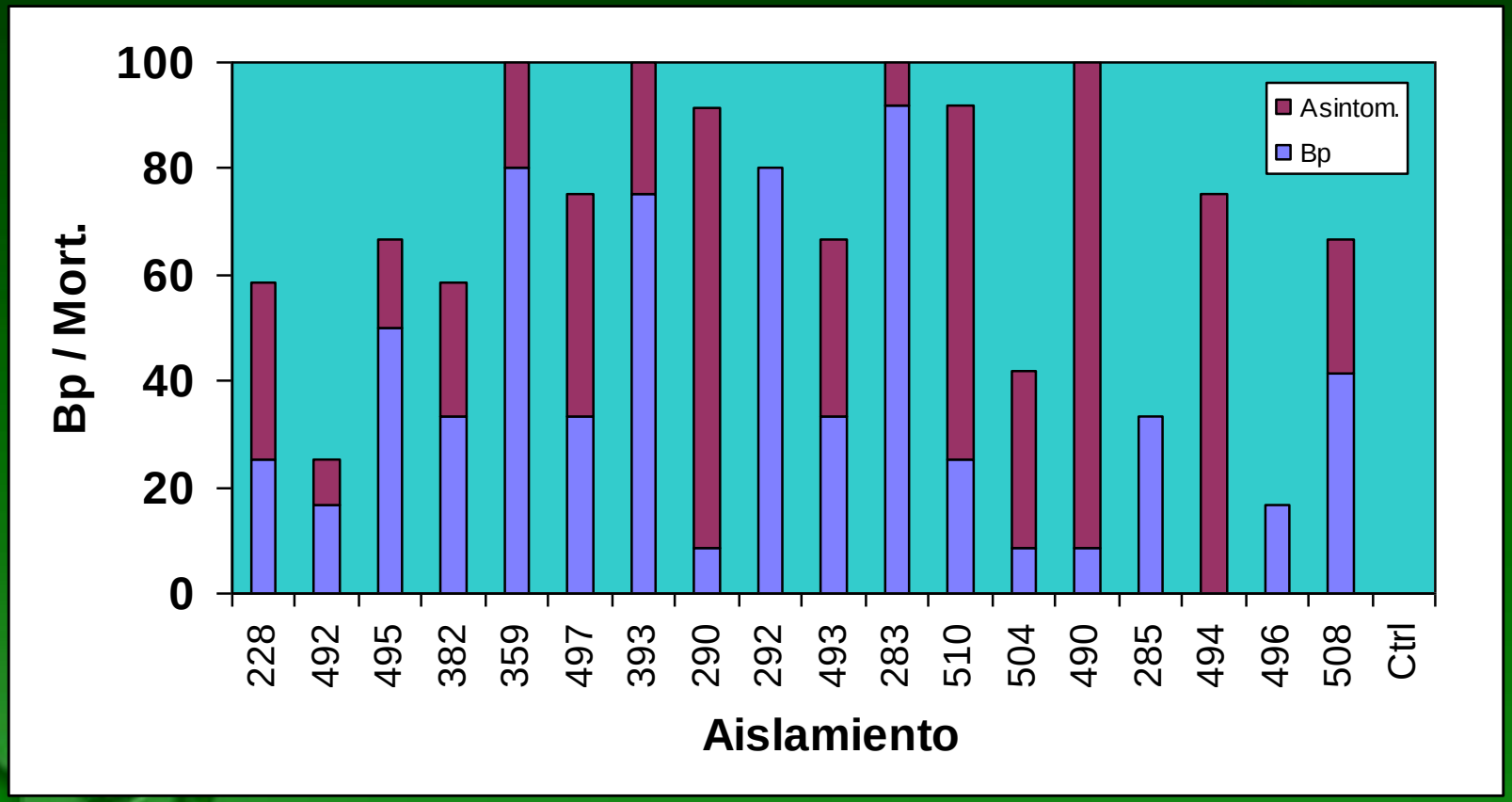
Reproduction

Extraction

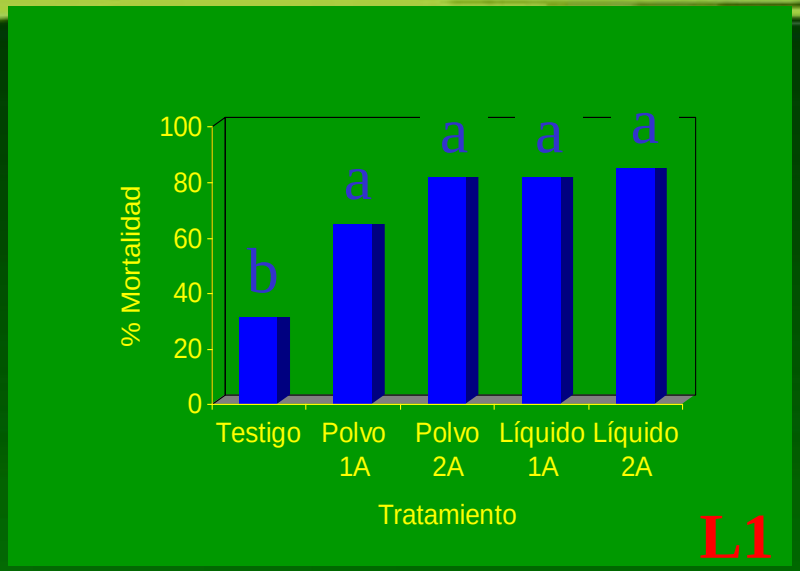


Storage

Screening of Bp strains against *Phyllophaga elenans* L3, 16DAI, via injection.

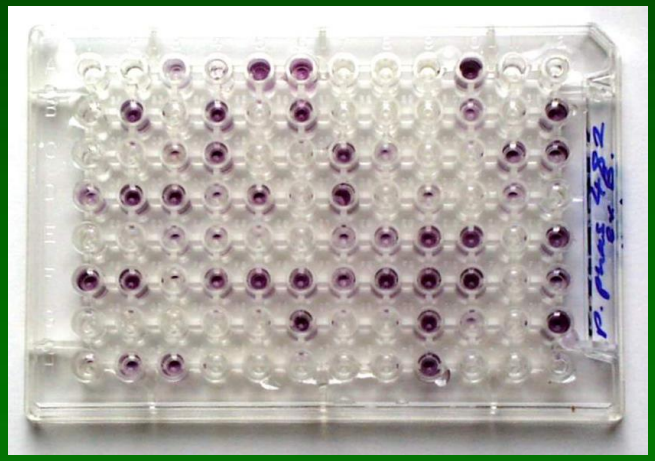


Mortality of *P. elenans*L1 y L2, 35 days after inoculation with *B. popilliae* (292) 1x10^9 spores/ml



Quality control / Identification kit

- **Descriptive methods :Bergey´s Manual: (Breed 1954)**
- **Metabolism of carbohydrates (Biolog)**
- **API (**Assimilation of carbohydrates; reduction of nitrates)



