Fungos entomopatológicos e outros patógenos no controle biológico de insetos

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Entomopathogenic fungi and other insect pathogens in biological control

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Proposed programme

- Pathogens causing diseases in insects:
  - entomopathogenic fungi,
  - entomopathogenic virus,
  - entomopathogenic bacteria,
  - entomopathogenic microsporidia.
- General aspects of entomopathogens.
- Successful examples:
  - Migratory locusts,
  - Scarabaeidae.

Entomopathogens

Pathogens

cause *infectious diseases* → taking effect to the organism by pathogenic micro-organisms or virus

One host individual is enough for successful replication,

they cannot search and cannot directly attack their host

Invertebrate pathology

- milestones in invertebrate pathology and microbial control:
  - 1834 Agostino BASSI - ("contagious microbes, causing infectious diseases")
  - 1835 A. BASSI – "Del mal segno, calcinaccio o moscardino, malattia …" = "white Muscardine" (⇨ later: *Beauveria bassiana*, described by Giuseppe Gabriel Balsamo-Crivelli and later revised by Jean Paul Vuillemin)

Beauveria bassiana

Invertebrate pathology

- milestones in invertebrate pathology and microbial control:
  - 1834 Agostino BASSI
  - 1835 A. BASSI
  - 1870 L. PASTEUR – “Études sur la maladie des vers à soie”
  - ….
  - 1949 E.A. STEINHAUS – “microbial control”
  - ….

Types of pathogens

- potential pathogens: are pathogenic but incapable of invading the insect host
- facultative pathogens: are pathogenic but they can live and multiply independently from insect host; e.g.: bacteria or fungi ⇨ **extra**-cellular development ! (in “medium”)
- obligate pathogens: require live insect hosts for survival and replication; e.g. virus or microsporidia ⇨ **intra**-cellular development ! (in “living cell”)

Epidemiology

- enzootic disease

  is a disease which is constantly in a population, usually in low prevalence

- epizootic disease

  is an outbreak of a disease, usually in large number of cases

Insect pathogens

Virus

Bacteria

Fungi

Microsporidia

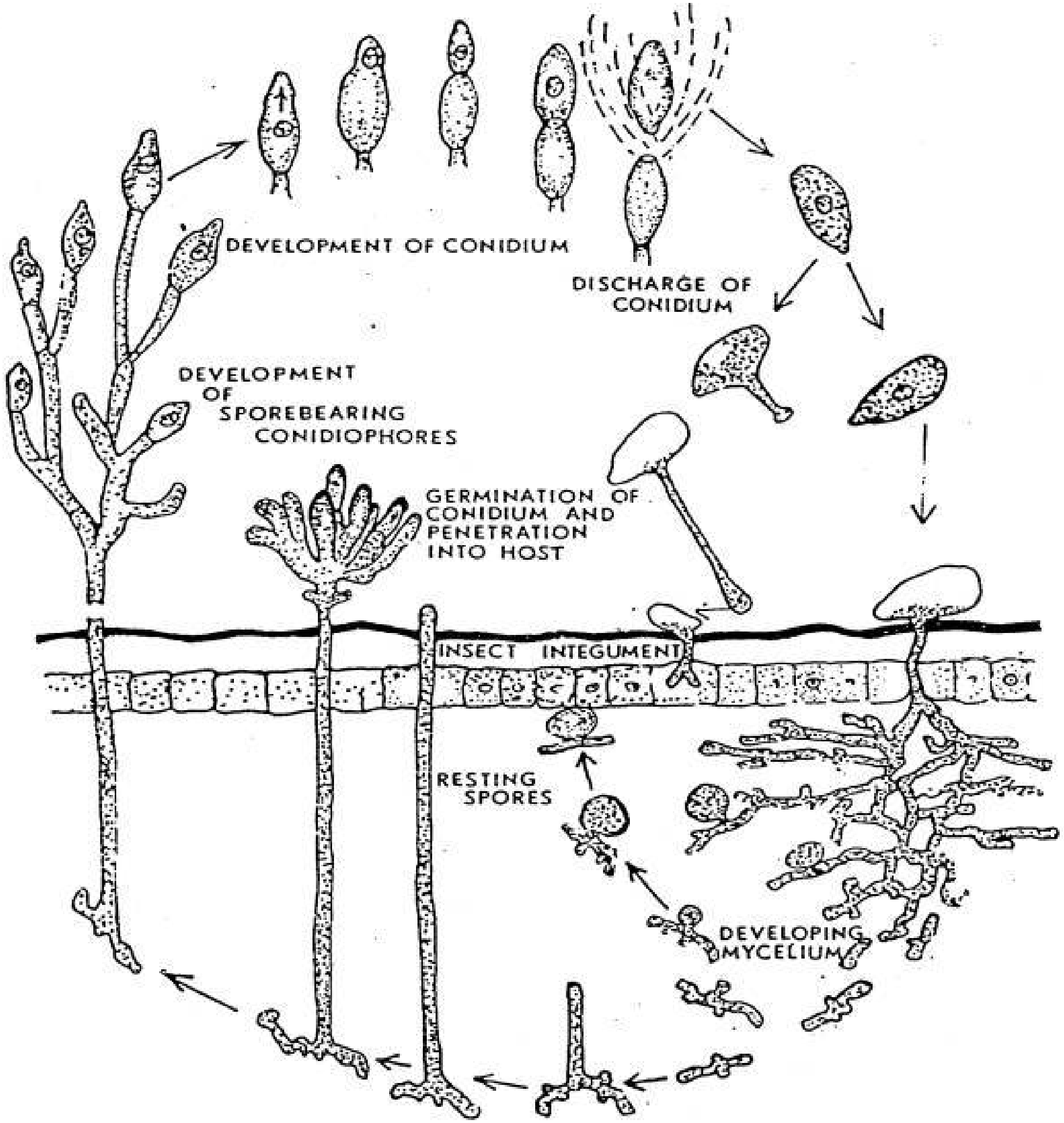
<u>Fungi</u> in insects

- Different fungi occur associated with insects:
  - symbiotic fungi (e.g. ambrosia beetles)
  - saprophytic fungi (“everywhere”)
  - **entomopathogenic fungi !**

Entomopathogenic Fungi (1)

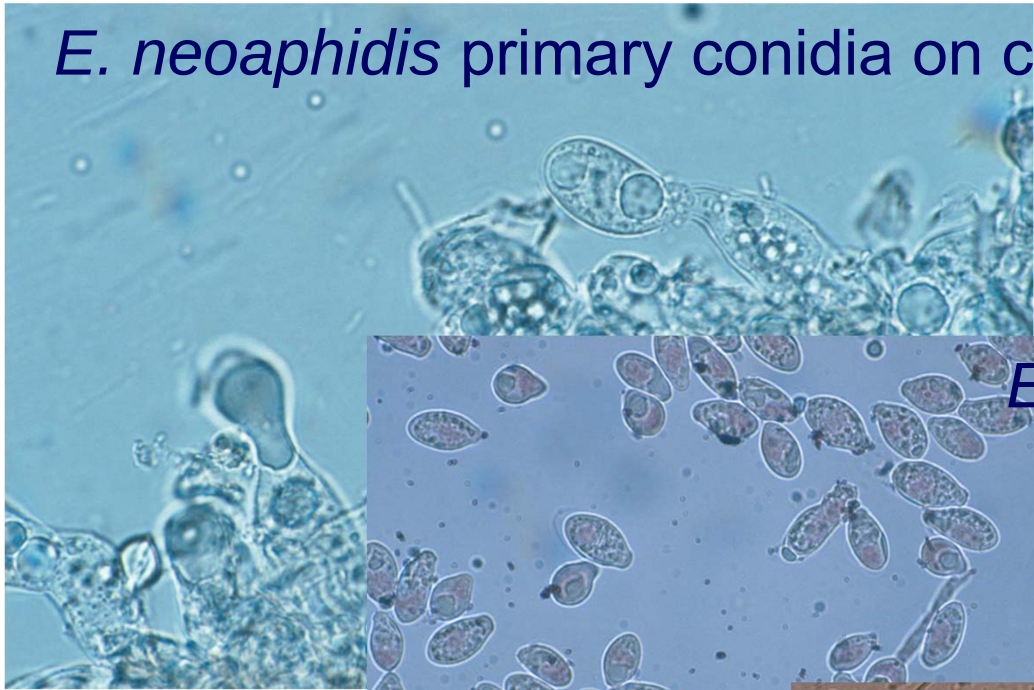
- Zygomycota
  - *Empusa* spp., *Entomophthora* spp., *Entomophaga* spp., *Zoophthora* spp., …
- Ascomycota
  - *Cordyceps* spp.
- former Deuteromycota are → mainly Ascomycota
  - *Beauveria* spp., *Metarhizium* spp., *Isaria* spp.

Entomophthorales infection

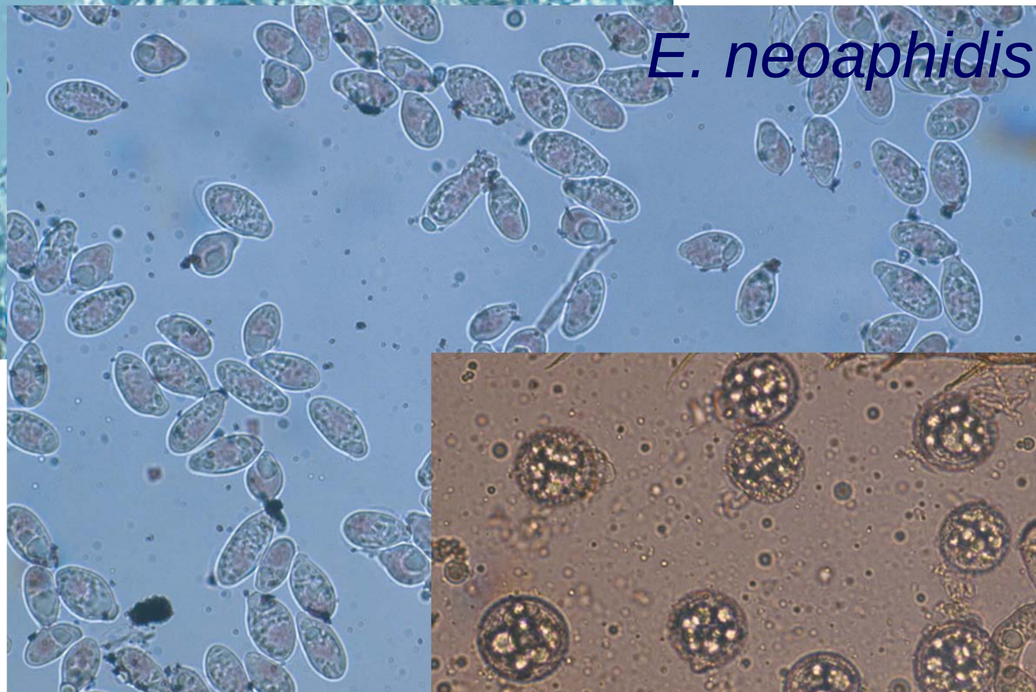


Anoecia corni infected with *Zoophthora aphidis*

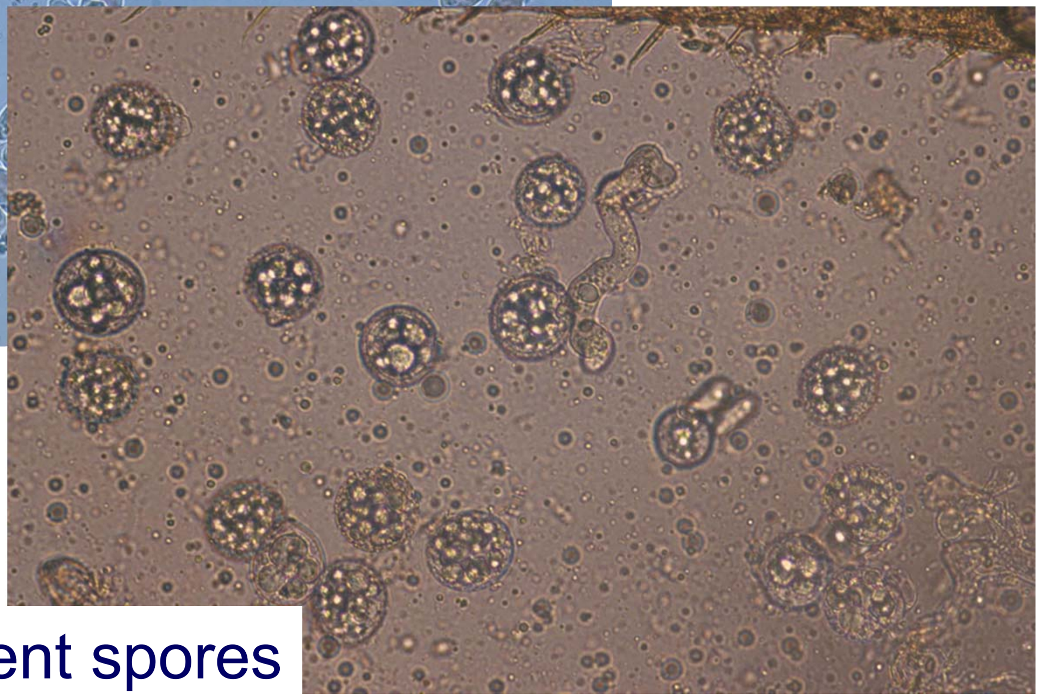
Myzus persicae infected with *Erynia neoaphidis*



E. neoaphidis primary conidia on conidiophores



E. neoaphidis primary conidia

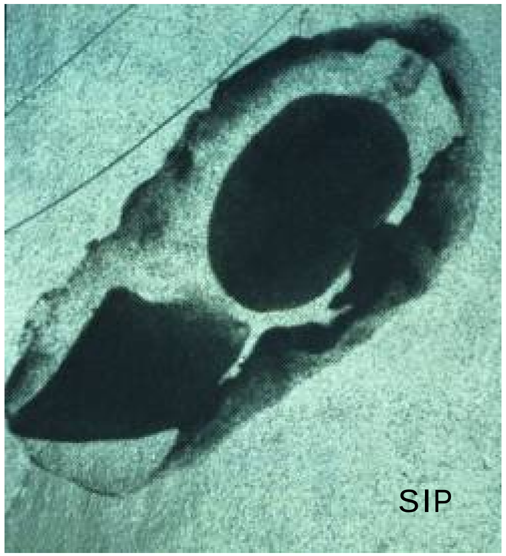


E. neoaphidis permanent spores

Control of *Lymantria dispar*

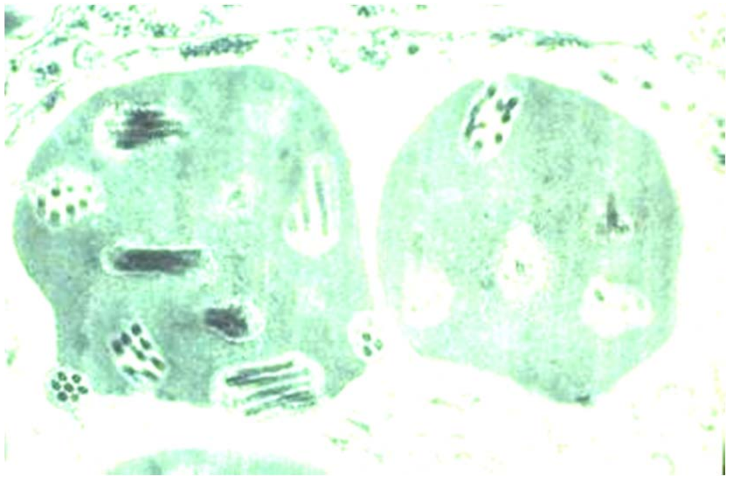
Arsenic; DDT, Carbaryl; Pyrethroids; Dimilin - no long term control!!!

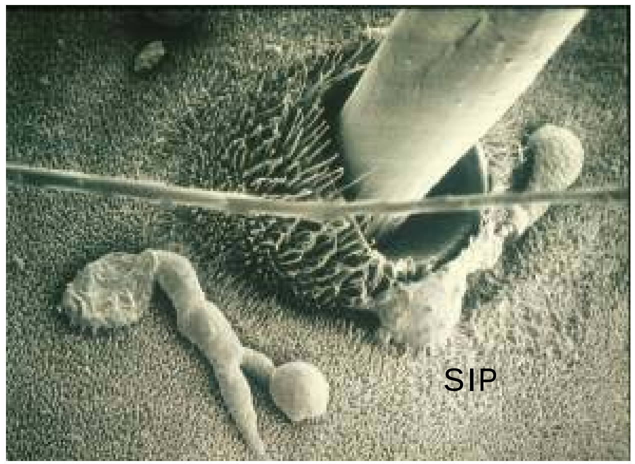
NPV and Bt applications were very successful



Control of *Lymantria dispar*

- Arsenic; DDT, Carbaryl; Pyrethroids; Dimilin - no long term control!!!
- NPV- and Bt-applications were very successful
- USA: best control effect using the entomopathogenic fungus: *Entomophaga maimaiga*





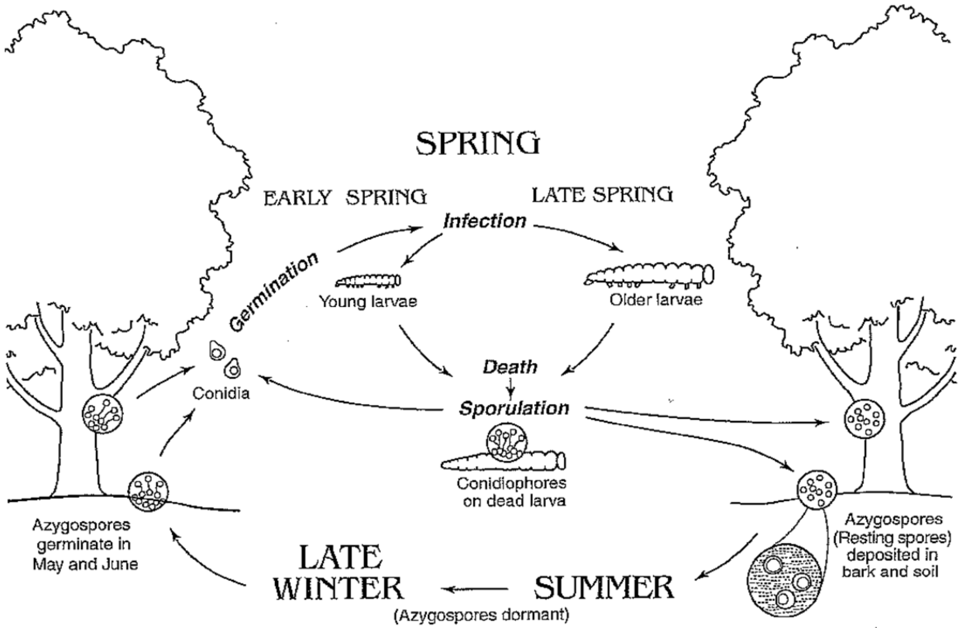


Fig. 12.2 Life cycle of the entomophthoralean fungal pathogen *Entomophaga maimaiga* infecting gypsy moth, *Lymantria dispar*. (Illustration by Frances Fawcett.)

Lymantria dispar + NPV

Lymantria dispar + *Entomophaga maimaiga*

Advantages + disadvantages of Entomophthorales

- **Selective** action of most species.
- **Rapid** epidemics in “colonies”.
- **Active discharge** of conidia.

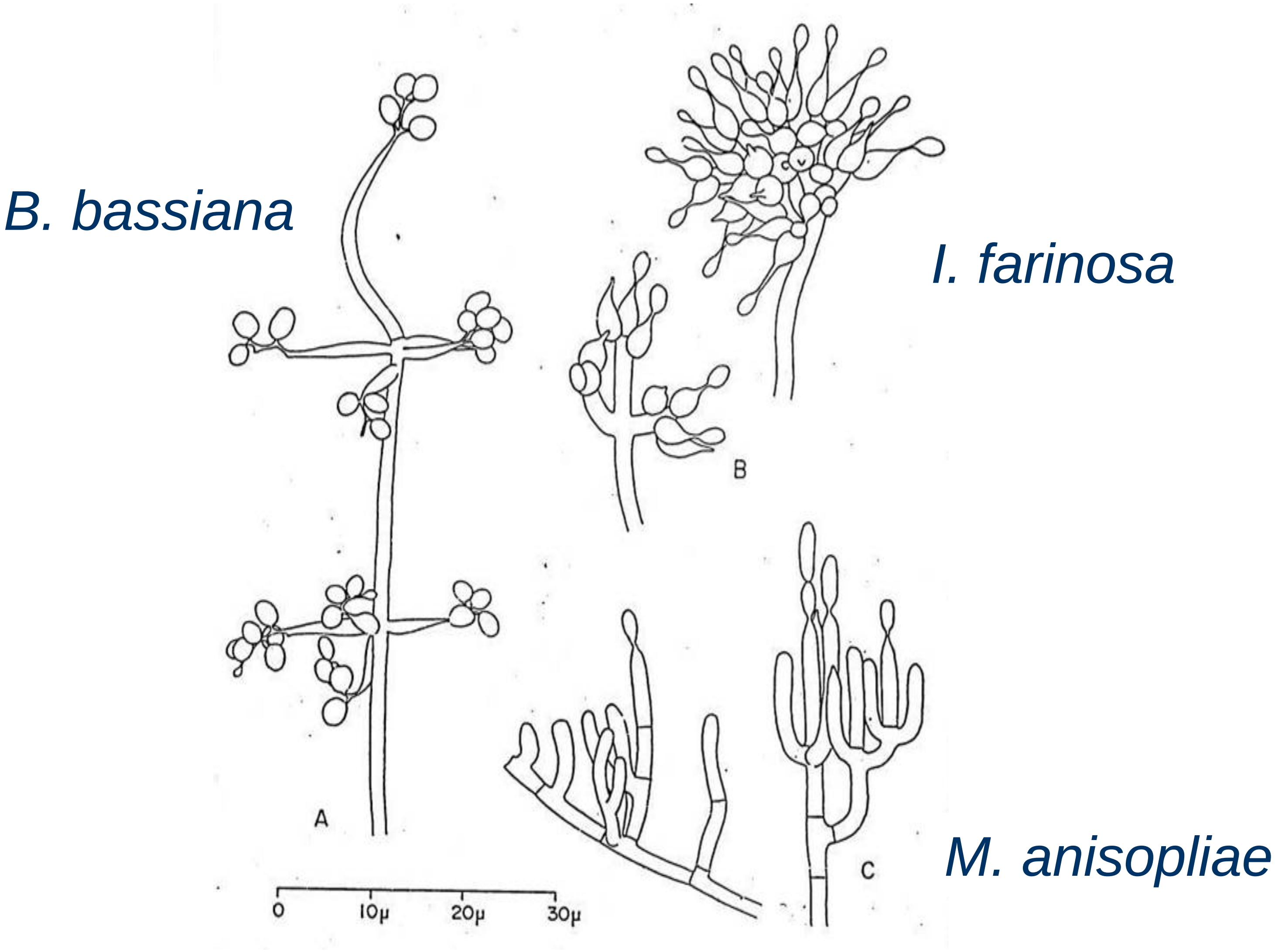
- Complicated in vitro mass production.
- No commercial product available on the market for the moment.

