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 Department of Forest- and Soil Sciences

## Insect pathogens – is there a potential for use in biological control of pest insects ?


**Rudolf Wegensteiner**  
 Department of Forest and Soil Sciences  
 Institute of Forest Entomology, Forest Pathology and Forest Protection  
 University of Natural Resources and Applied Life Sciences, BOKU Vienna

  
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## Pathogens in Insects

Virus  
 Bacteria  
 Fungi  
 Microsporidia  
 (Protozoa)


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## Entomopathogenic Virus

- **Virion = infective particle:**
  - ↳ - RNA or DNA,
  - cannot multiply or grow without a living cell,
  - has no autonomous metabolism.

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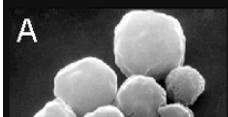
## Entomopathogenic Virus

- ↳ without occlusion bodies:
  - ⇒ Togavirus, Rhabdovirus, .....
- ↳ enclosed in occlusion bodies:
  - ⇒ cytoplasm polydervirus (cpv)
  - ⇒ nucleopolydervirus (npv)
  - ⇒ granulosis virus (gv)
  - ⇒ entomopoxvirus (epv)

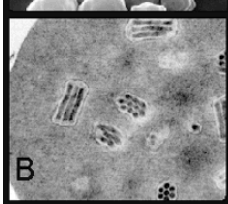
occlusion bodies must be dissolved in the gut !

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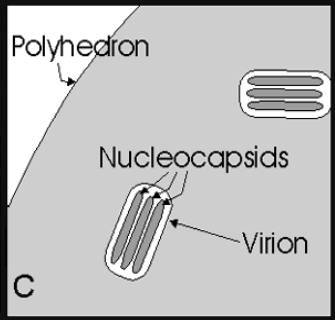
  
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**A**




**B**



**C**

Labels in diagram: Polyhedron, Nucleocapsids, Virion

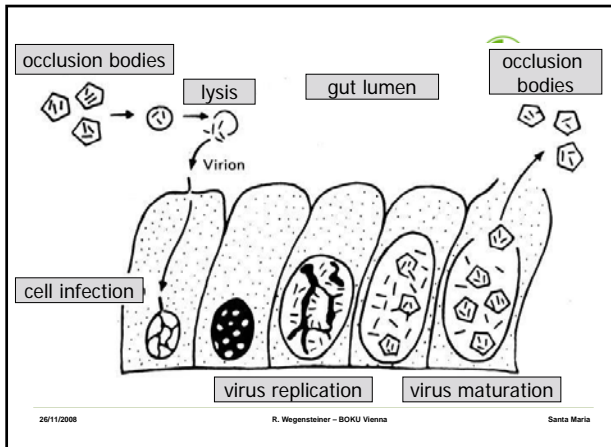
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## Entomopathogenic Virus

- **virus multiplies in living cells only !**
  - ↳ type host or alternate host
  - ↳ cell lines
- **virus multiplies in cytoplasm or in nucleus**
  - ↳ fusion of viral membrane with cell membrane and nucleus membrane (viral core + capsid = nucleocapsid; enclosed in envelopes).

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**Virus infections result in**

- Larvae move slowly, without coordination.
- Larvae are "sluggish".
- Larvae may show changes in colour.

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**Entomopathogenic Bacteria**

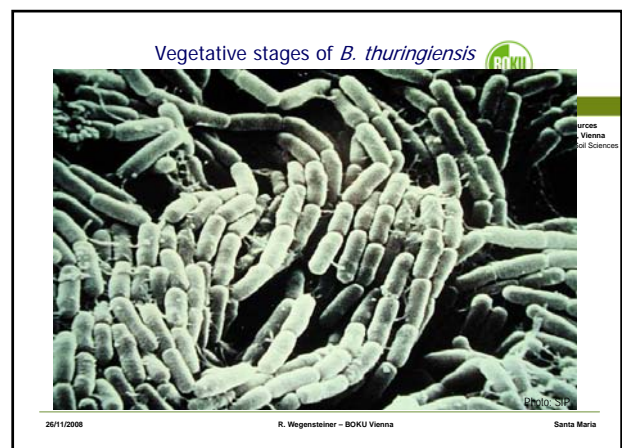
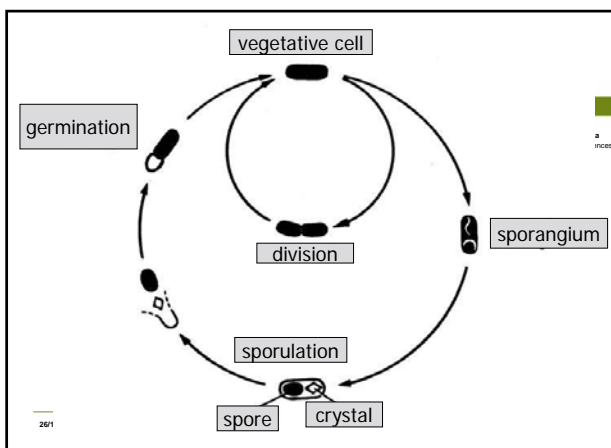
- *Bacillus* spp. (forming spores !)
- ↳ *Bacillus thuringiensis* (e.g.):
  - ⇒ *Bacillus thuringiensis kurstaki*
  - ⇒ *Bacillus thuringiensis israelensis*
  - ⇒ *Bacillus thuringiensis tenebrionis*