- **APIZYM (enzimes)**

- **Molecular methods**

Entomopathogenic fungi

- Agostino Bassi, 1834
- >700 species/100 genus
- Multipple environments
- Commercial formulations

Epizootics

Cordiceps

Nomurea

Entomophthora

Verticillium (*Lecanicillium*)

Hirsutela

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Difficult to mass produce

Widely used

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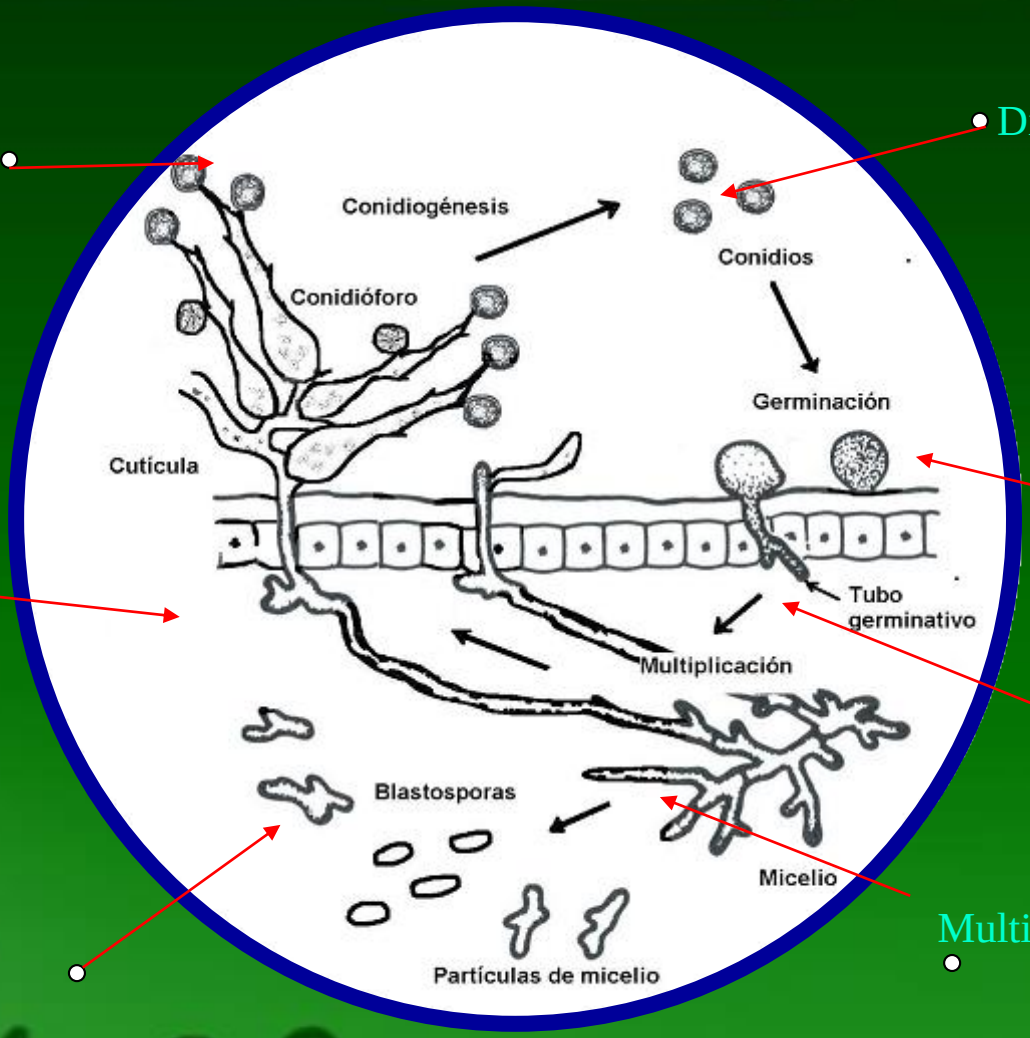
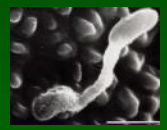
Life cycle of an entomopathogenic fungus (Deuteromicete)