Ascomycota

Cordyceps tuberculata

Cordyceps militaris

Isaria (Paecilomyces) farinosa


B. bassiana
I. farinosa
M. anisopliae
A
B
C
0
10μ
20μ
30μ

Entomopathogenic Fungi (2)

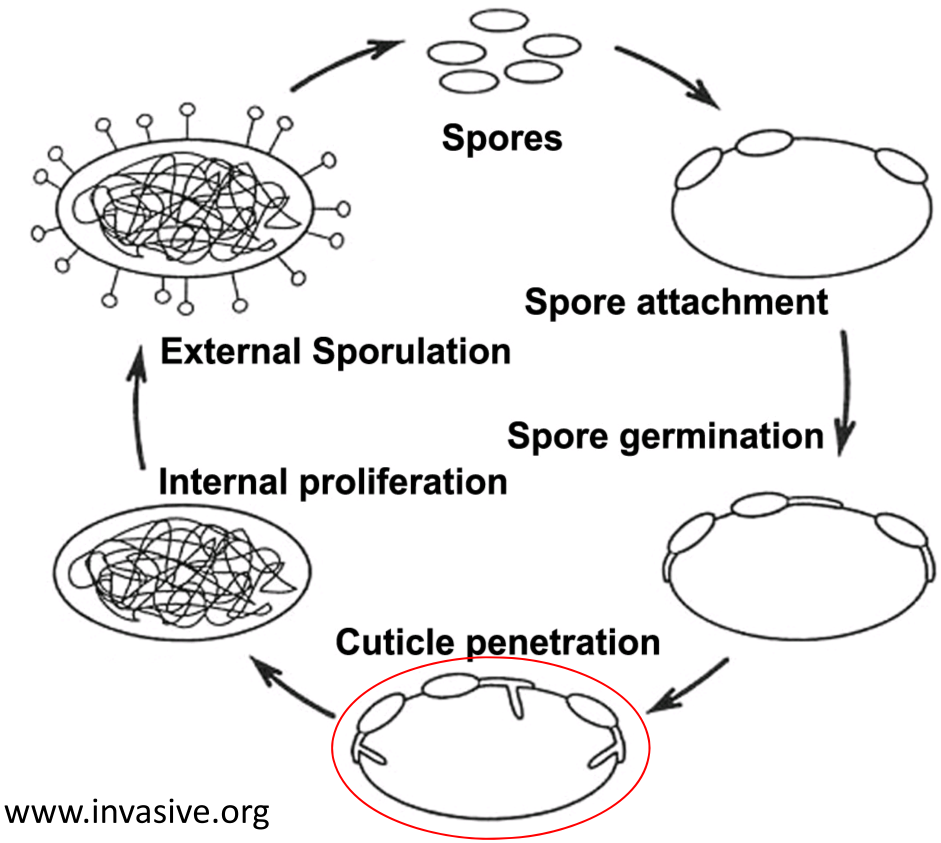
"parasitic" (pathogenic) phase:

- spore inoculation on cuticle surface
- infection via integument
- development in the whole insect (<u>blasto</u>spores)

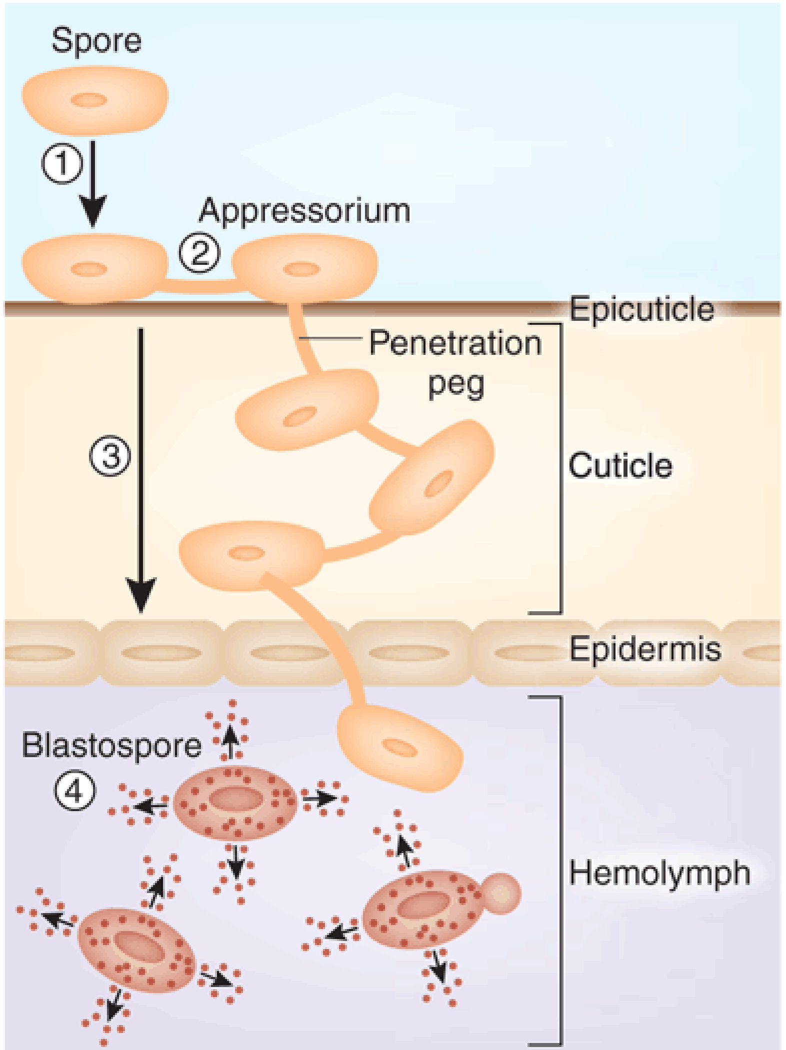
insect dies ! ➔ "saprophytic" phase:

- colonisation of the whole host
- penetration of cuticle and production of <u>conidio</u>spores

Course of infection of EPF

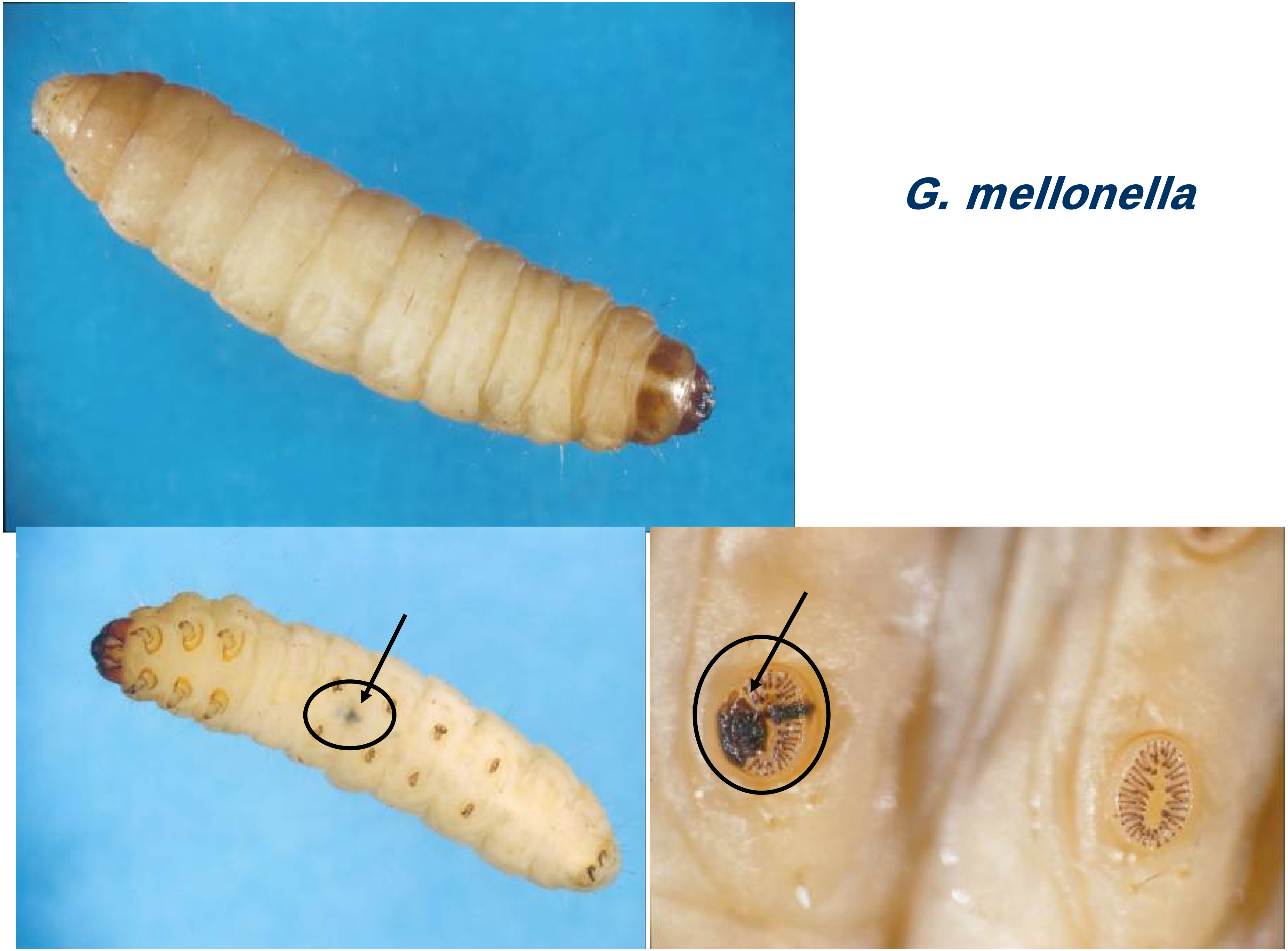


www.invasive.org



www.nature.com

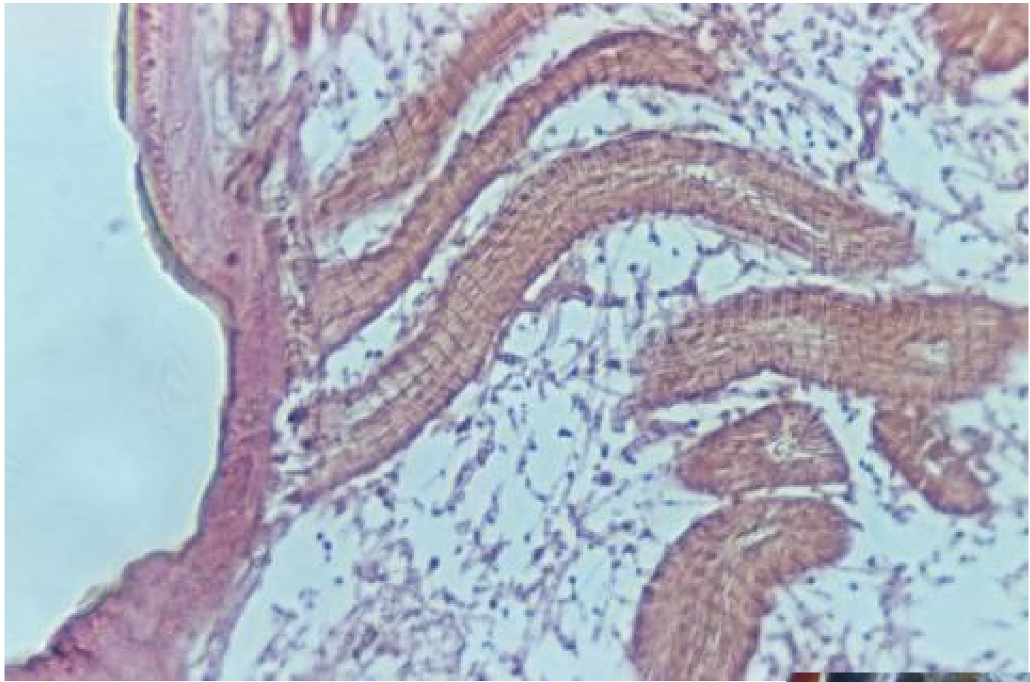
Galleria mellonella + Beauveria bassiana

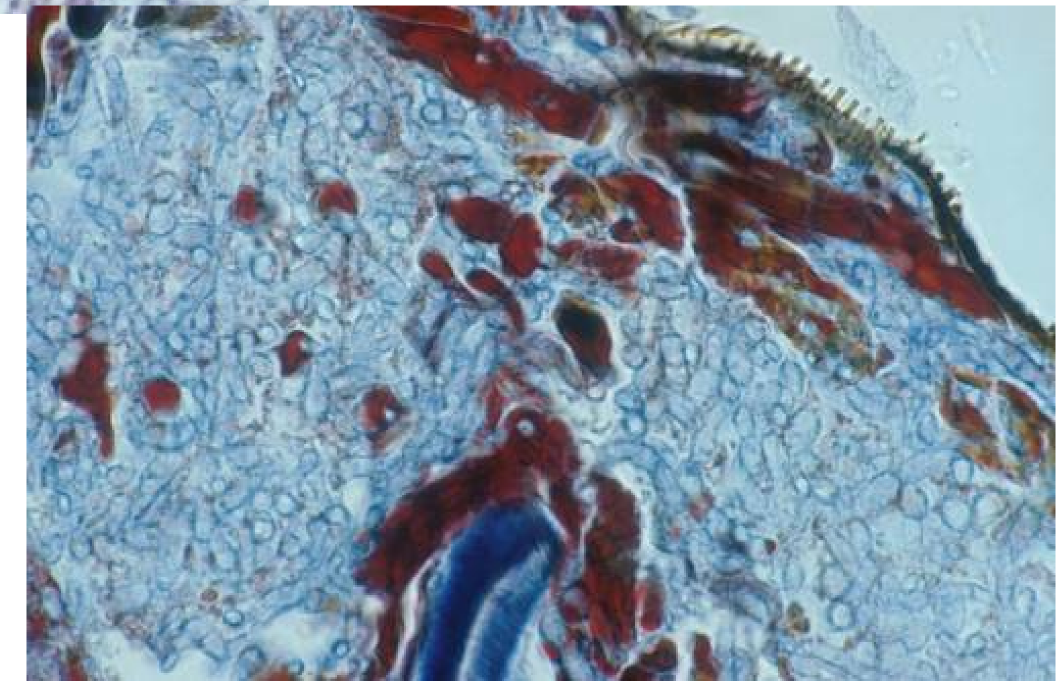


G. mellonella

Elateridae larvae infected with *M. anisopliae*

Ips typographus
+
Beauveria bassiana





Entomopathogenic fungus ”filling“ a host insect

Melolontha melolontha + Metarhizium anisopliae

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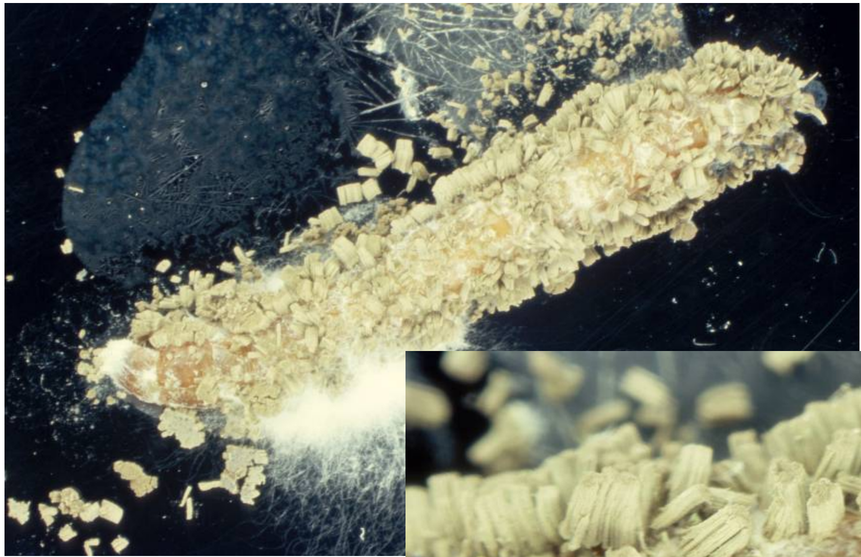
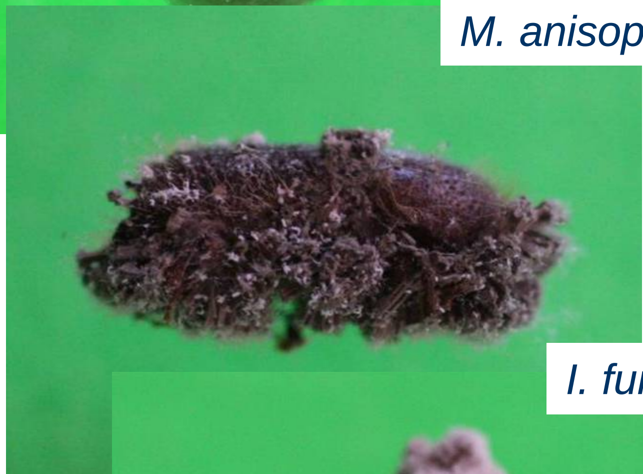
Metarhizium anisopliae on
Tenebrio molitor