Sporulation

Disemination



Germination

death

Penetration

Toxin production

Multiplication

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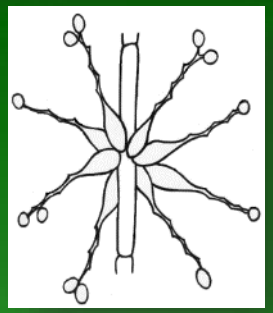
Beauveria bassiana



Conidiogenous cells:

- base swollen
- conidia borne on denticles

Denticulate rachis is the ***only*** essential diagnostic feature defining this genus

Controls:

Coleoptera-Lepidoptera

Homoptera-Hemiptera

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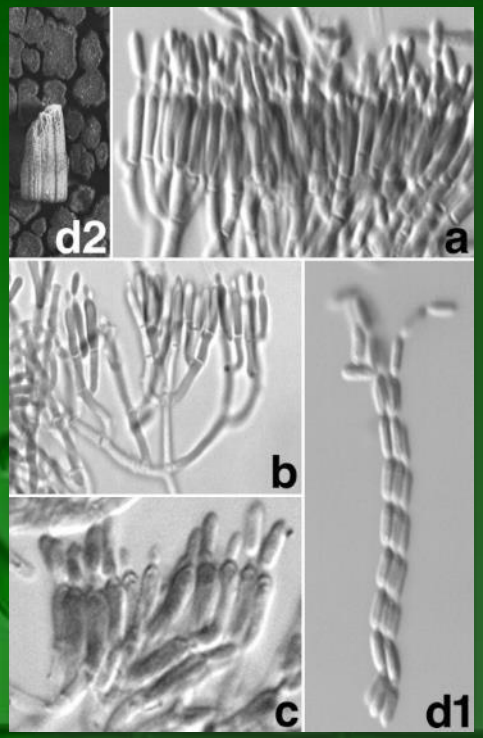
Control of *Cosmopolites sordidus* with *Beauveria bassiana*

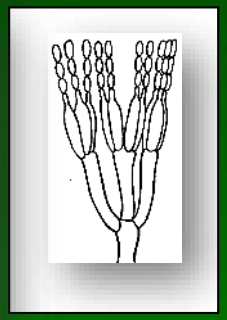
Application method: Pseudostem traps

Approx dose:

10^9 conidia/trap

Metarhizium anisopliae





Conidia:

- in laterally adherent chains (forming prismatic columns or solid plates)
- cylindrical (with waist)

Conidiophores:

- Candelabrum-like branching

Controls:

Coleoptera-Lepidoptera

Homoptera-Hemiptera

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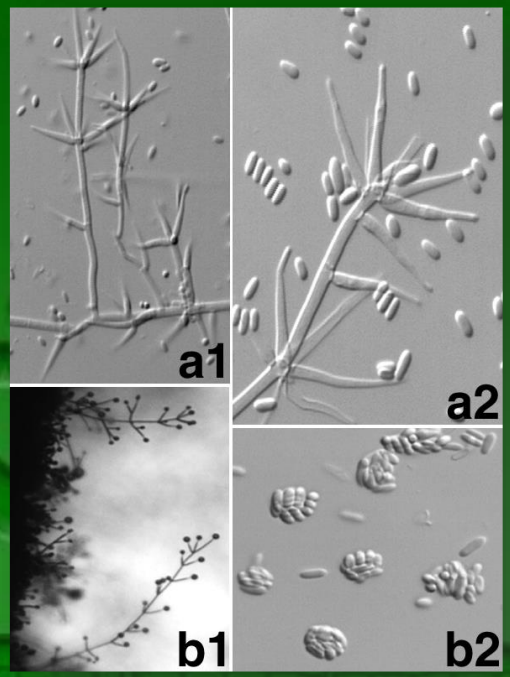
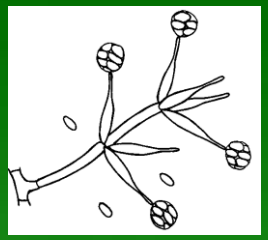
Lecanicillium lecanii

Conidiophores bear pairs or whorls of awl-like phialids

Conidia born in apical slime balls





Controls:

Aphids, scale insects

Whitefly, Coffee leave rust

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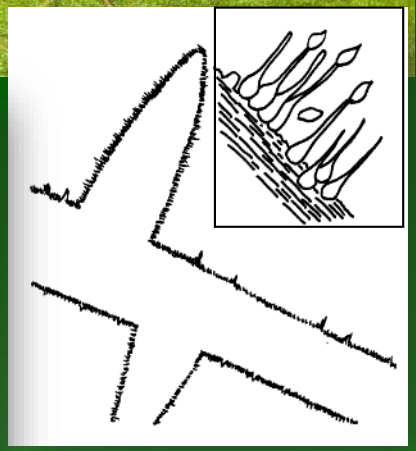
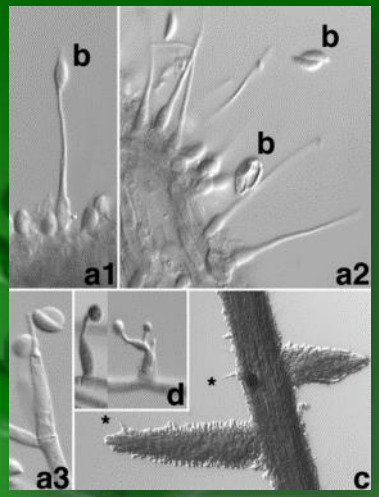
Hirsutella spp.





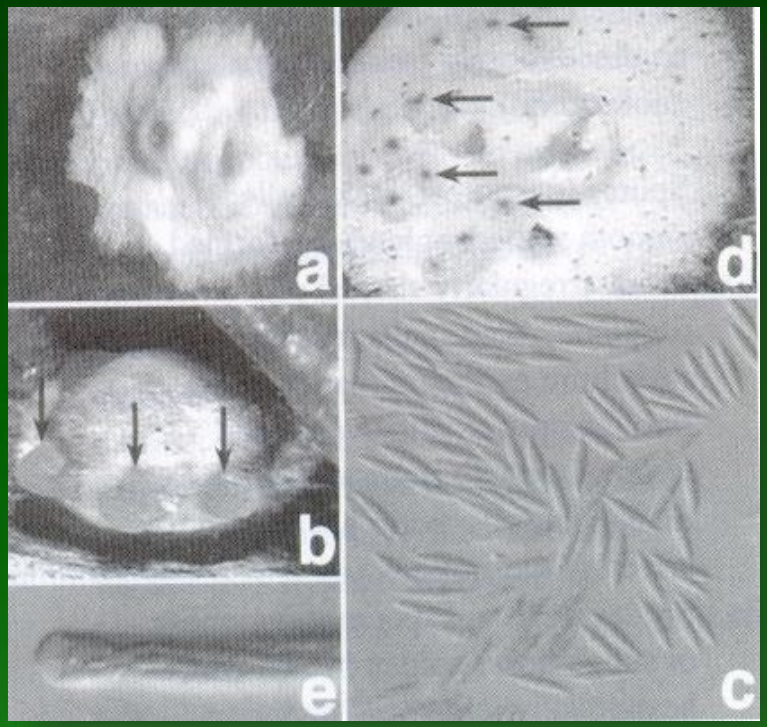
- Conidiogenous cell with
- swollen base and long, narrow neck
- Conidia usually in small slime drop or with slime coating
- Synnematous

Controls:

Mites,

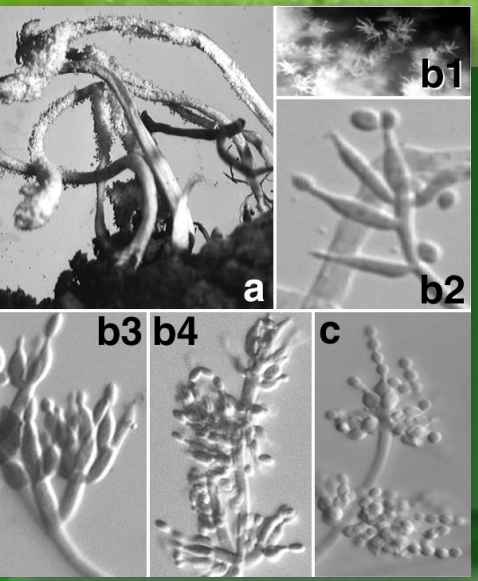
Coleoptera-Homoptera

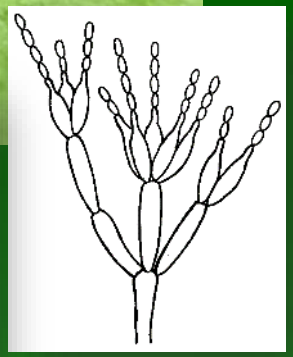
Aschersonia



- Conidia in ascas (limospores)
- Attacks scale insects

Paecilomyces fumosoroseus





- Phialids with distinct neck
- Conidia in chains in divergent clusters
- Synnematous or mononematous
- Last monograph was Samson (1974)
- Samson now moving to reclassify Section Isarioidea back into *Isaria*

Controls:

Hemiptera, Coleoptera, Lepidoptera

P. lilacinus: Nematodos

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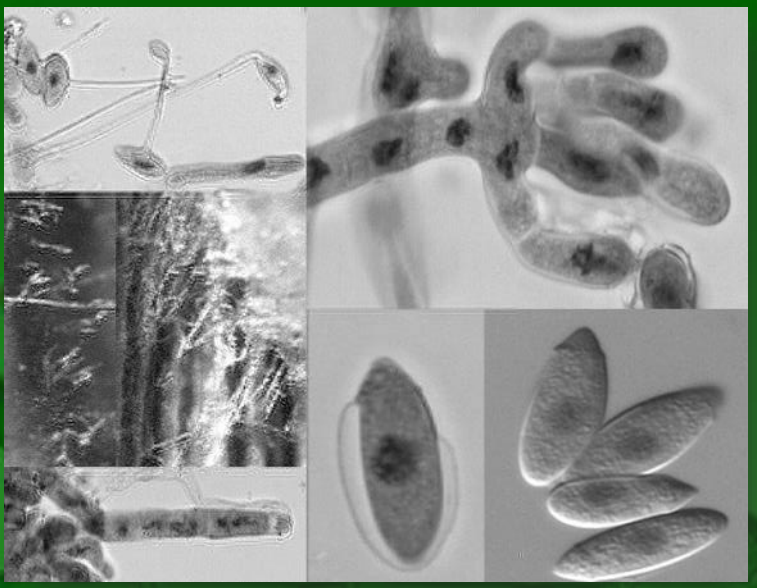
Zygomycetes

- **Entomophthorales**
  - More than 20 genus
    - Can cause epizootics
    - Presence of rhizoids and papila conidia



Controls:
Diptera, Ortoptera, Homoptera, Mites

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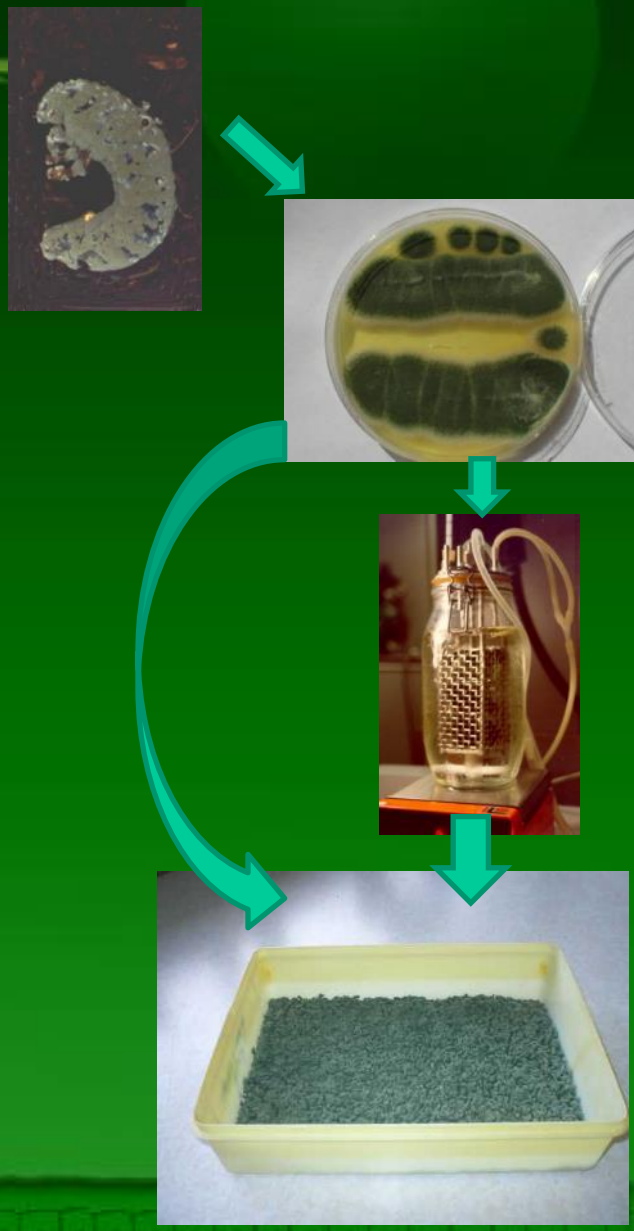
What affects their efficacy?

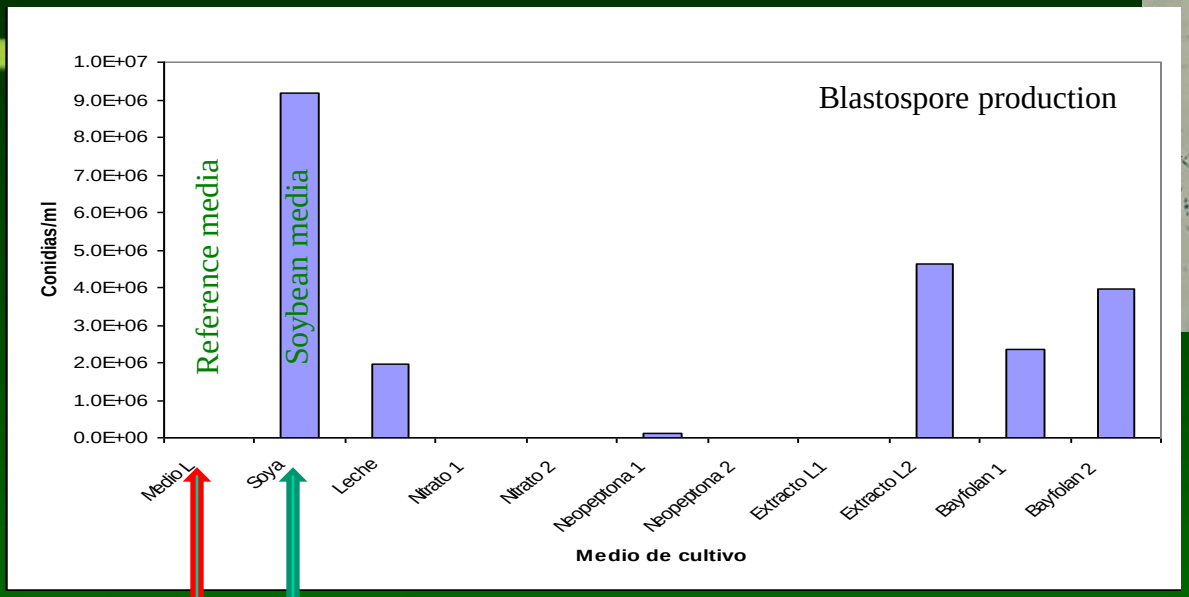
- UV radiation
- Mixing with fungicides
- Alkaline pH
- High temperatures
- Poor quality of spraying