B. bassiana



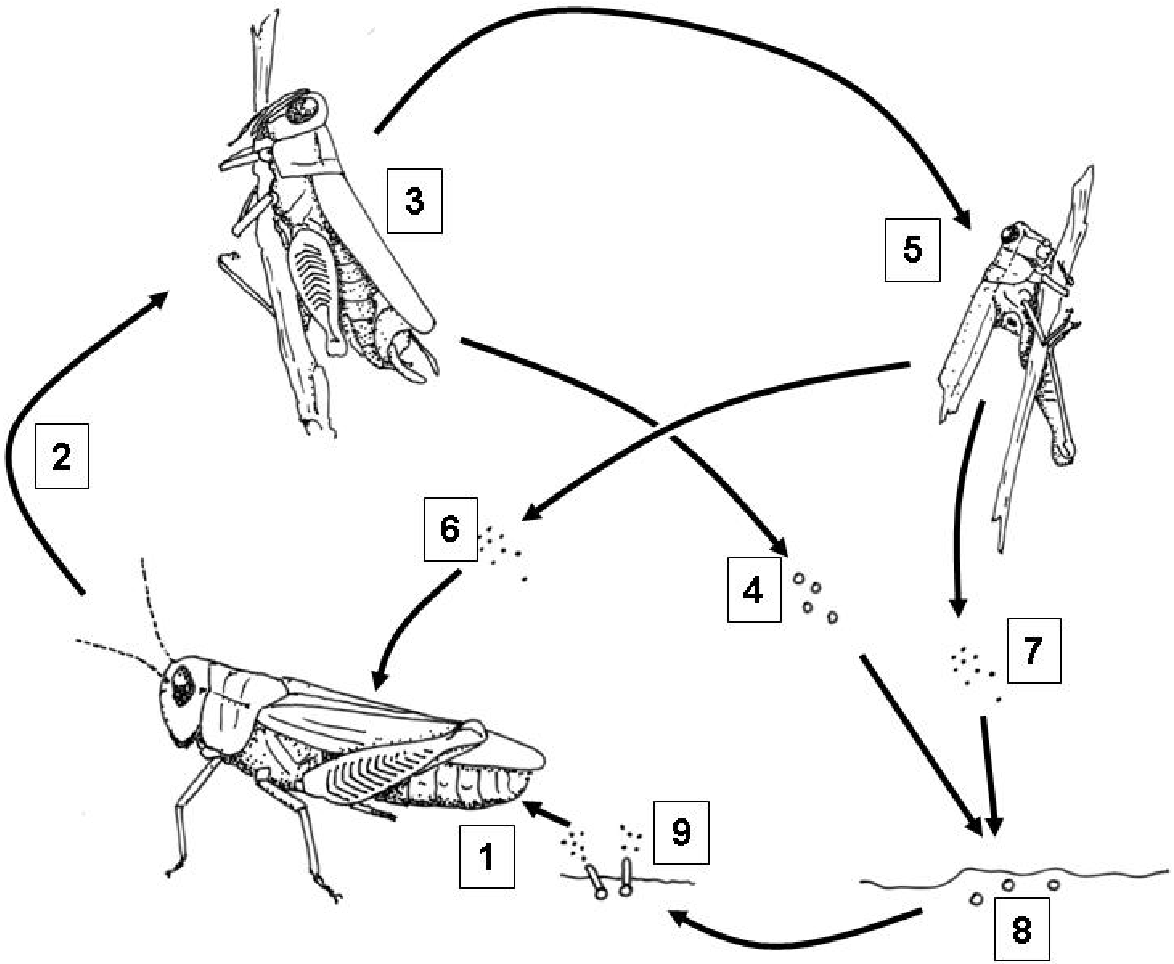
M. anisopliae

I. fumosorosea

B. bassiana

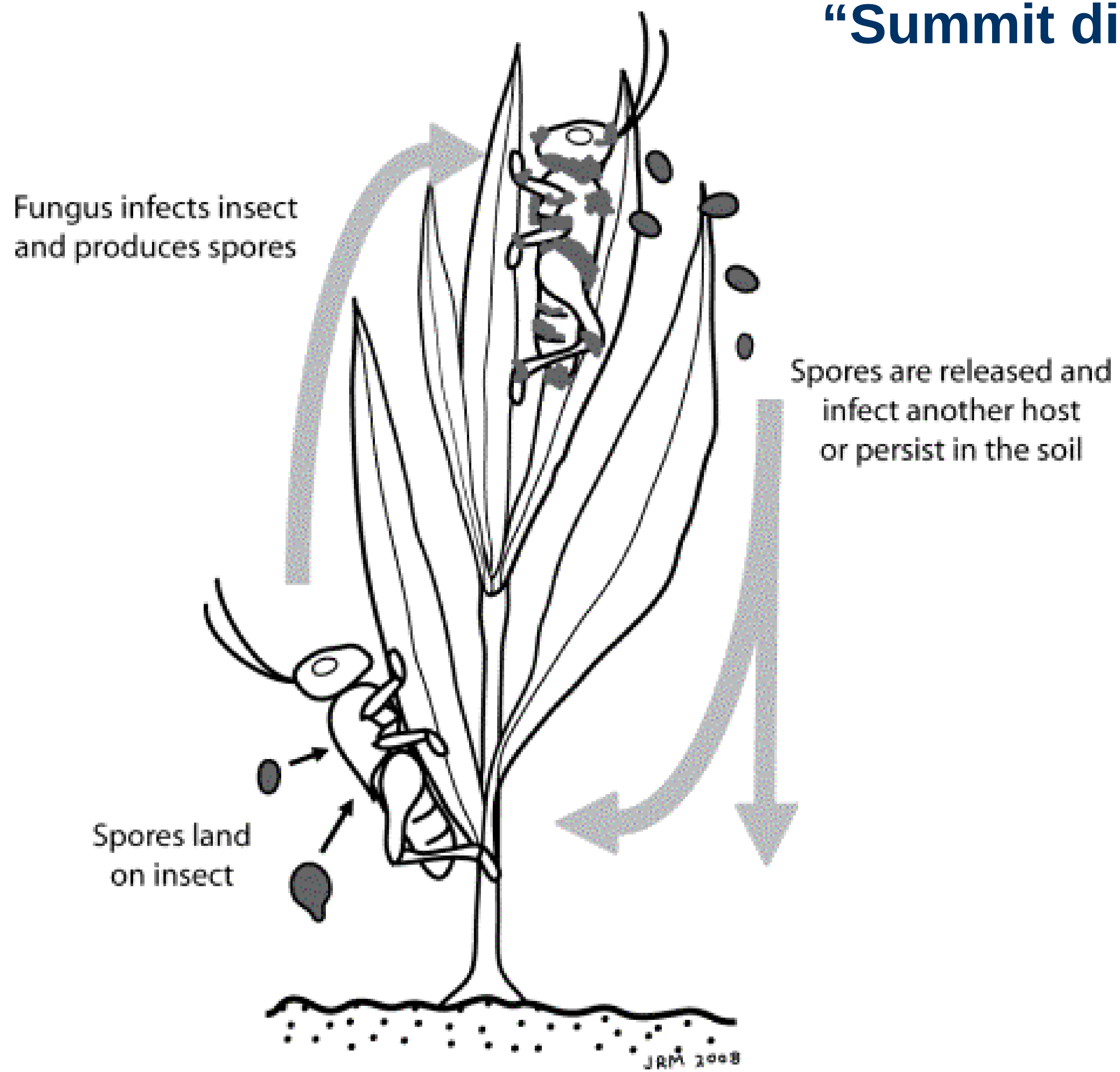
I. fumosorosea

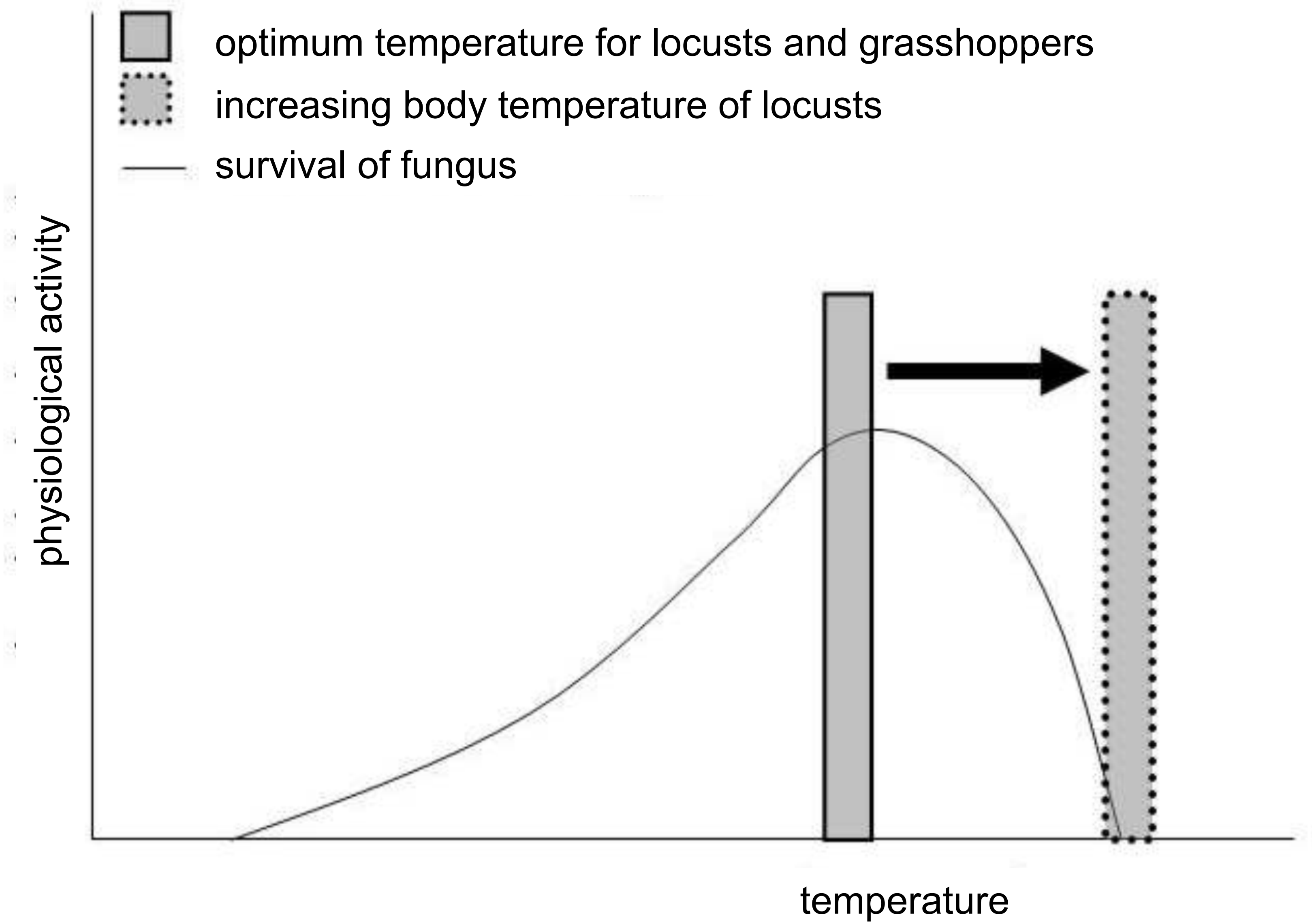
Entomophaga grylli



J. Zettel, Articulata 23, 2008, 43-58

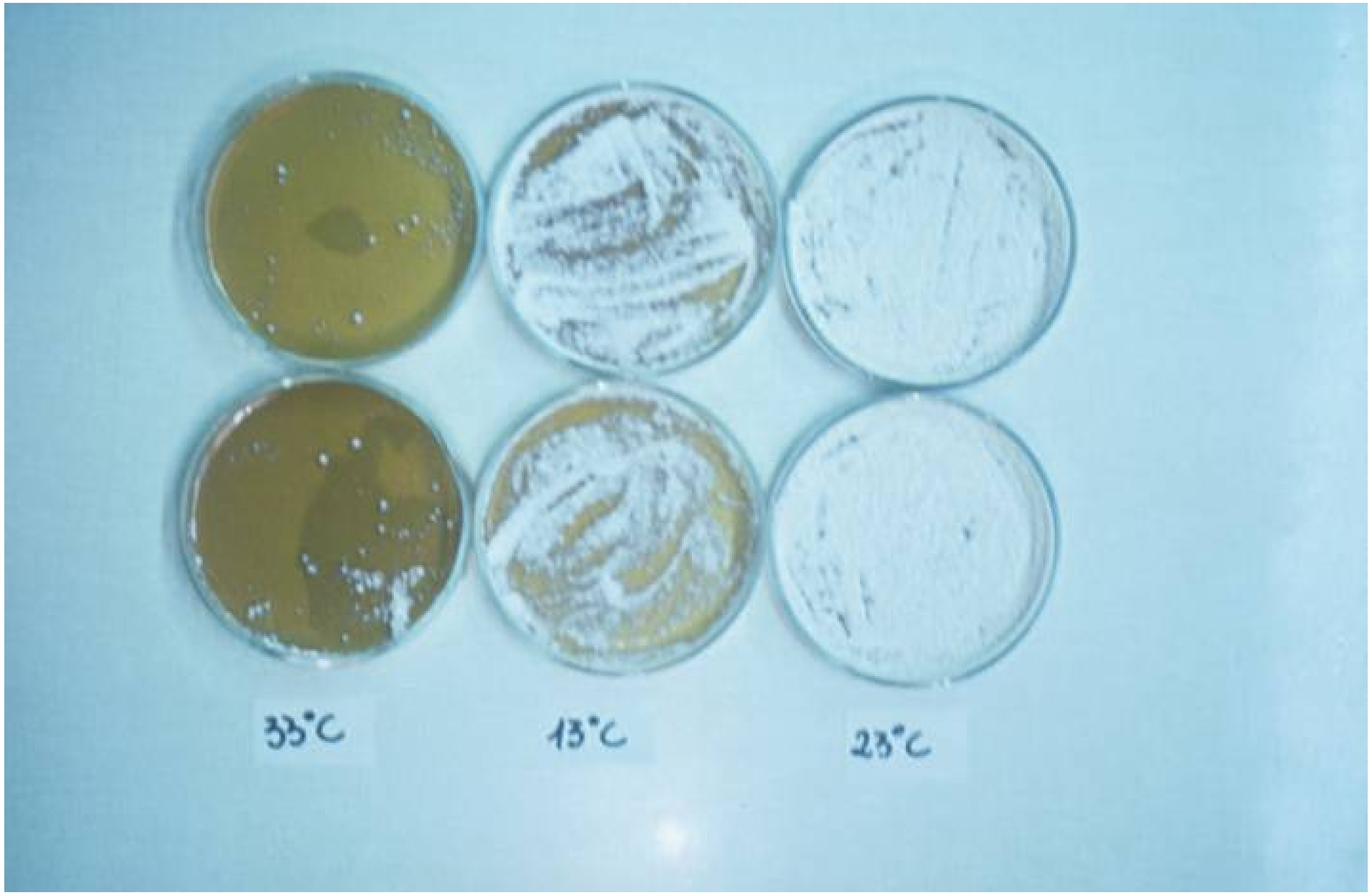
“Summit disease”



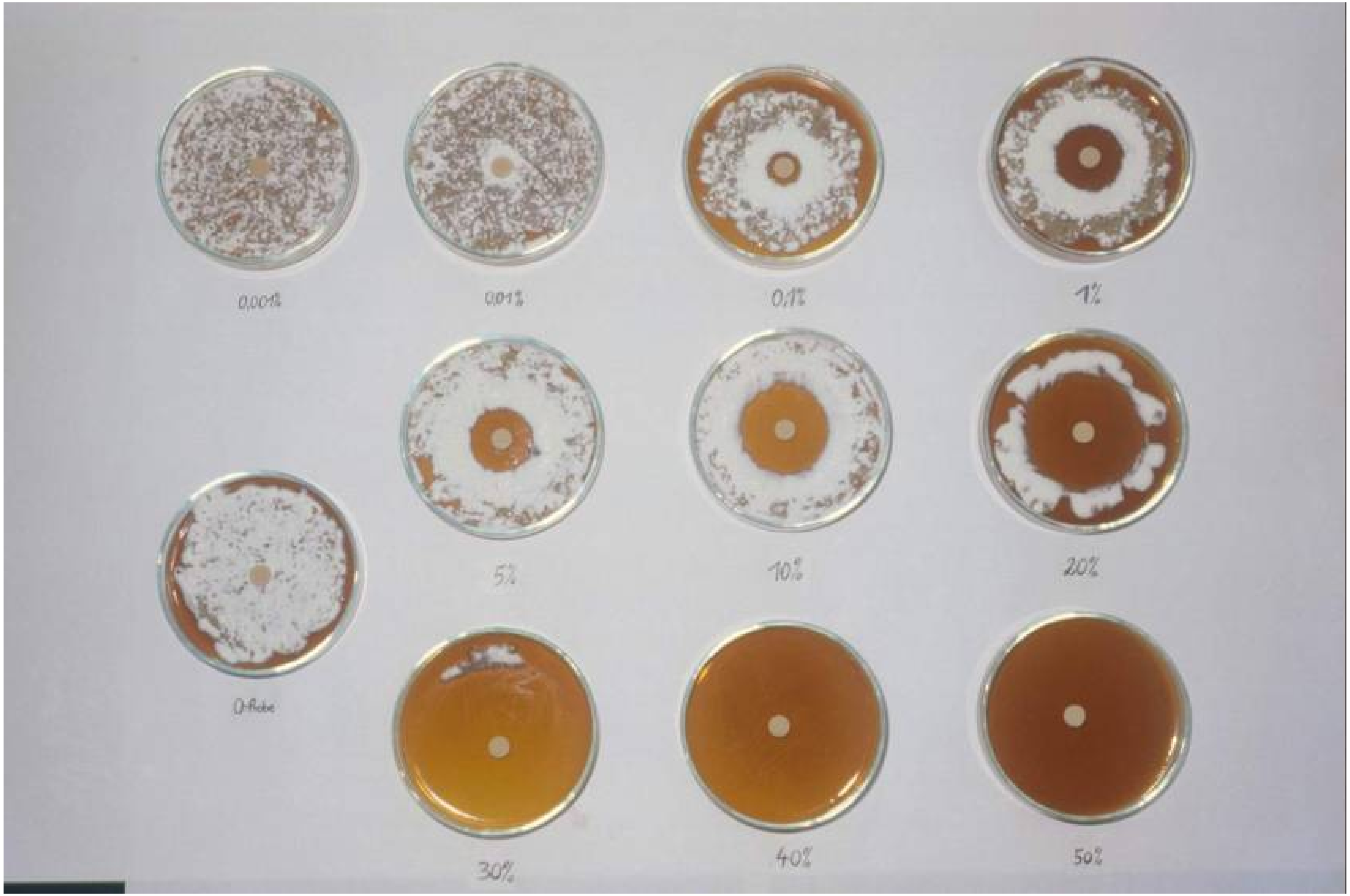


J. Zettel, Articulata 23, 2008, 43-58

Fungal growth depends on temperature



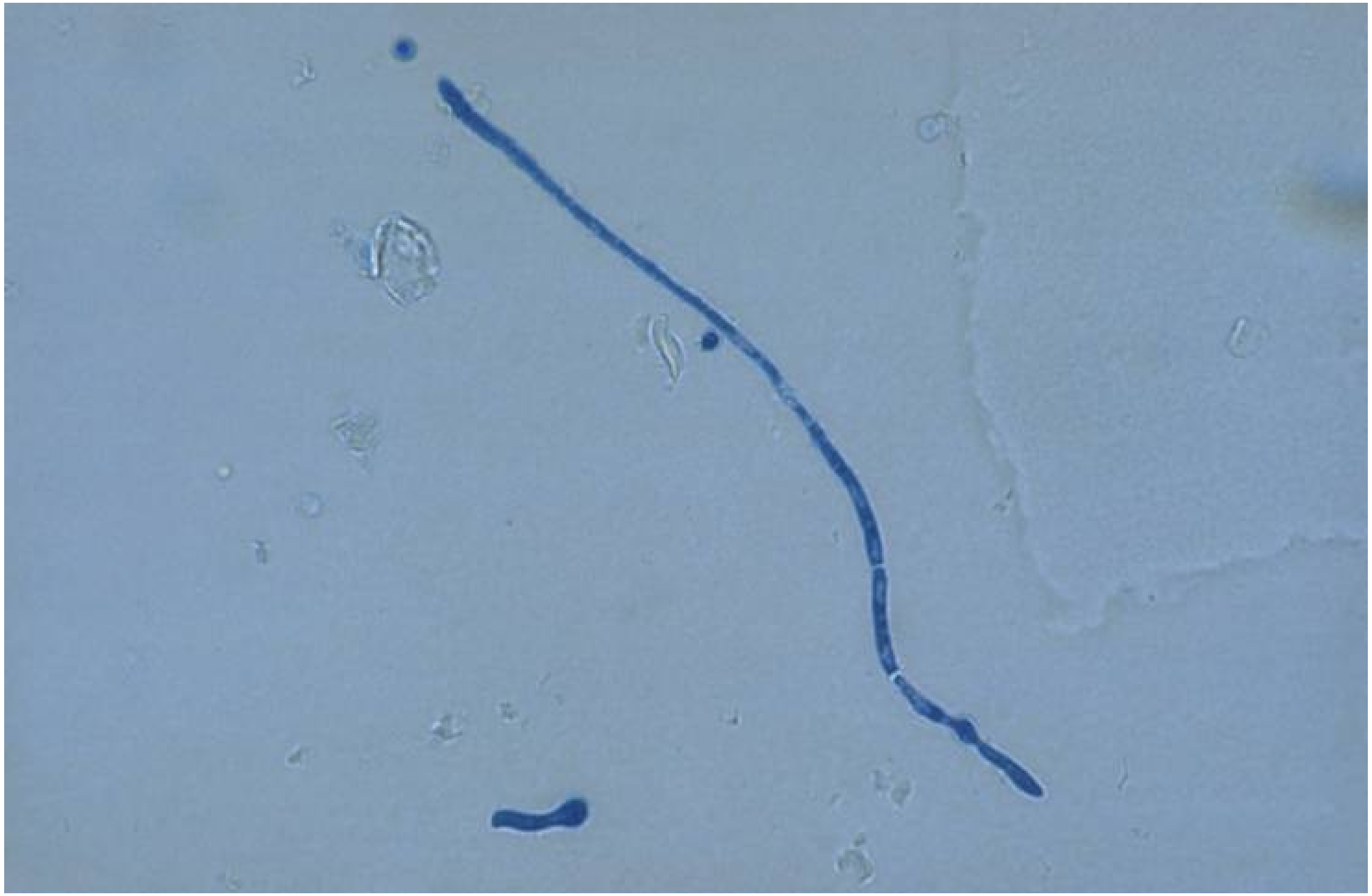
Germination and hyphal growth inhibition of *B. bassiana* by formic acid



Effects of “contaminants”

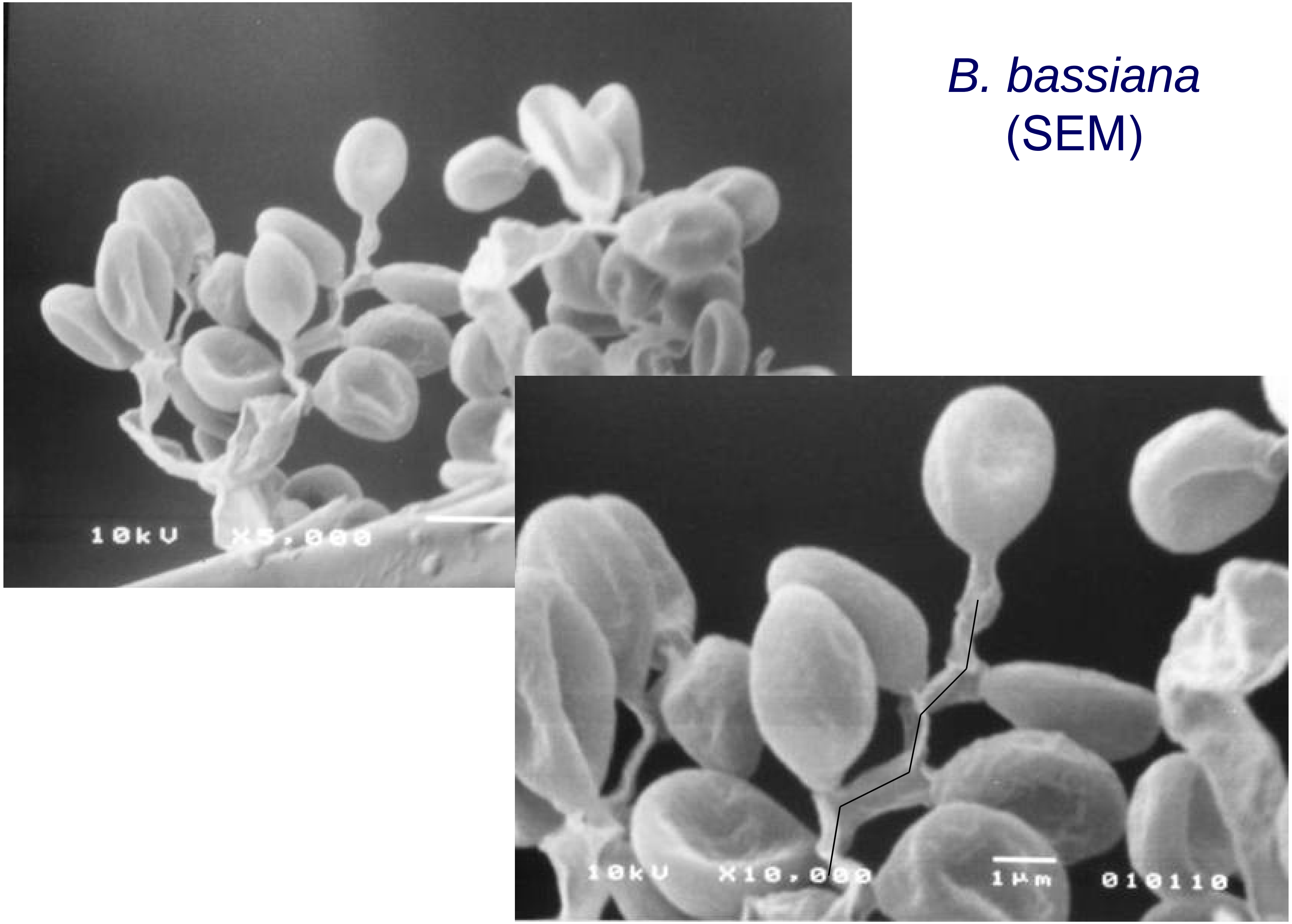
Spore germination

Spore germination and fungal growth



Fungal growth

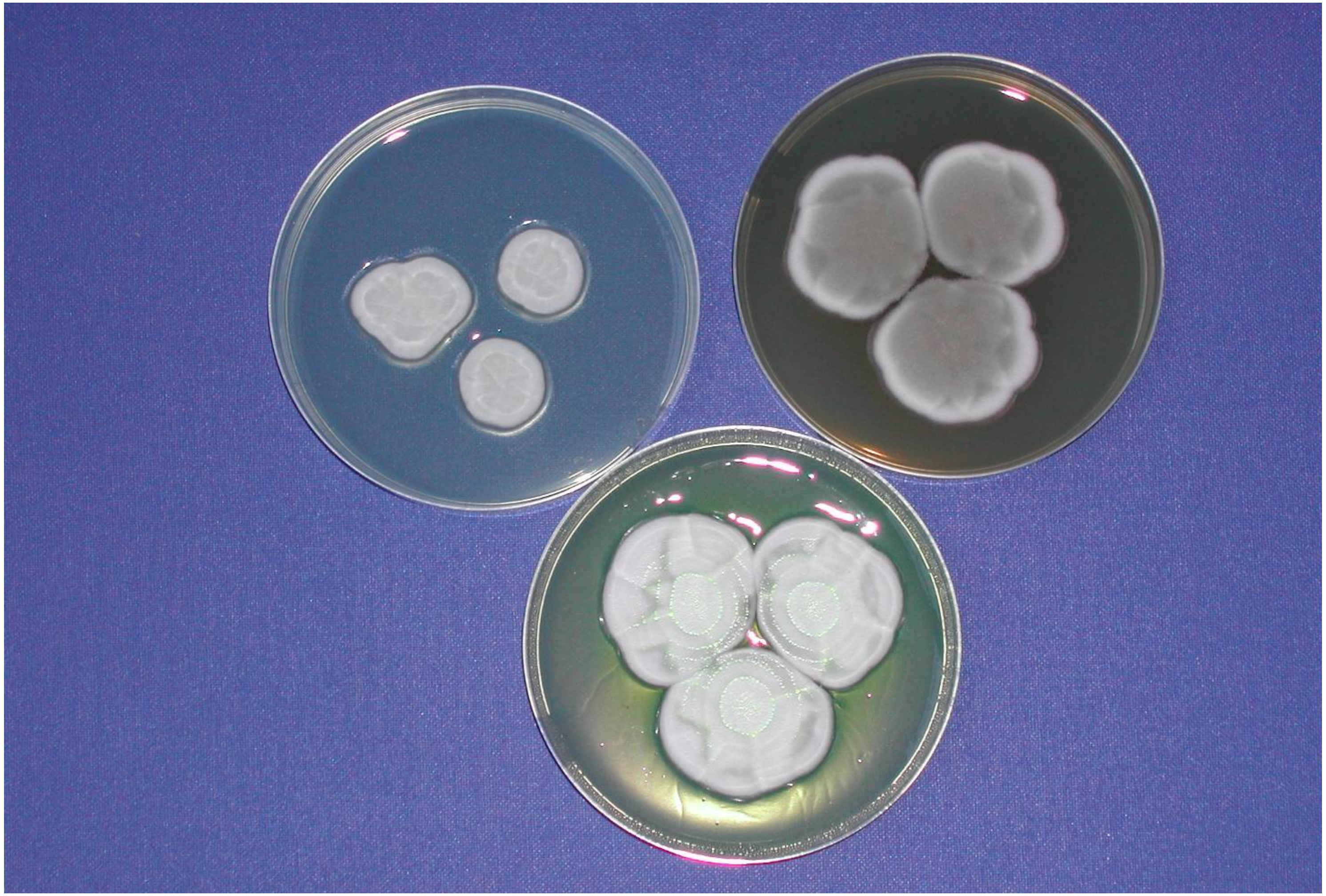
B. bassiana
(SEM)



zig-zag rachis

B. bassiana grown <u>on</u> “Agar”

Colonies grow differently depending <u>on</u> medium



Culture flasks and culture tubes

B. bassiana in liquid medium

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Conidiospore production on liquid medium

Conidiospore drying and formulation

M. anisopliae grown <u>on</u> crashed oat or wheat bran

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Fungal infections result in:

- larvae move slowly, without coordination ?
- at the beginning infected larvae are “slack” – dark spots on cuticle ?
- dead larvae are semisolid to firm (“cheese like”) ?
- larvae show fungal growth on surface and changes in colour ?

Time to death: depending on insect species, conidia dose, temperature, humidity, … >4 days

Mycoinsecticides (1)

worldwide approximately 170 commercial products registered!

- *Aschersonia aleyrodis*
- *Beauveria bassiana + B. brongniartii*
- *Hirsutella thompsonii*
- *Isaria fumosorosea + Isaria* sp.
- *Lecanicillium longisporium + L. muscarium*
- *Lecanicillium sp.*
- *Metarhizium anisopliae*
- *Metarhizium anisopliae* var. *acridium*
- *Nomuraea riley*
- *Sporothrix insectorum*
- *Conidiobolus thromboides*
- *Lagenidium giganteum*

Mycoinsecticides (2)

e.g.:

– *Beauveria bassiana*

- **Boverol,** Fytovita (Czech Republic)
- **Boveral OF,** Intrachem BIO Italia SpA (Italy)
- **Naturalis-L,** Andermatt Biocontrol AG (Switzerland)
- **Naturalis,** Intrachem BIO Italia SpA (Italy)
- **Ostrinil,** Natural Plant Protection (NPP) (France)
- ….

Mycoinsecticides (3)

e.g.:

– *Beauveria brongniartii*

- **Beauveria Schweizer,** Eric Schweizer Samen AG (Schweiz)
- **Betel,** Natural Plant Protection (NPP) (Frankreich)
- **Engerlingspilz, Beaupro** Andermatt Biocontrol AG (Schweiz)
- **Melocont Pilzgerste,** Agrifutur (Italien)
- …

Mycoinsecticides (4)

- *Metarhizium anisopliae*
  - **BIO 1020 NEU,** Taensa (USA)
  - **Metarhizium Schweizer,** Eric Schweizer Samen AG (Switzerland)
- *Isaria fumosorosea (Paecilomyces fumosoroseus)*
  - **PreFeRal WG,** Biobest Biological Systems (Belgium)
- *Lecanicillium muscarium* (formerly: *Verticillium lecanii)*
  - **Mycotal,** Koppert B.V. (The Netherlands)
  - **Vertalec,** Koppert B.V. (The Netherlands)
- *… and other species*

Mycoinsecticides in Brasil

(40 commercial products)

- *Metarhizium anisopliae* (against Hemiptera: Cercopidae; Acari: Ixodidae):
  - Metarril E9
  - Metarril 1037
  - Metarriz
  - Methavida
  - Biotech
  - …
- *Beauveria bassiana* (against Coleoptera: Curculionidae; Acari: Tetranychidae):
  - Boveril PL 63
  - …
- *Sporothrix insectorum* (against Hemiptera: Tingidae):
  - …

Biological Control of Locusts and Grasshoppers - Windows Internet Explorer

http://www.lubilosa.org/

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Biological Control of Locusts and Grasshoppers

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Biological Control

LUBILOSA GREEN MUSCLE

+++For US visitors: "Green Muscle is not regi

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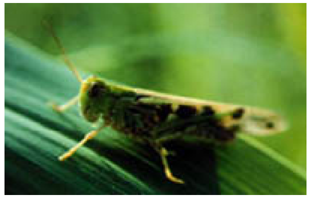
A serious pest

Locusts and grasshoppers often cause extensive and serious damage to crops in many parts of Africa and Asia. Locusts are well known for their potential of invading cropping areas in swarms of millions of individuals leaving behind devastated fields and plantations. Luckily, these invasions are infrequent and may be followed by long periods of recession. In contrast, grasshoppers form a more chronic problem causing serious yield losses in most years.

Locust and grasshopper control: The environment at risk



Locust and grasshopper control is currently carried out with chemical pesticides. For many years, the product of choice was dieldrin, a persistent pesticide well suited for barrier treatment. However, concern about its negative impact on the environment caused it to be prohibited in most countries. Most modern pesticides replacing it are much less persistent and have therefore to be applied more frequently in blanket treatments and in larger volumes. So, even though they are less toxic than dieldrin, their environmental impact may well be worse. During the last major Desert Locust outbreak 1986-1989, donors spent 300 million $US, and 1.5 million litres of pesticides were applied. The international community became more and more concerned about this issue and has initiated the development of alternative control methods.

The biological solution

LUBILOSA has developed a mycopesticide called GREEN MUSCLE® based on the spores of the insect pathogenic fungus *Metarhizium anisopliae* var. *acridum*. This fungus, which appears to be specific to species of short-horned grasshoppers (Acridoidea and Pyrgomorphoidea), is widely distributed in Africa and under favourable climatic conditions, can cause local epidemics in grasshopper or locust populations. Its biological and physical properties make this fungus an ideal candidate for augmentative biological control. Spores of *M. anisopliae var. acridum* can be easily mass-produced.

The state of the art

GREEN MUSCLE® is available either as dry spore powder or as oil miscible concentrate. It is applied as an oil suspension and can be

Fertig Internet 100%

Start Novell GroupWise ... Biological Control ... Microsoft PowerP... DE Desktop durchsuchen 08:22

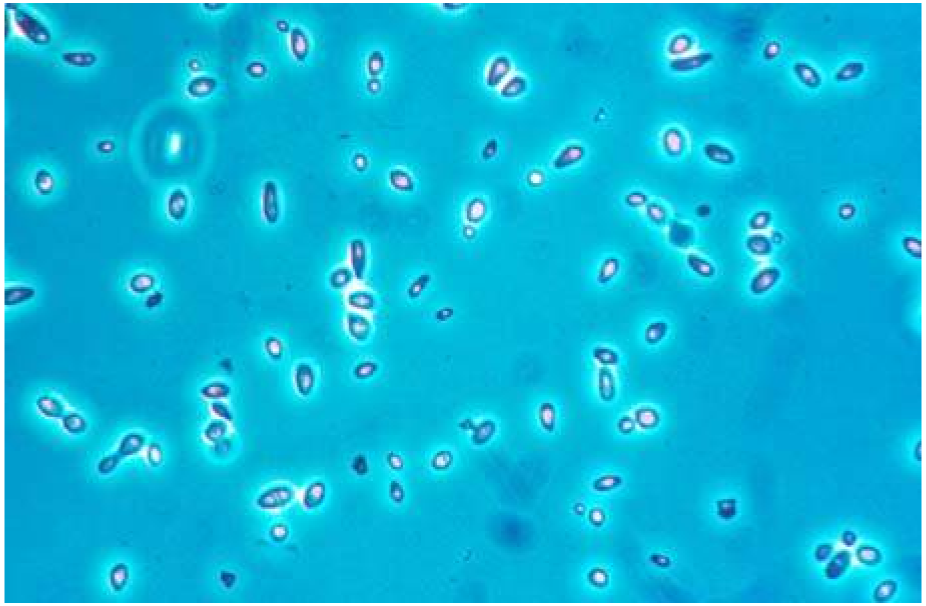
Metarhizium spp.

(entomopathogenic fungi!)

- Conidiospores attach to the cuticle ⇨ germ penetrates the integument ⇨ fungus develops in the host ⇨ after host‘s death ⇨ penetration of integument and formation of conidiospores;
- Adults and larvae are sensitive, spraying “hoppers” (to reduce feeding);
- Mass production is easy.

Metarhizium anisopliae var. *acridium*

"LUBILOSA"



Metarhizium anisopliae var. *acridium*
(or *Metarhizium flavoviride)*

Control of migratory locusts

- Within the LUBILOSA project the mycopesticide GREEN MUSCLE® was used based on the spores of the insect pathogenic fungus *Metarhizium anisopliae* var*. acridum*
- This fungus acts specific in species of locusts (Acridoidea and Pyrgomorphoidea), is widely distributed in Africa and under favourable climatic conditions, can cause local epidemics in grasshopper or locust populations.
- Its biological and physical properties make this fungus an ideal candidate for augmentative biological control.
- Spores of *M. anisopliae var. acridum* can be easily mass-produced.

GREEN MUSCLE®

- GREEN MUSCLE® is available either as dry spore powder or as oil miscible concentrate.
- It is applied as an oil suspension and can be sprayed using standard ultra low volume spinning disk spray equipment.
- The efficacy of GREEN MUSCLE® has been demonstrated in many field trials carried out by the programme and its collaborators over the past ten years, including aerial application at an operational scale.
- GREEN MUSCLE® is being recommended by the pesticide referee group of FAO


M. melolontha
SIP

Vorarlberg, May 2011

Melolontha melolontha

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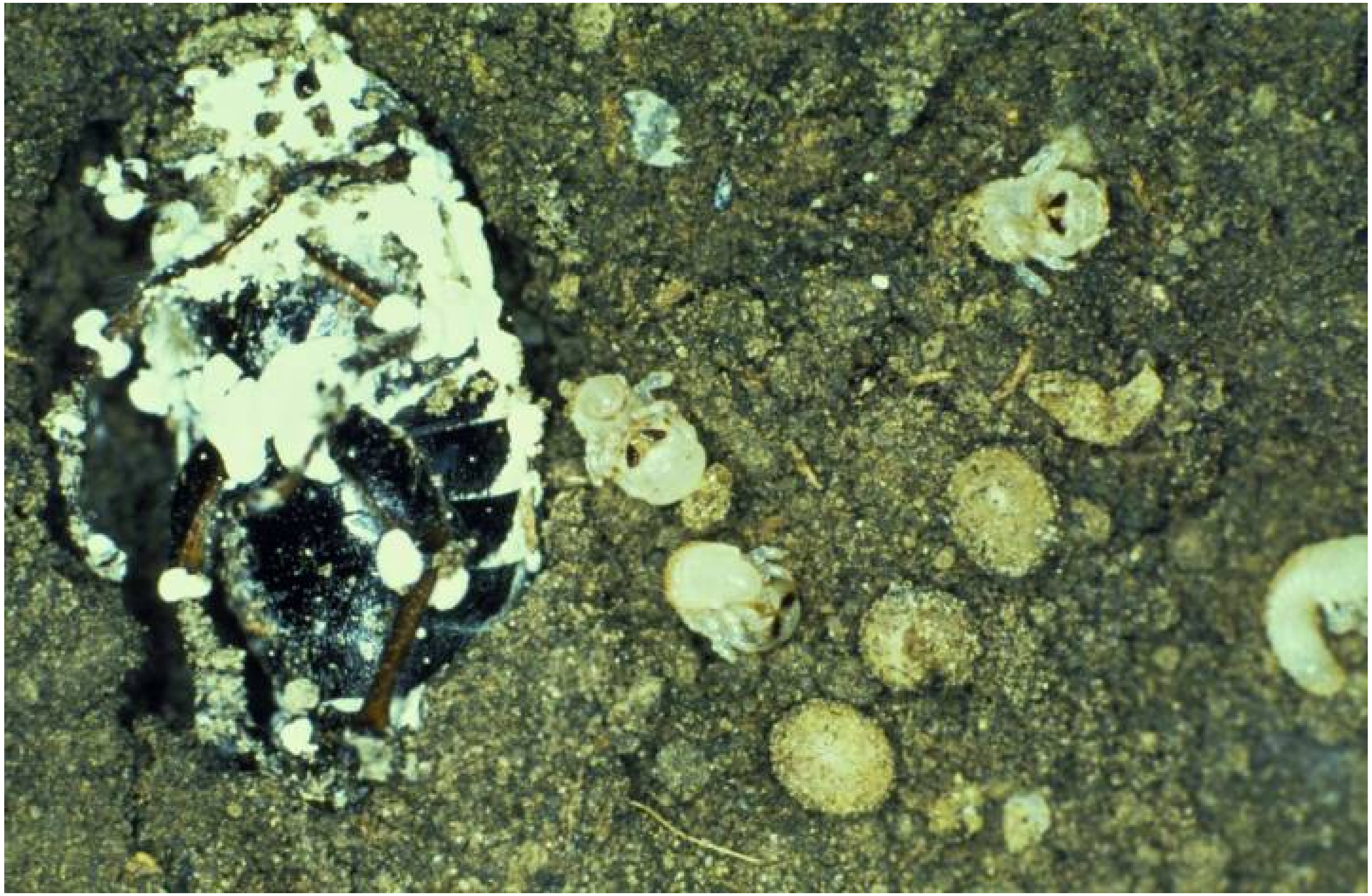

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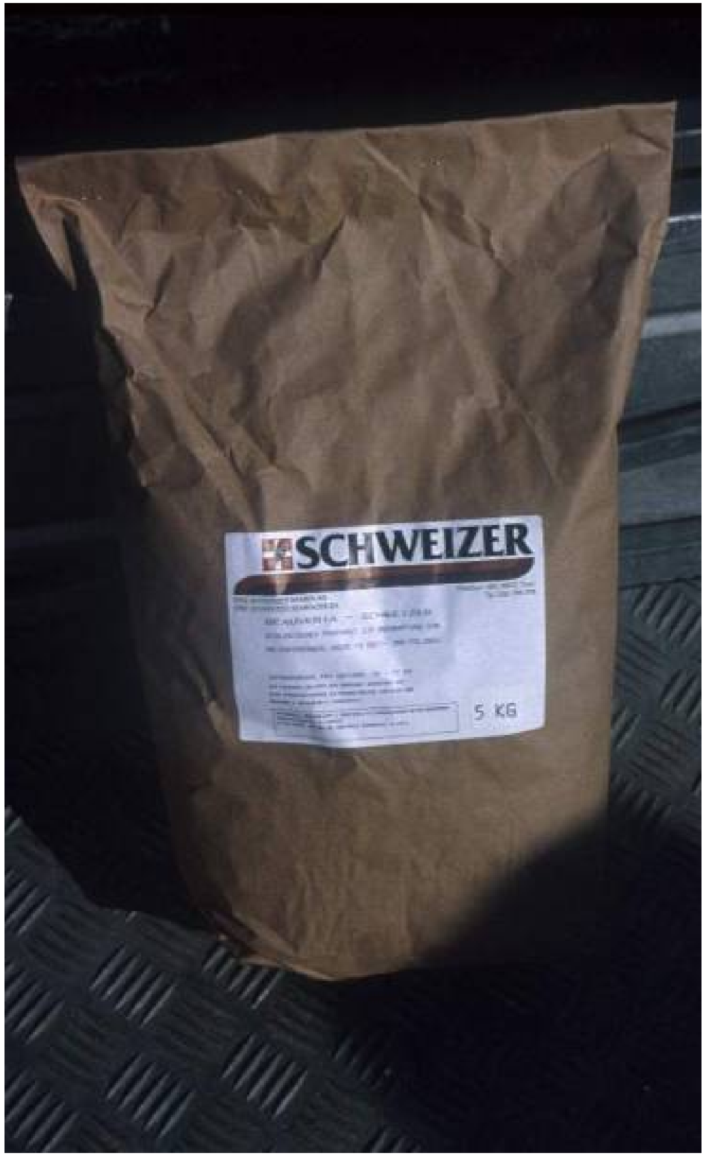
SIP

Control of ***Melolontha* spp.**

- Soil dwelling species:
  different Scarabaeidae, other species ?
- Area:
  farmland (corn, potatoes), orchards, vineyards, forests.
- Control measures:
  adult beetles or larvae (or both).
- Microbials

Entomopathogenic fungus: *Beauveria brongniartii*

- Conidiospores attached to the cuticle – germ penetrates the integument – development in the host – after host‘s death – penetration of integument and formation of conidiospores.
- Adults and larvae are sensitive.
- Mass production is easy.