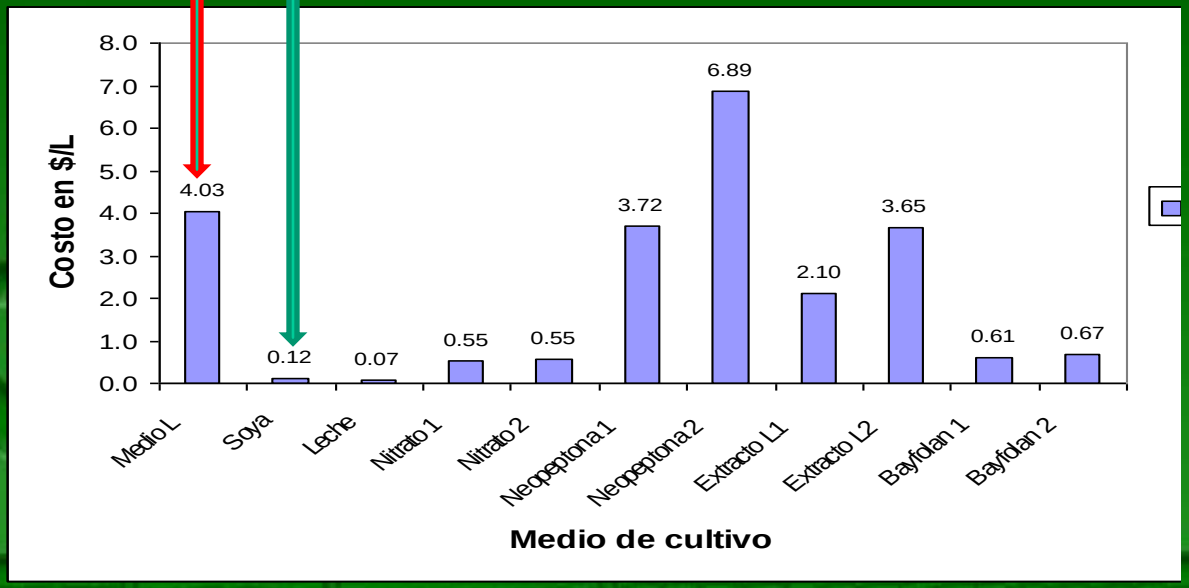
How to produce entomopathogenic fungi

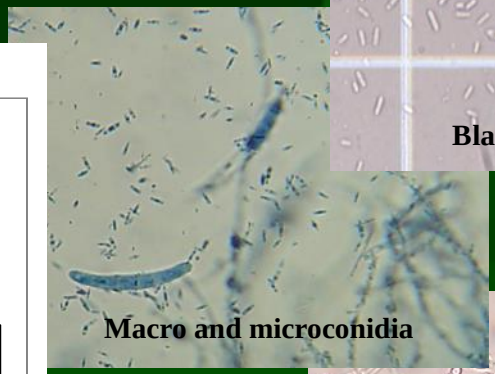
1. Isolate from insect or directly from soil using bait insects or media with antibiotics
2. Use monosporic isolates to avoid contaminants and assure genetic homogeneity
3. Inoculate the solid substrate (rice) with a conidial suspension of aprox. $1x10^6$ con/ml, 10ml per 100g of substrate in polypropylene bags.
4. Incubate at 24-26 ^{0}C for 10 to 15 days
5. For large production, multiply the inoculum with a 3 day liquid fermentation in nutrient broth
6. Some fungi produce low amount of conidia or need a longer period of time to produce.

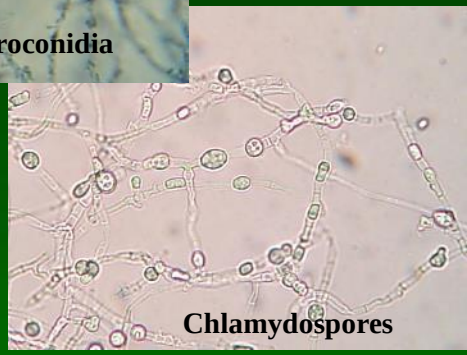


Production of blastospores vs cost/litre using different liquid media









Advantages

- Faster than solid fermentation (3-7 days)
- Works for fungi of difficult production
- Some produce better in low cost media

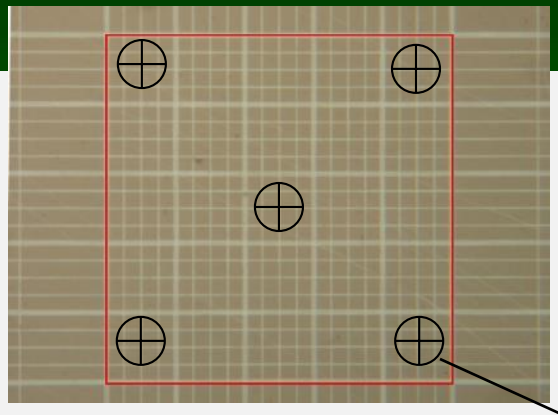
Disadvantages

- Higher risk of contamination
- Need to adjust media accordingly
- Some fungi do not produce blastospores

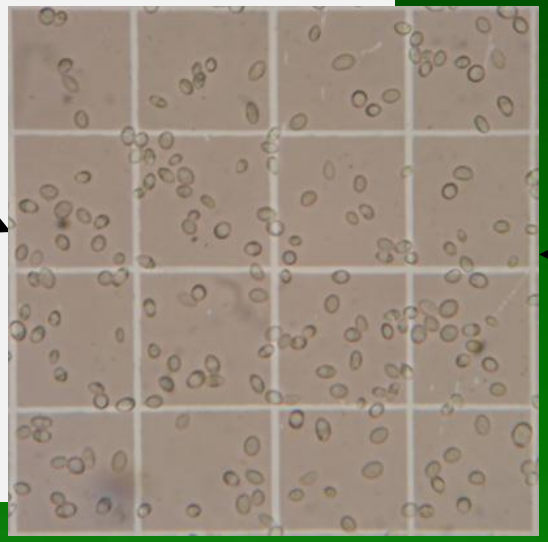
Quality Control

Check for:

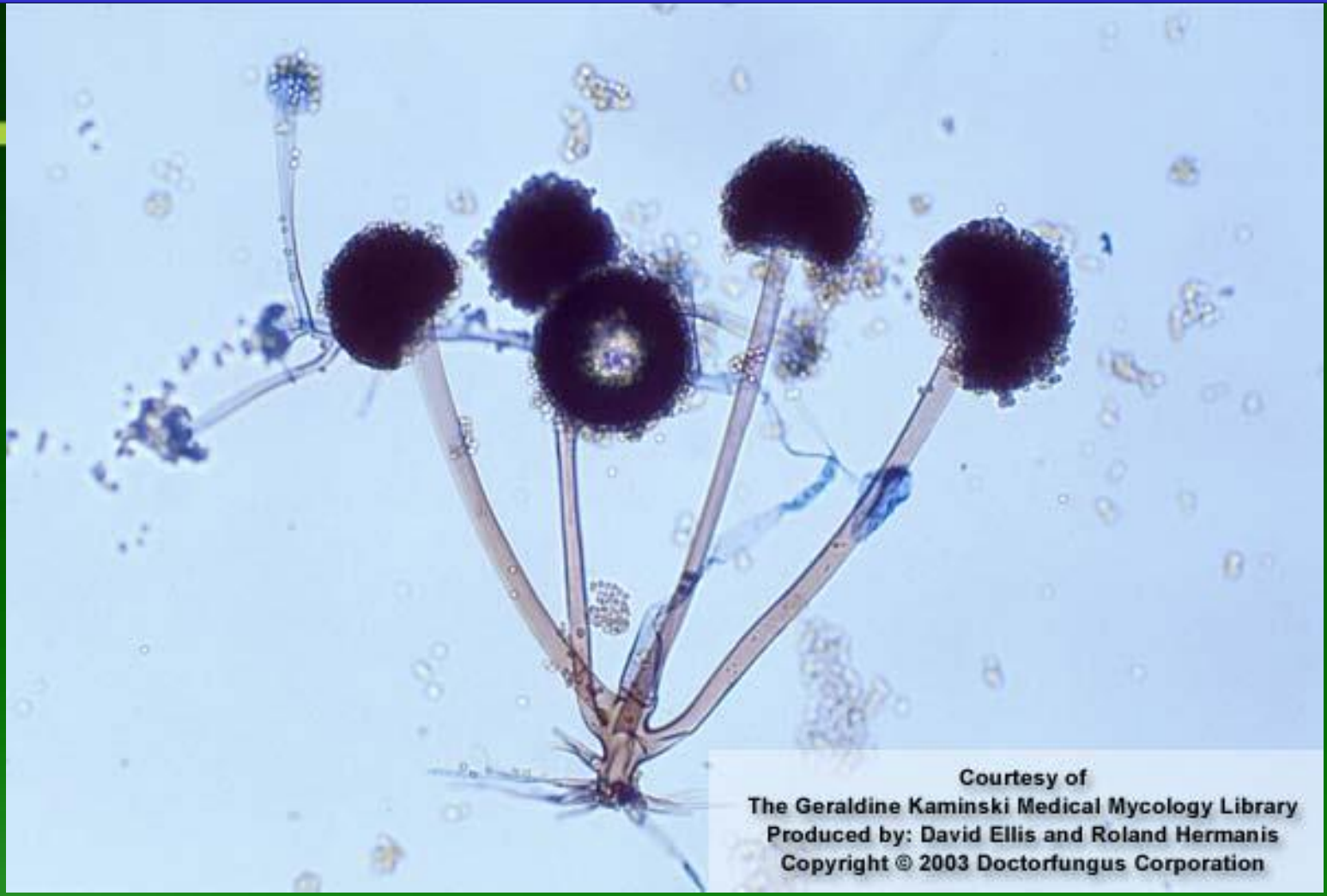
- Concentration
- Viability
- Virulence
- **Contaminants**



Calculate concentration using a haemacitometer



Rhizopus oryzae



- **Genus/Species:** *Rhizopus oryzae*
- **Slide Reference #: GK 587**
- **Image Type:** Microscopic Morphology
- **Disease(s):** Systemic Zygomycosis (Mucormycosis)

Rhizopus oryzae

- **Slide Reference #: GK 582**
- **Disease(s):** Rhinocerebral zygomycosis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Thank you