B. brongniartii grown on barley

B. brongniartii preparation

B. brongniartii application in an orchard

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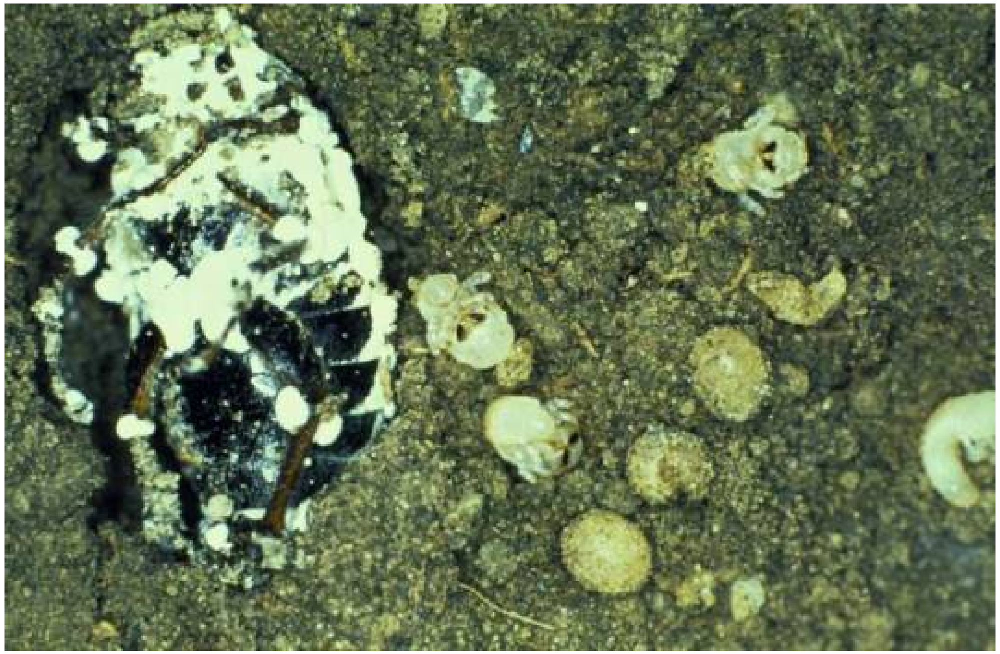
Machine for *B. brongniartii* application in turf grass on steep slopes

Melolontha melolontha

Melolontha melolontha
+ Beauveria brongniartii



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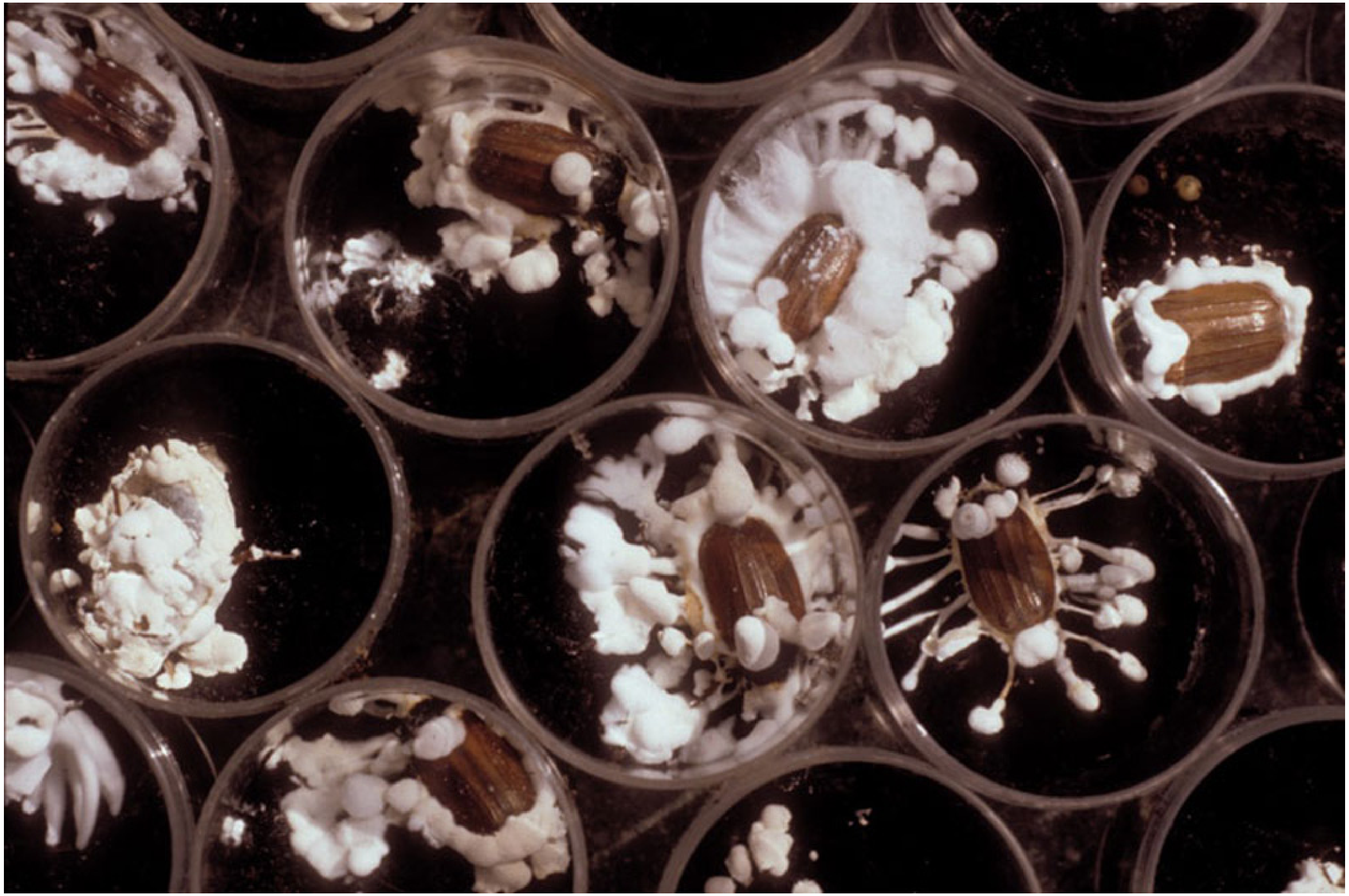
Melolontha melolontha + Beauveria brongniartii



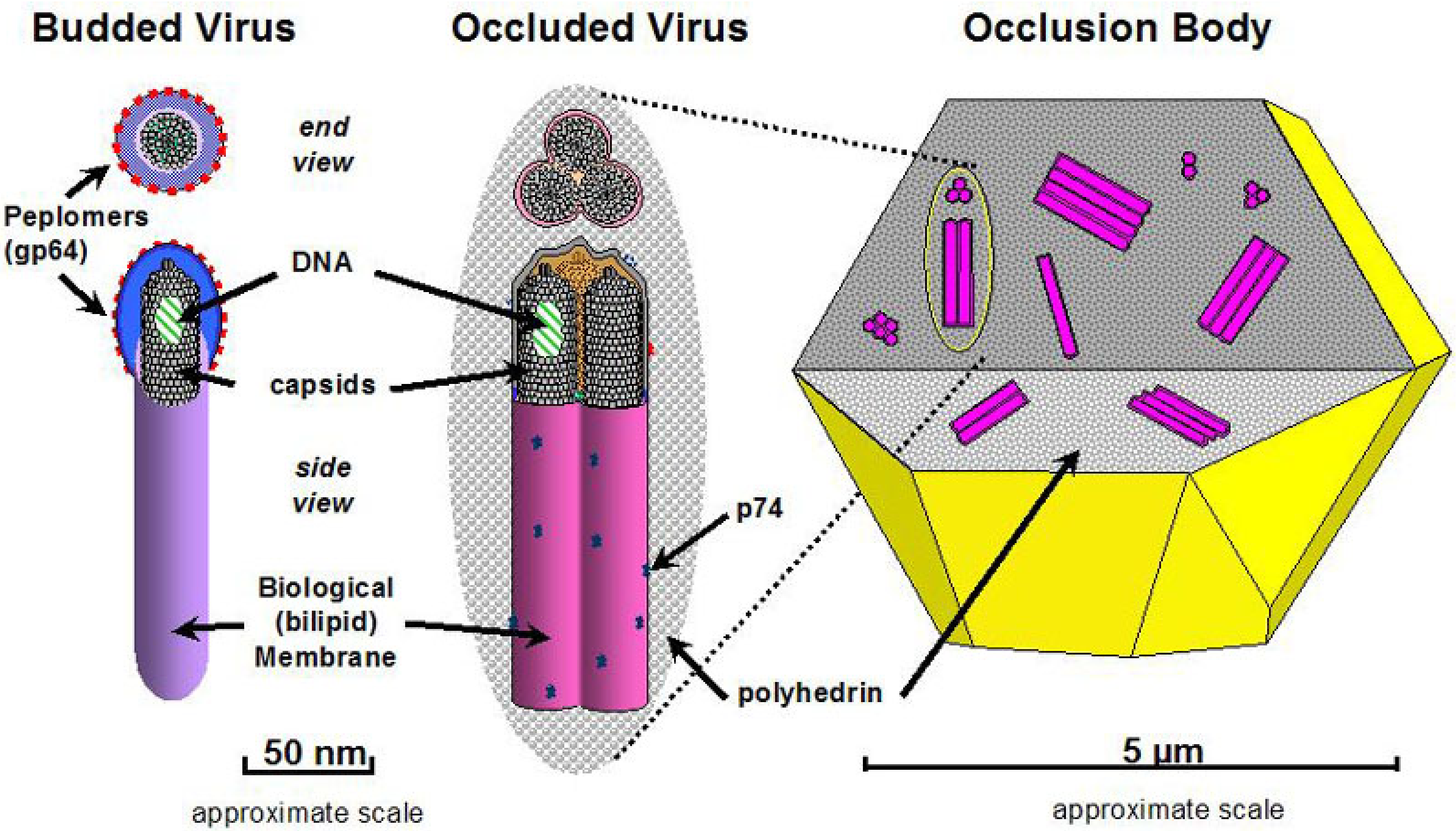
Entomopathogenic Virus

- Virion = infective particle:

  ⇘

  - RNA or DNA,
  - cannot multiply or grow without a living cell,
  - has no autonomous metabolism.

Baculovirus
Multicapsid nucleopolyhedrovirus



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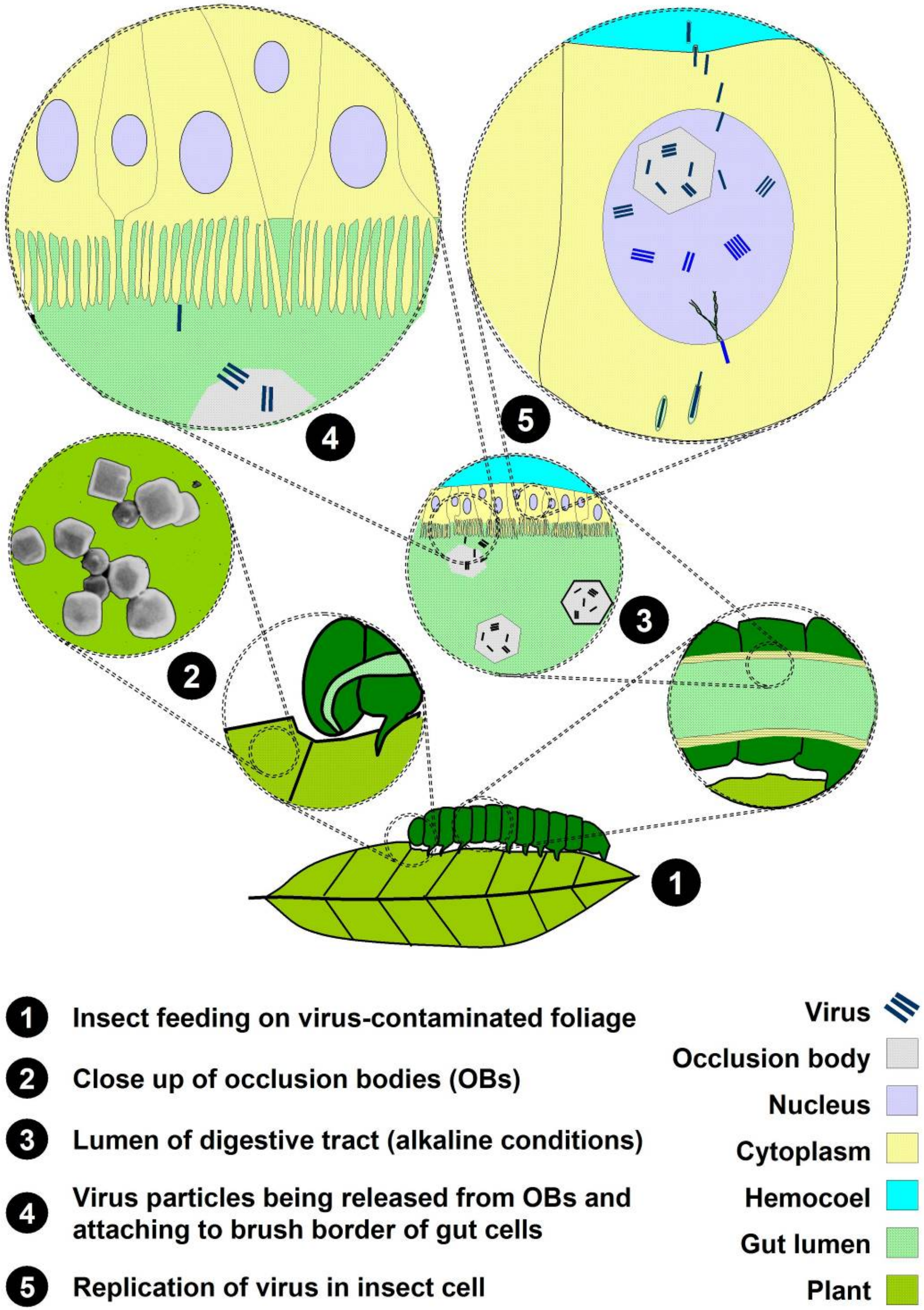


1 Insect feeding on virus-contaminated foliage
2 Close up of occlusion bodies (OBs)
3 Lumen of digestive tract (alkaline conditions)
4 Virus particles being released from OBs and attaching to brush border of gut cells
5 Replication of virus in insect cell
Virus
Occlusion body
Nucleus
Cytoplasm
Hemocoel
Gut lumen
Plant

Virus infections result in:

- Larvae move slowly, without coordination ?
- Larvae are “sluggish” ?
- Larvae show changes in colour ?

Time to death: depending on virus dose, temperature, … >4 days

Virus in Brasil

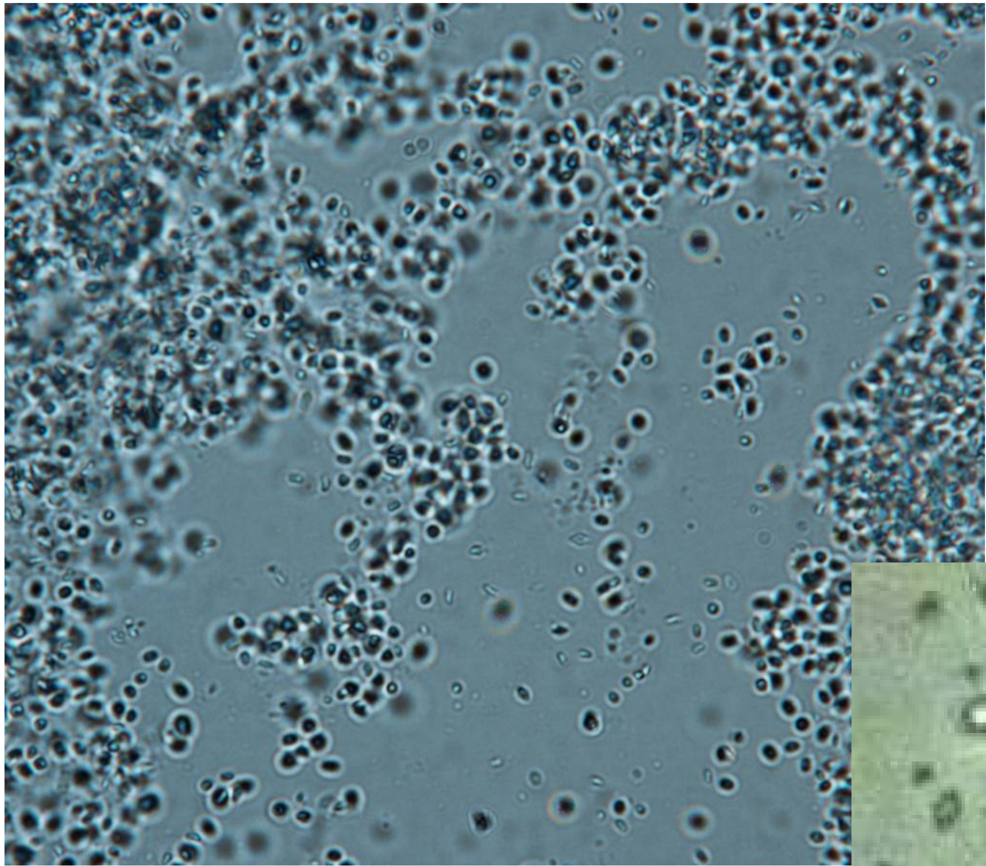
- *Anticarsia gemmatalis* (AgNPV):
  - Baculo-Soja
  - Baculovirus Nitral
  - Coopervirus PM
  - Protégé
  - …

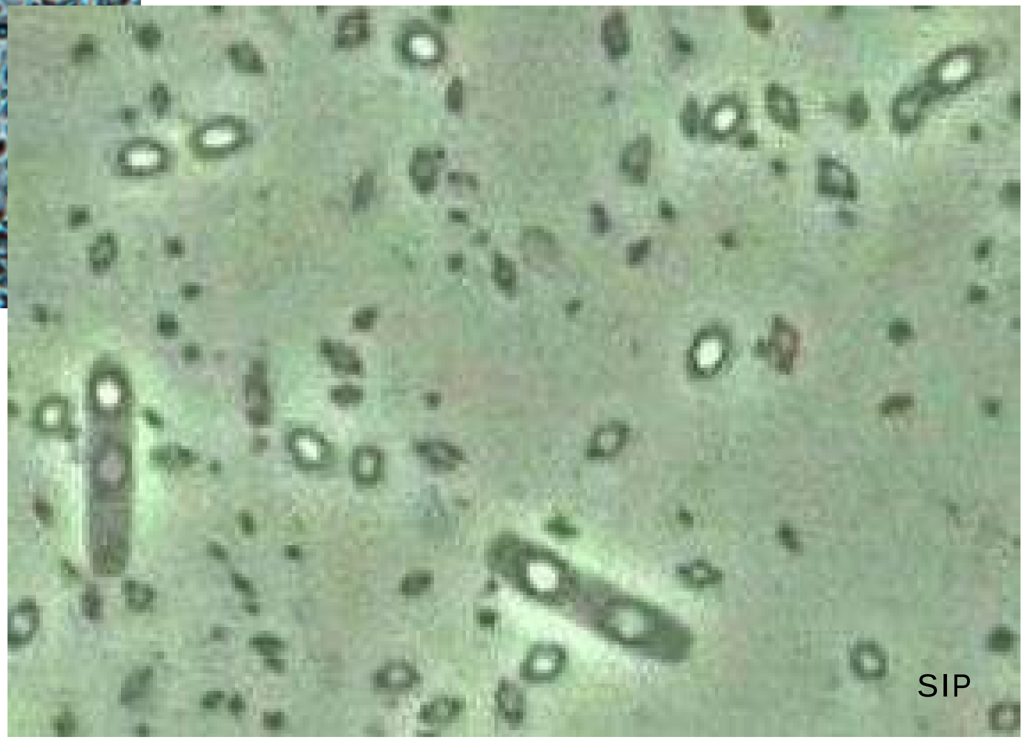
Bacteria in insects (Prokaryota)

- Within the mid-gut bacteria can be found frequently – most are not harmful to insects !
- But *Bacillus thuringiensis*:

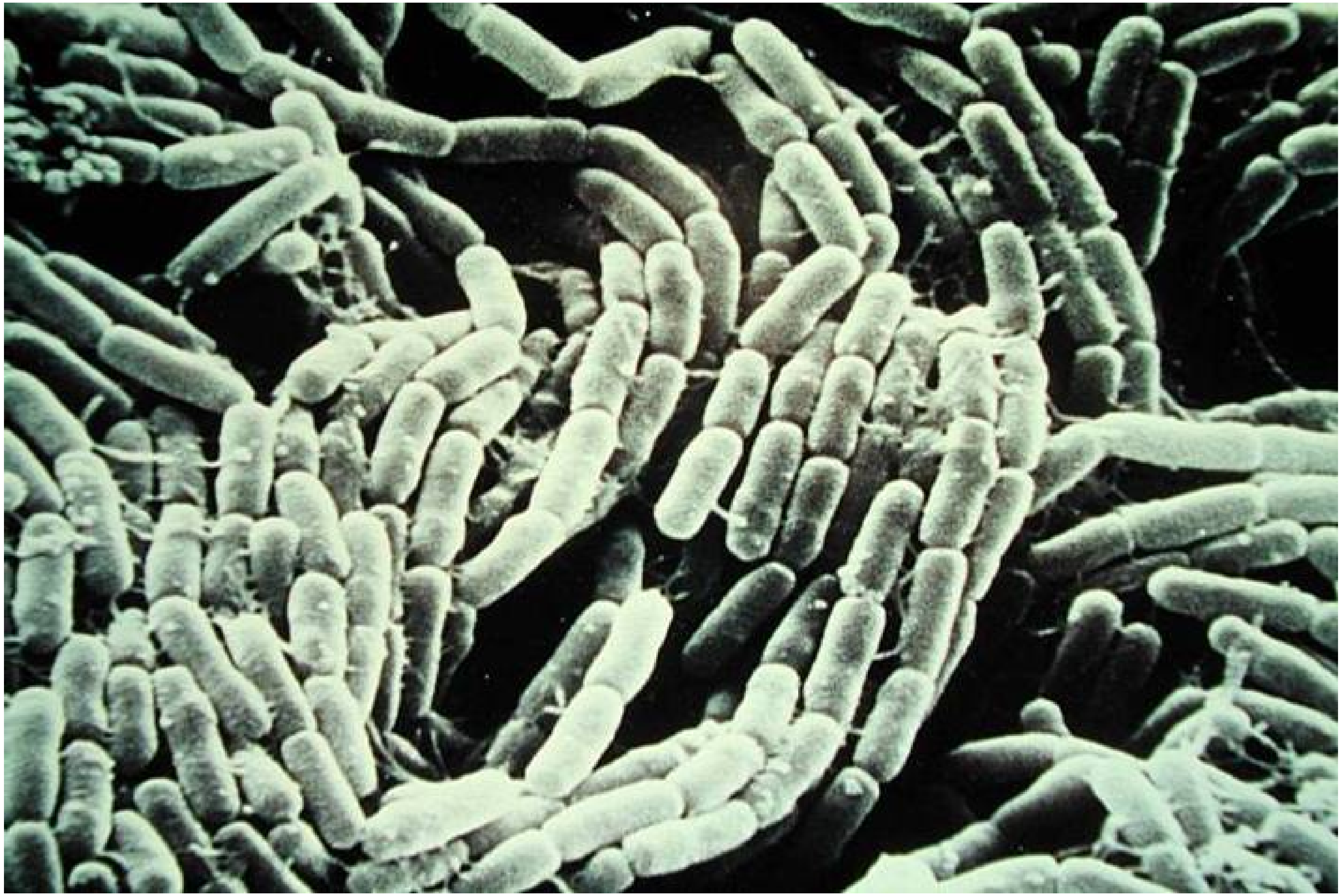
  ↳ in case of "partial damage" of the gut epithelium, gut content trespasses into haemocoel and causes "septicaemia" ⇨ death of insect !

Bacillus thuringiensis

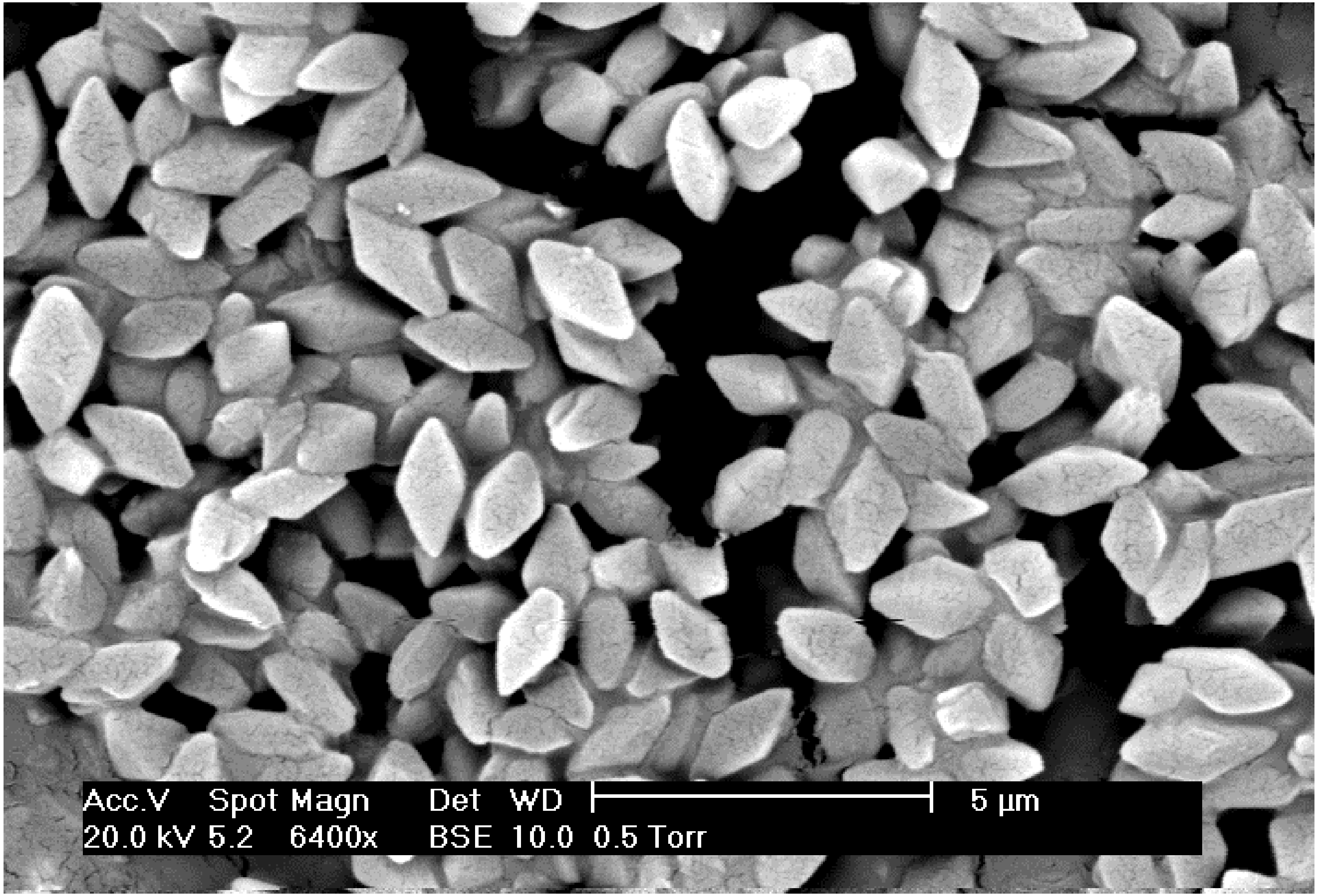




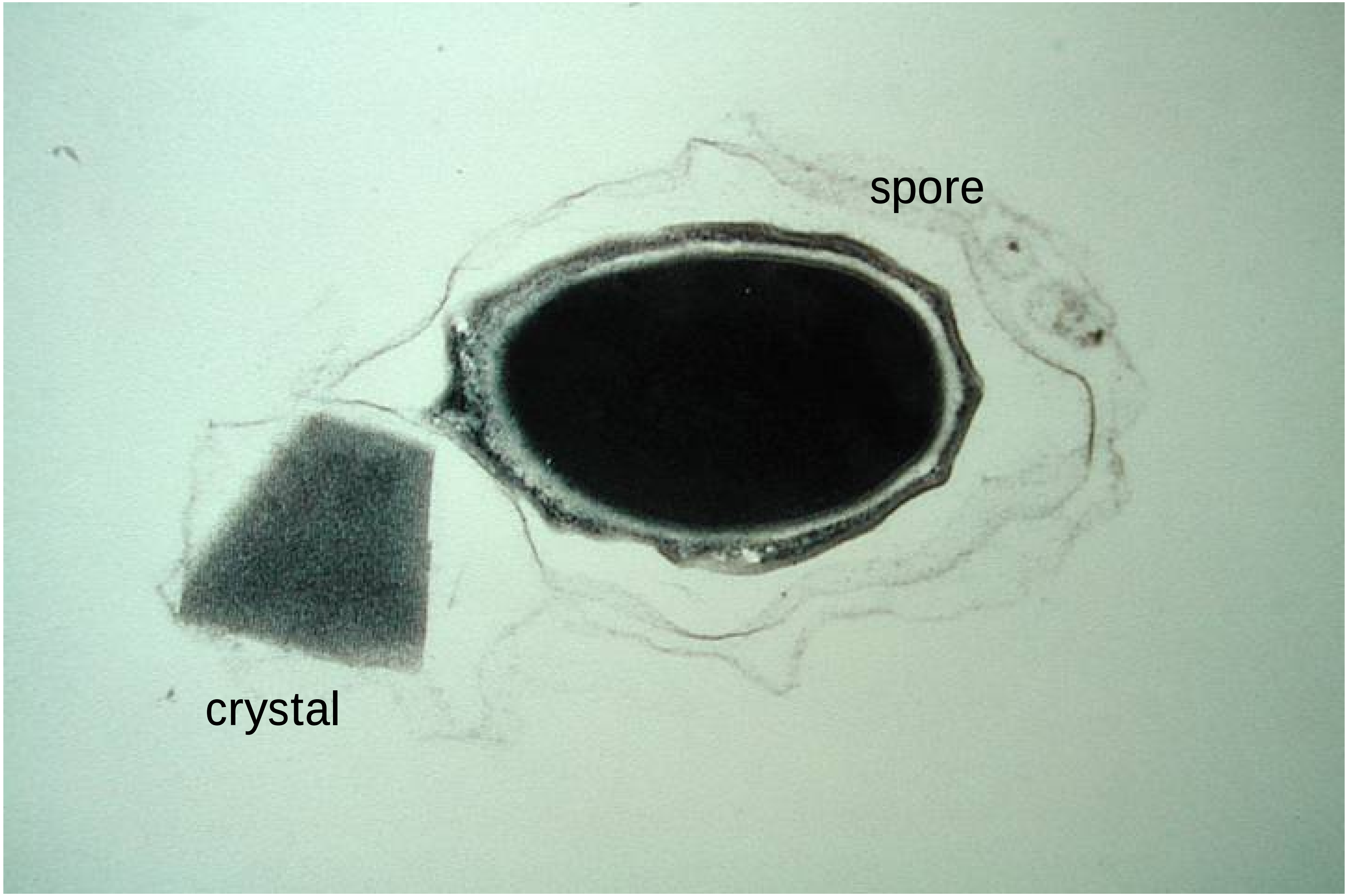
Vegetative cells of *B. thuringiensis*

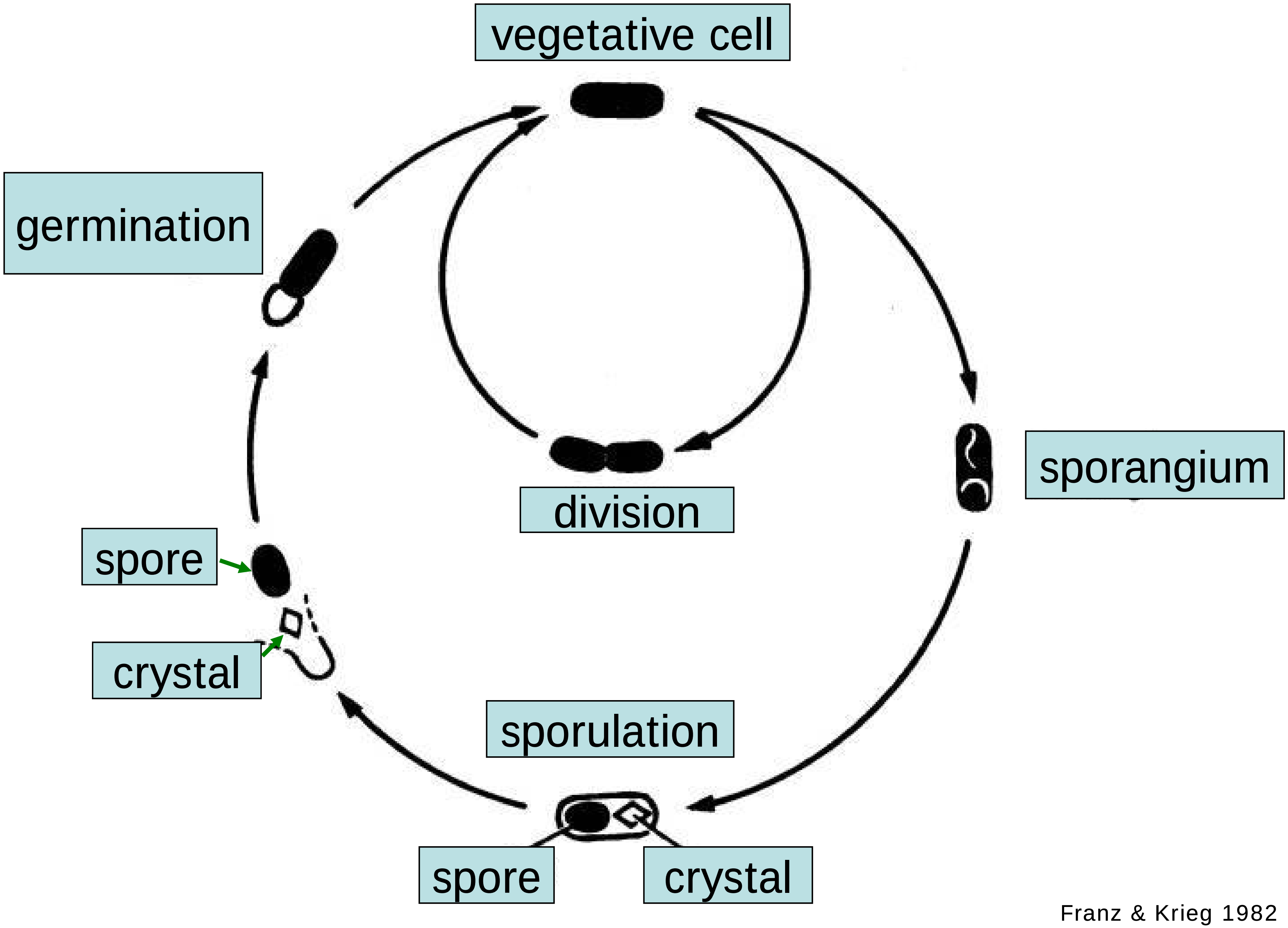


Parasporal bodies (crystals) of B.t.

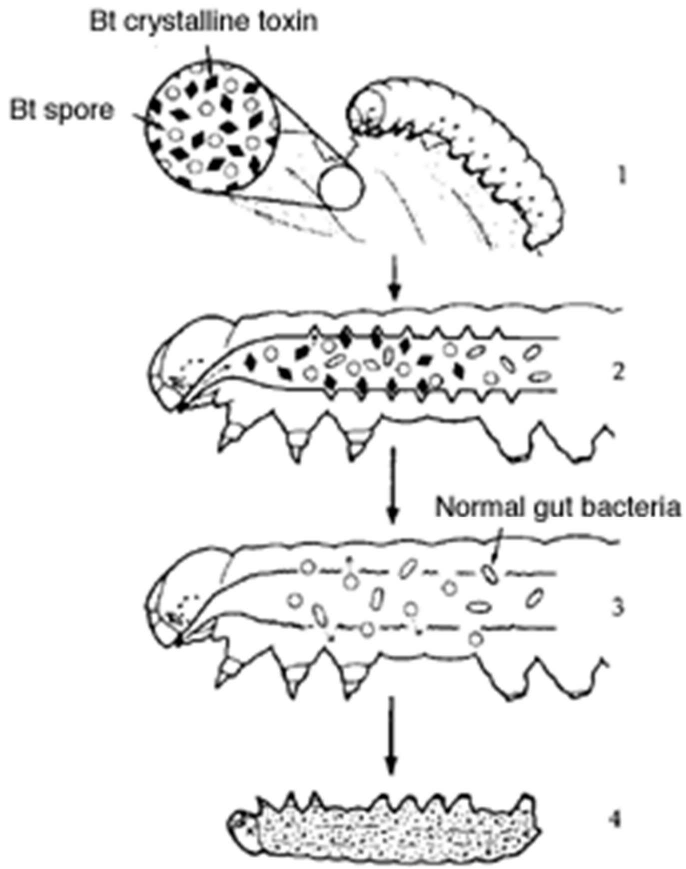


SIP

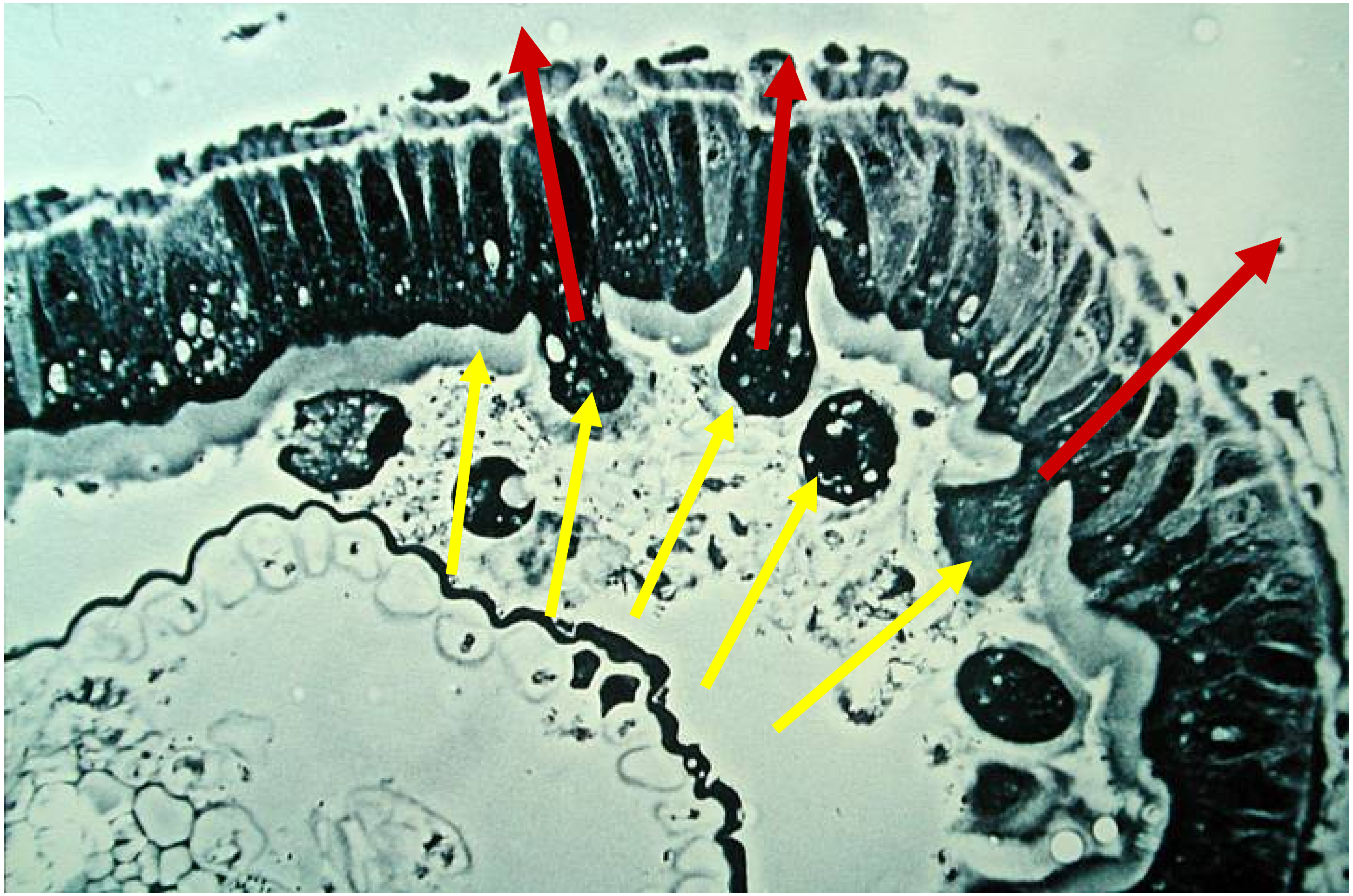

spore
crystal


vegetative cell
germination
sporangium
division
spore
crystal
sporulation
spore
crystal
Franz & Krieg 1982

Action of *Bacillus thuringiensis* var. *kurstaki* on caterpillars



1) Caterpillar consumes foliage treated with Bt (spores and crystalline toxin).
2) Within minutes, the toxin binds to specific receptors in the gut wall, and the caterpillar stops feeding.
3) Within hours, the gut wall breaks down, allowing spores and normal gut bacteria to enter the body cavity; the toxin dissolves.
4) In 1-2 days, the caterpillar dies from septicemia as spores and gut bacteria proliferate in its blood.



SIP

Different Bt-crystal protein genes (→ patho-types)

- Pathotype A = pathogenic to Lepidoptera larvae
- Pathotype B = pathogenic to Nematocera larvae
- Pathotype C = pathogenic to Chrysomelidae larvae

- **Serovar**: “KUR”, “AIZ”, “ISR”, “TEN”, …

Bacterial infections result in:

- Larvae move slowly, without coordination ?
- Larvae are “sluggish” ?
- Larvae show changes in colour ?

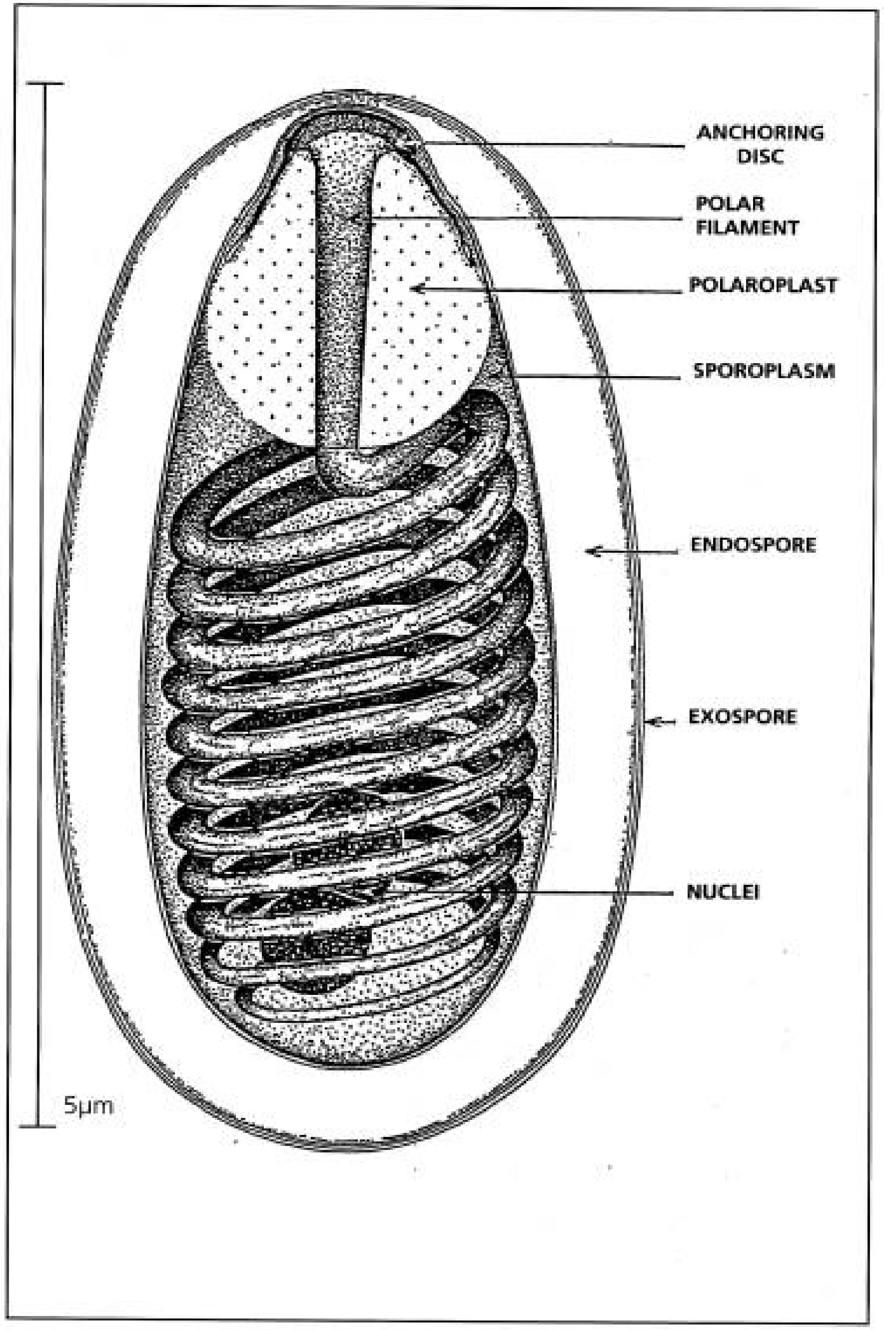
Time to death: depending on bacterial/crystal dose, temperature, … <4 days

Bacteria in Brasil

- *Bacillus thuringiensis* (against several Lepidoptera):
  - Ponto Final
  - Agree
  - Bac-Control
  - Bactur
  - Dipel
  - Thuricide
  - Xentari
  - …

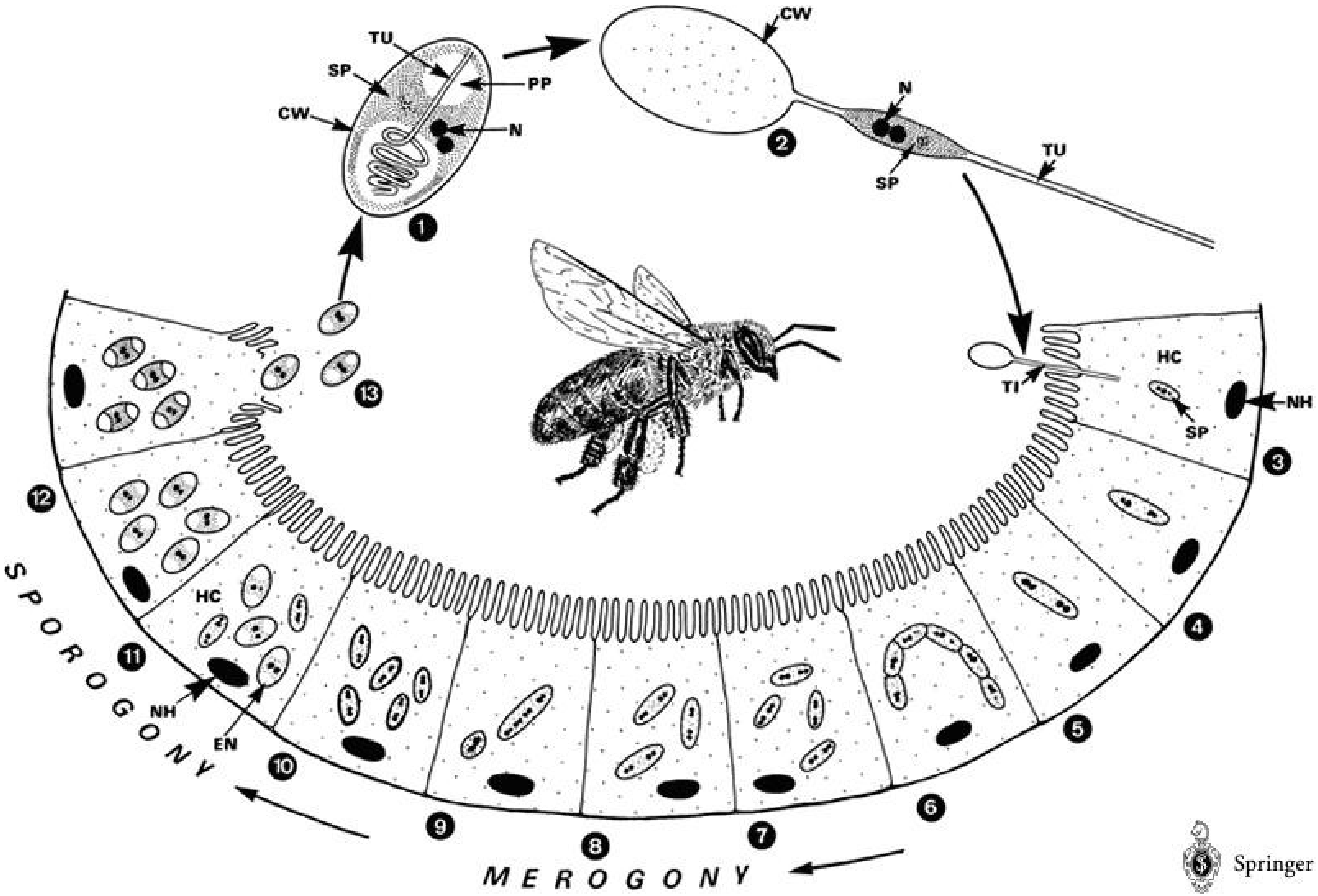
”Pébrine disease“
(= Microsporidium):
Nosema bombycis
in the silkworm, *Bombyx mori*,
with horizontal and
vertical transmission
(Pasteur 1870)

Louis Pasteur
(1822-1895)



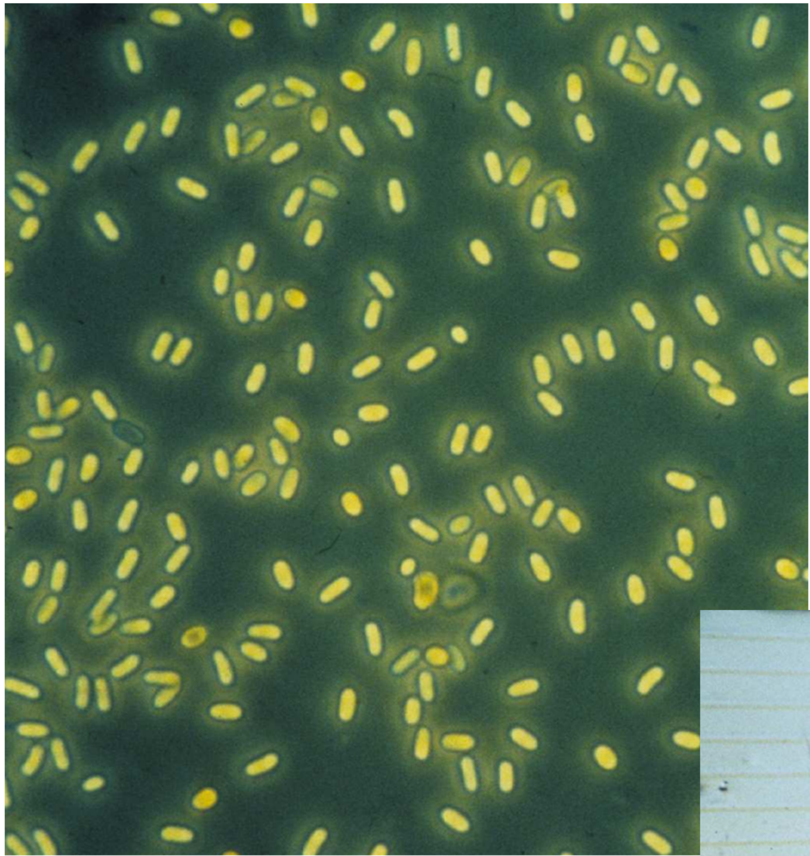
Graph of a microsporidian spore *(Paranosema locustae)*

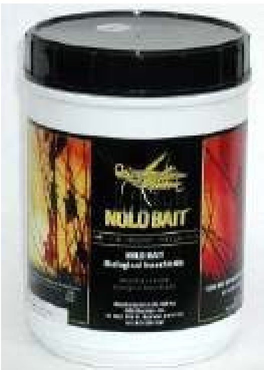
Life cycle of *Nosema apis*

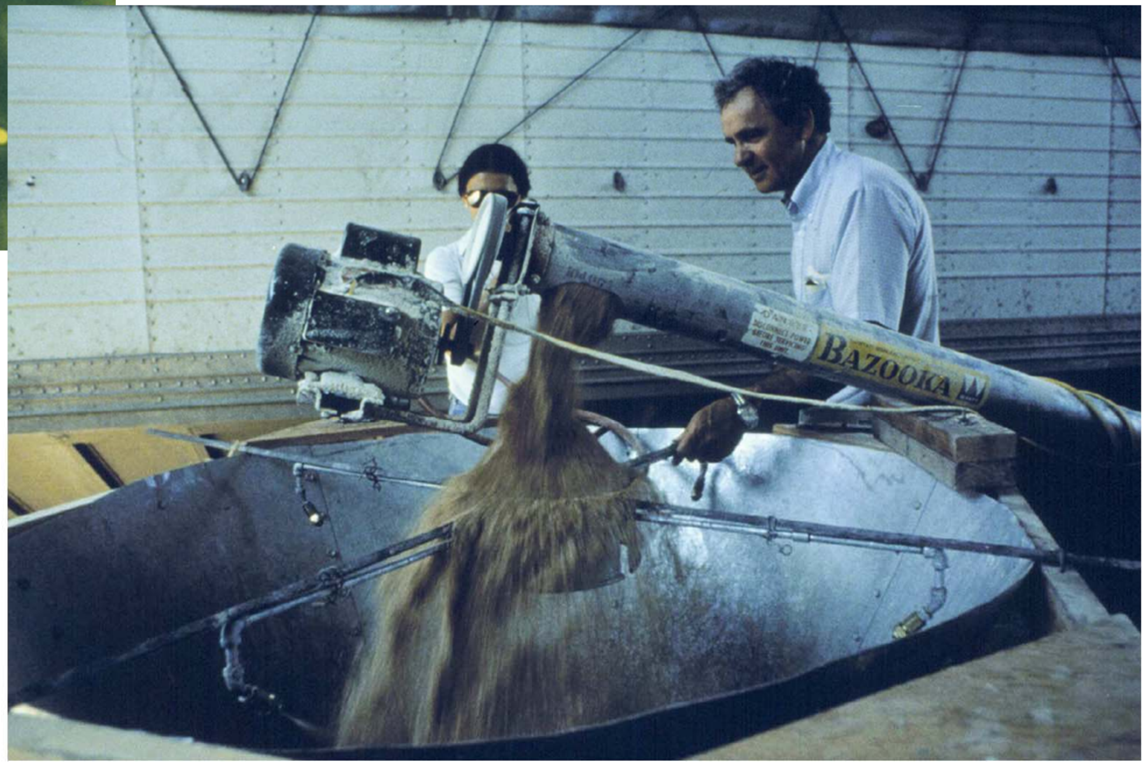


Springer

NOLOBAIT: *Paranosema locustae*







Paranosema (Nosema) locustae

- NOLO BAIT

  Nosema locustae,

  in combination with a bait (wheat bran)

NOLO BAIT

BIOLOGICAL INSECTICIDE

For use in suppressing grasshoppers and Mormon Crickets

Active Ingredient: *Nosema locustae Canning** ...0.05%
Inert Ingredients: ...99.95%
Total: ...100.00%

*Contains at least one billion viable spores per 454 grams (1.0 pound)

Net contents: ________ Date Formulated: ________

Lot# ________

NOLO BAIT™

FOR ORGANIC PRODUCTION

NOLO BAIT BIOLOGICAL INSECTICIDE

Nosema locustae
Biological Insecticide

Manufactured in the USA by:
M&R Durango, Inc.
6565 Hwy. 172, Ignacio, CO 81137
Tel: 970-259-3521

KEEP OUT OF REACH OF CHILDREN

SEE FIRST AID AND PRECAUTIONARY STATEMENTS ON BACK PANEL

EPA Registration #46149-2
EPA Establishment #46149-CO-001

http://www.goodbug.com/nolobait.html#FAQ

Microsporidian infections result in:

- Larvae move slowly, without coordination ?
- Infected larvae are “slack” ?
- Larvae show changes in colour ?

Time to death: depending on spore dose, temperature, … often chronic infection → long time to death

Entomopathogenic Protozoa

- …
- Class Rhizopoda

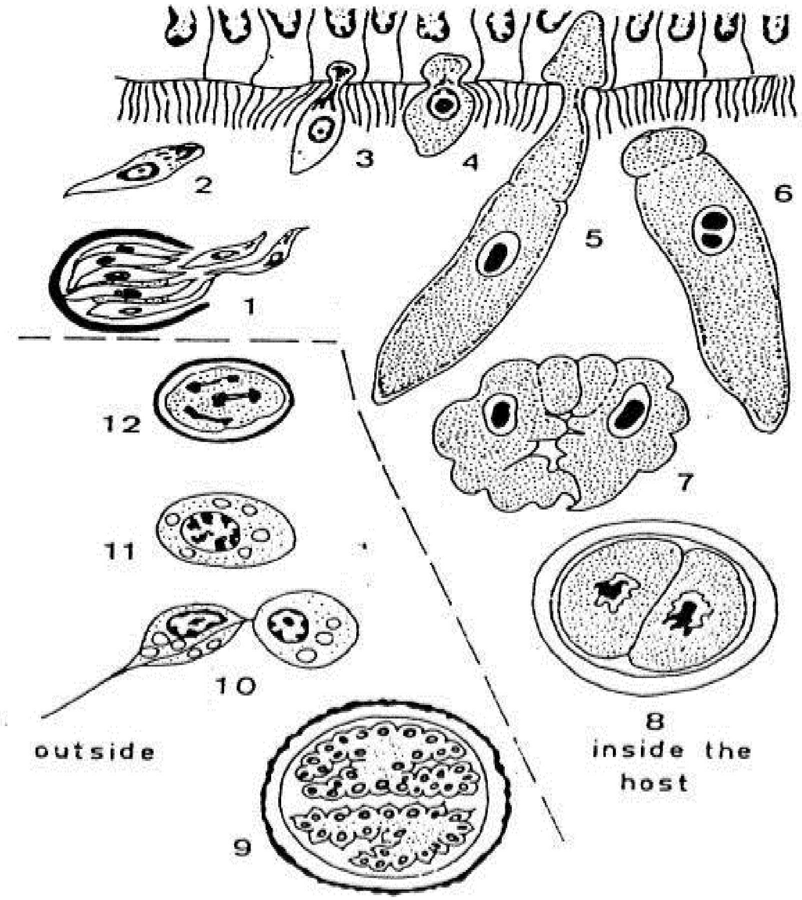
  Amoebina
- Class Sporozoa

  Gregarinida

  Neogregarinida

↳especially those forming cysts or spores (“environmental stages”) !

Developmental cycle of *Gregarina* sp.



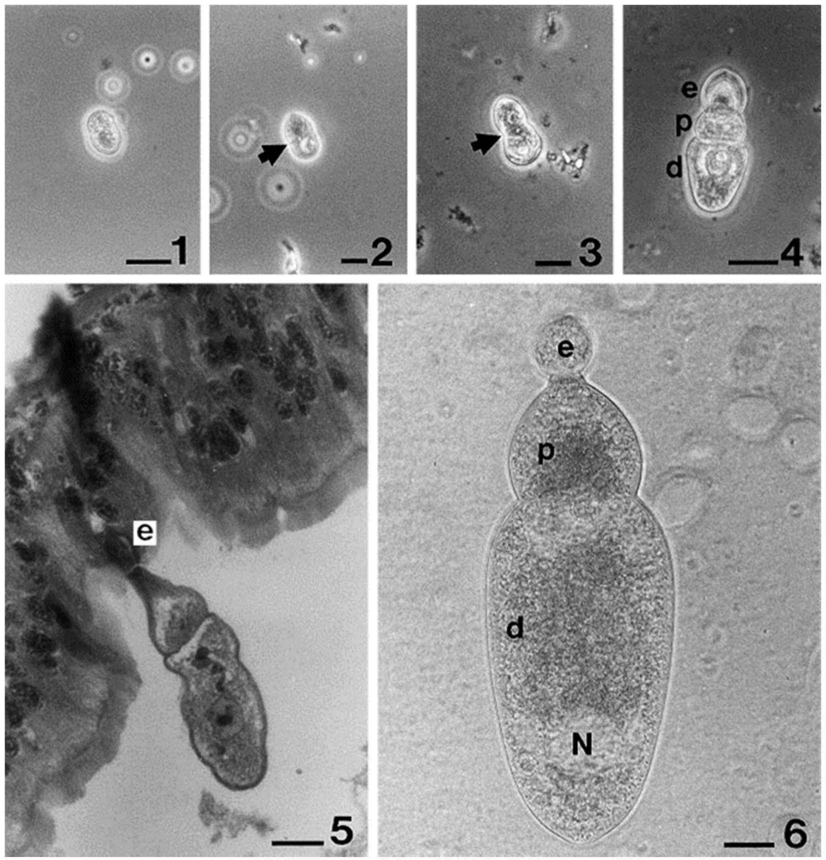
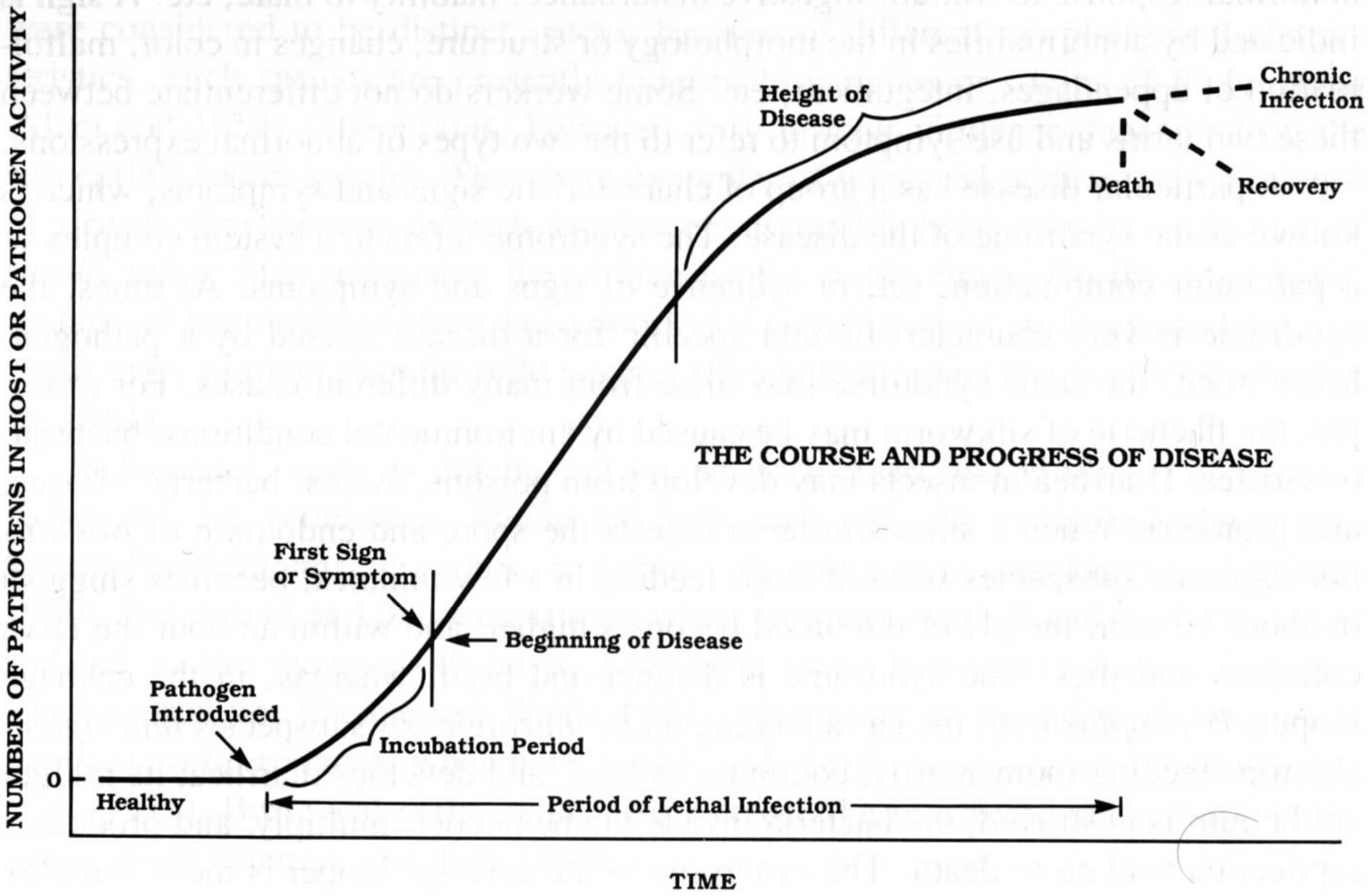


FIGURE 3–2



The course of infection by microorganisms in an insect host.

Horizontal transmission (1)

oral ingestion of (infectious) virus occlusion bodies or spores of bacteria, of microsporidia or protozoa (found in food or liquid), or spores of fungi via cuticle inoculation

- sufficient quantity of occlusion bodies or spores !
- appropriate conditions in the alimentary tract (pH, enzymes, …), or on cuticle (humidity, …) !

Horizontal transmission (2)

after reproduction and maturation ⇨ occlusion bodies or spores are released:

- in faecal excrements (throughout the lifetime of an infected host),
- by regurgitation,
- through secretion in larval silk,
- by cannibalistic feeding on weak or moribund infected individuals or on cadavers,
- after disintegration of infected tissues following the death of the host,
- fungal spores on surface of the cuticle of cadavers.

***Vertical* transmission**

in most cases maternally mediated ("trans ovum")

pathogens are transferred to egg stage:

- within the ovary, in the egg
- on the surface of the egg (consumed by host larvae at eclosion)

pathogens are transferred to adult stage:

- in social living insects,
- in feeding communities (fortuitous),
- by contact with fungal spores on surface of the cuticle of cadavers (of the parental generation)

Transmission *by vectors*

by other Insects (e.g. Hymenopteran parasites), other Arthropods (e.g. mites) or other animals (e.g. earthworms)

- during sting with ovipositor (egg laying or wounding for host feeding),
- by hosts not susceptible to special pathogens.

Dispersal of pathogens ?

The capacity to disperse is a key factor !

- Passive dispersal of inoculum:
  contaminated living individuals (adults) disperse pathogens to conspecific males and females or larvae; wind, rain, …
- Dispersal by use of vectors.
- Active dispersal of inoculum:
  mobility of pathogens (e.g. Entomophthorales) ?
  "Dispersal strategies" ?
- Changes in behaviour of infected insects.

Host range of pathogens

- *monovalent* pathogen species = infecting
  one host species
- *oligovalent* pathogen species = infecting
  some (± related) host species
- *polyvalent* pathogen species = infecting
  several (many) host species

Tolerance and resistance

- age tolerance of insects:
  L1-larvae are ± more sensitive than older larval instars or adults (in most cases) !
- resistance of insects:
  there are always resistant individuals within a population – using always the same strain of a pathogen ➔ sensitive individuals will be killed, tolerant or resistant individuals will survive !

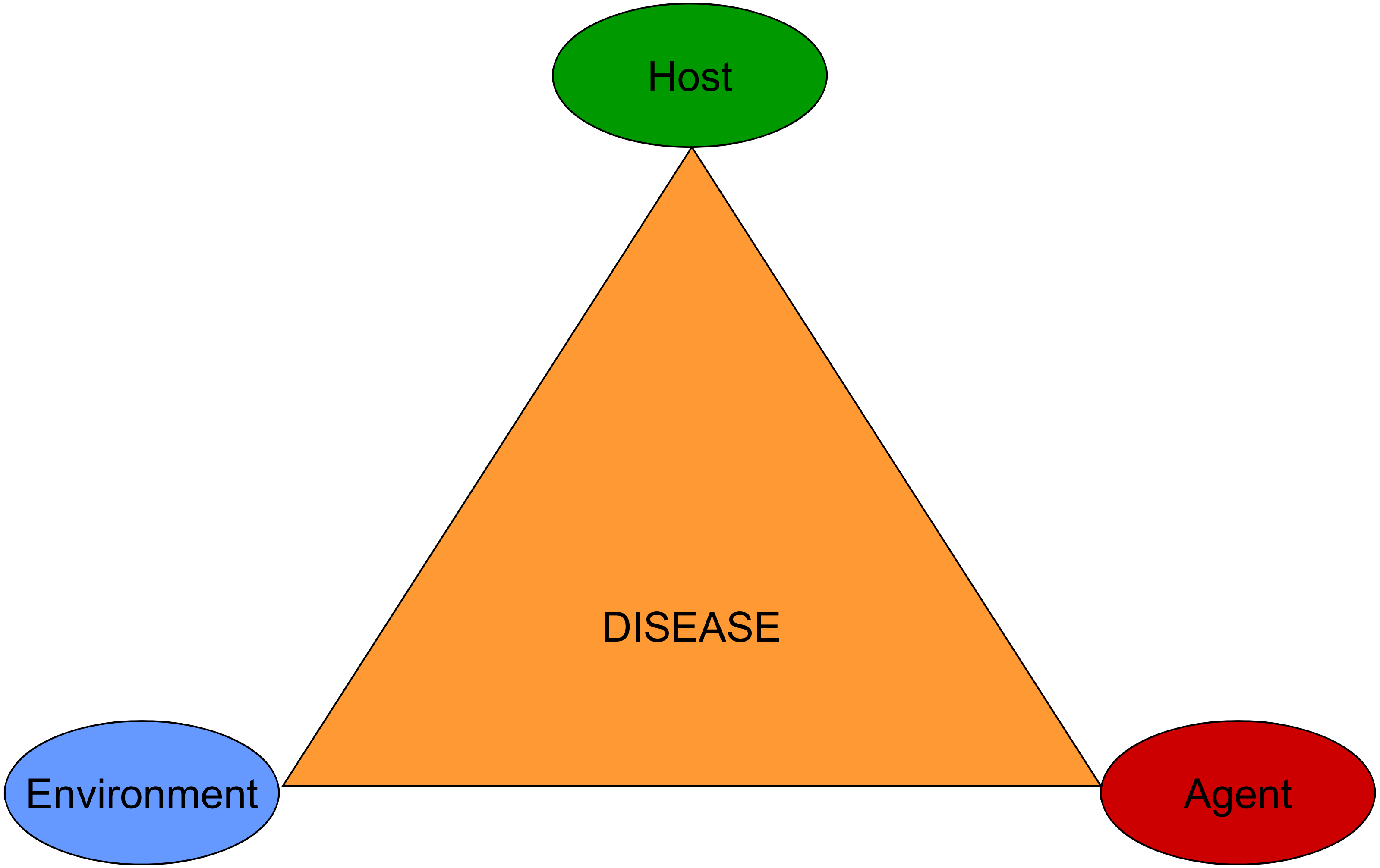
Key Factors in Epizootiology

- Presence of a pathogen population.
- Presence of a host population (abundance!).
- Effective means of pathogen transmission.
- Further biotic factors (other natural enemies, indifferent species, ...).
- Limiting abiotic factors.
- Interactions ...

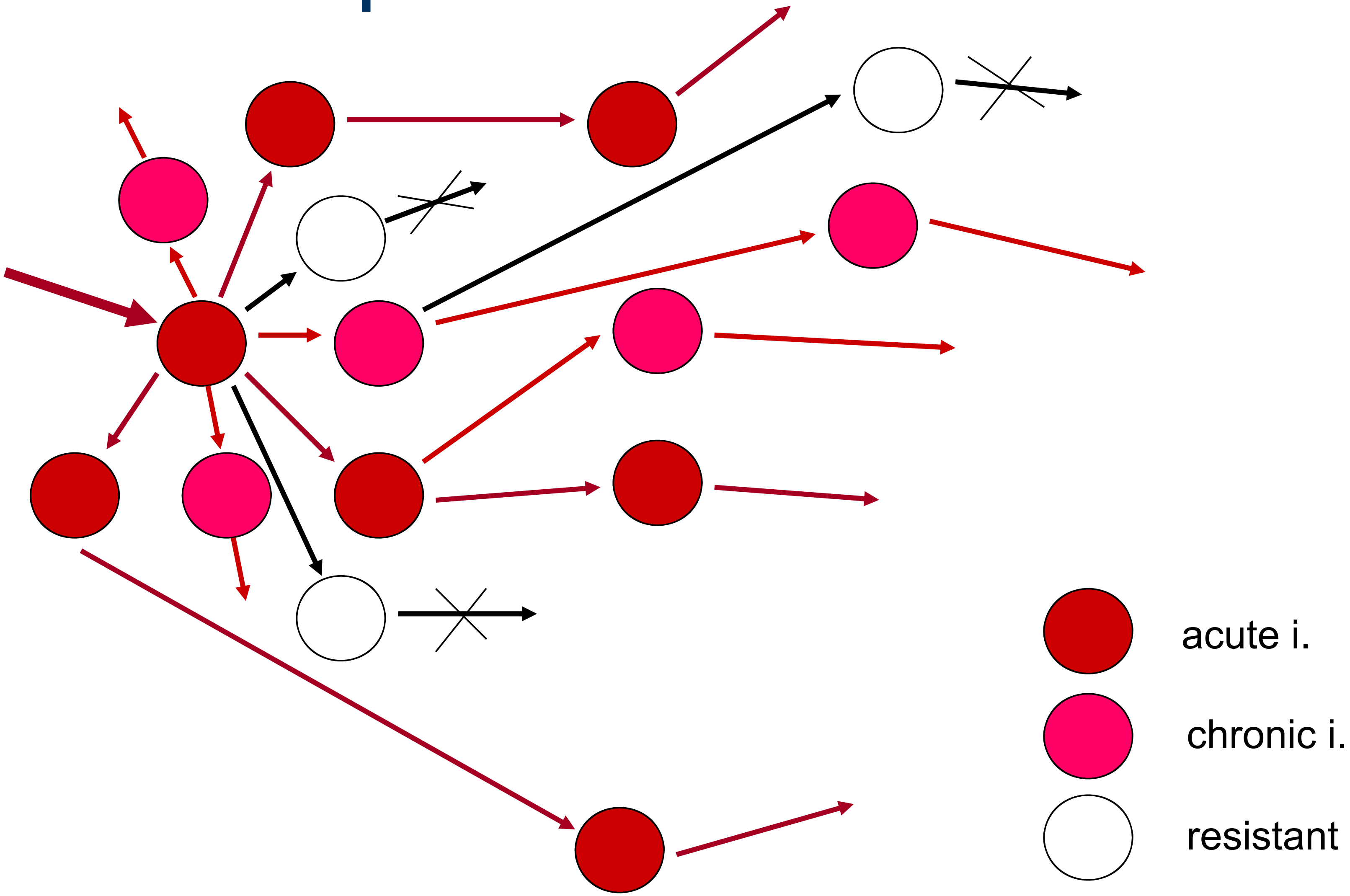
Pathogens are sensitive to

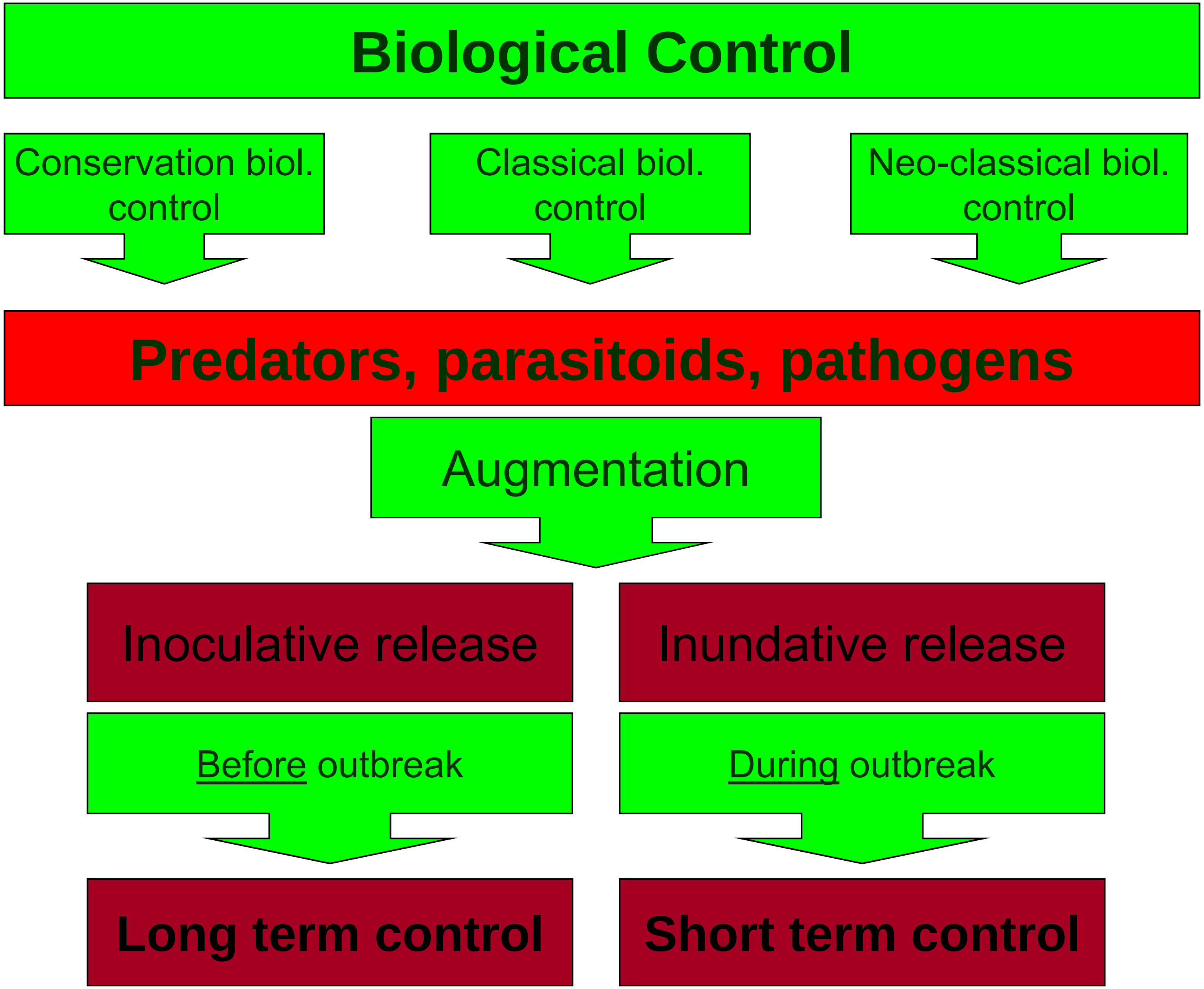
- UV radiation
- desiccation
- high temperature
- chemicals
- ....

“Disease triangle”



Disease spread



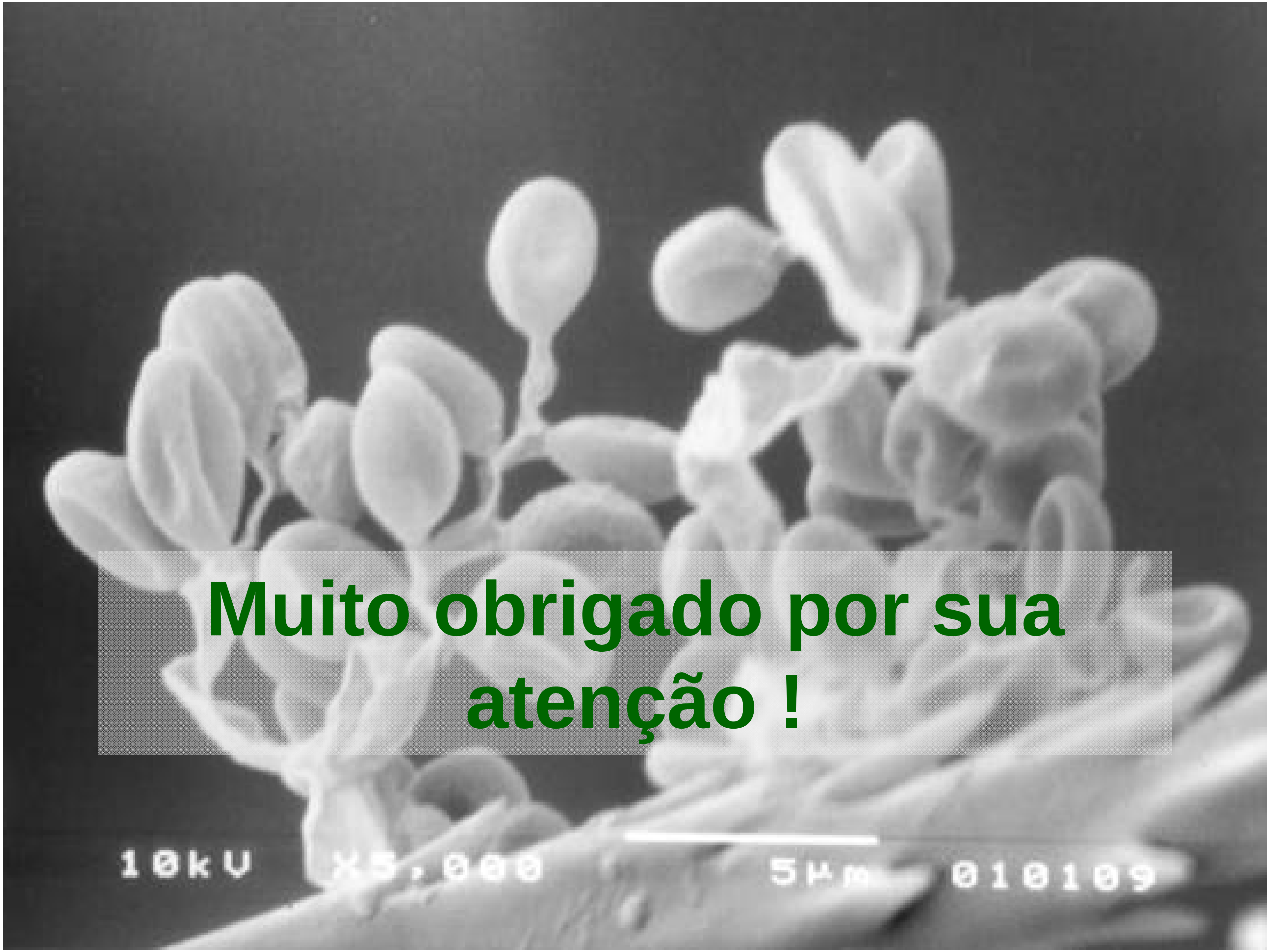

Biological Control
Conservation biol. control
Classical biol. control
Neo-classical biol. control
Predators, parasitoids, pathogens
Augmentation
Inoculative release
Inundative release
Before outbreak
During outbreak
Long term control
Short term control

Advantages of microbials

- The specific activity of microbials generally is considered highly beneficial (little or no direct impact upon parasitoids and predators) = specific to the target organism (or a limited number of hosts).
- Microbials are harmless to vertebrates and plants essentially nontoxic to people, pets and wildlife.
- No toxic residues, environmental friendly.
- Possibility of long-term control.

Disadvantages of microbials

- Inactivation by environmental factors, microbials are susceptible to degradation by sunlight.
- High specific activity of microbials only to target organism might limit their use on crops where problems with several pests occur; rel. high costs especially for obligate pathogens (niche markets).
- Strict timing of application for maximal effect.
- Relatively long period of lethal infection; since microbials do not kill rapidly, users may incorrectly assume that it is ineffective a day or two after treatment.


Muito obrigado por sua atenção !
10kV
X5,000
5µm
010109

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rudolf.wegensteiner@boku.ac.at , www.boku.ac.at