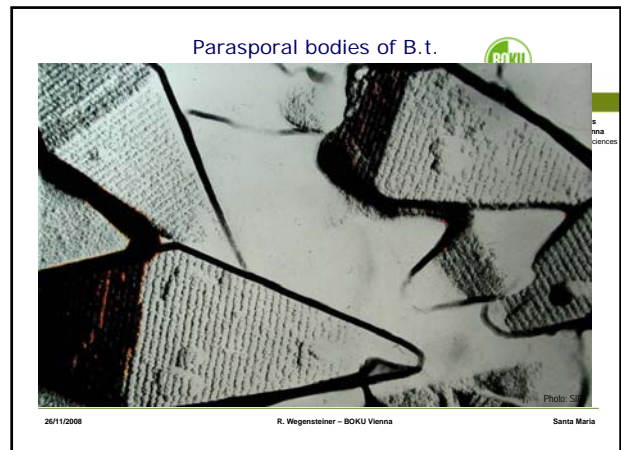
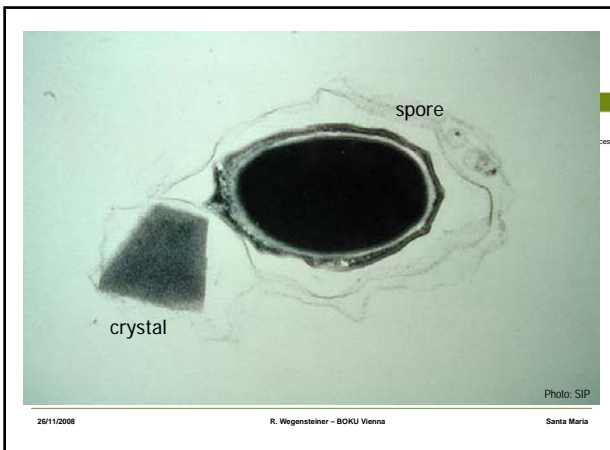
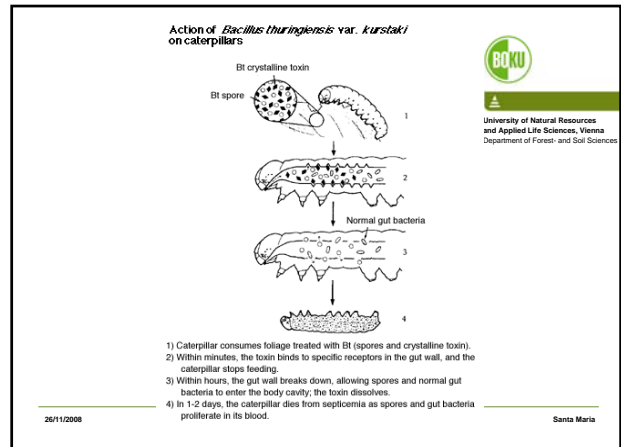
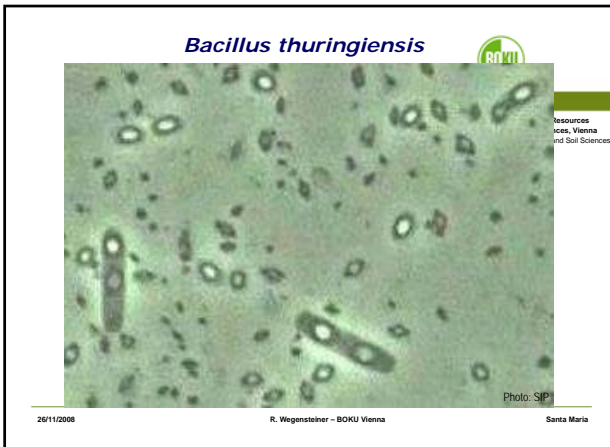
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**Entomopathogenic Bacteria**

- vegetative stages
- spore and parasporal crystal
- ↳ germ enclosed in spore
- ↳ crystal = protoxin (activated by protease; alkali conditions):
- crystal-toxin intoxicates gut epithelium → hypertrophy → "septicaemia"

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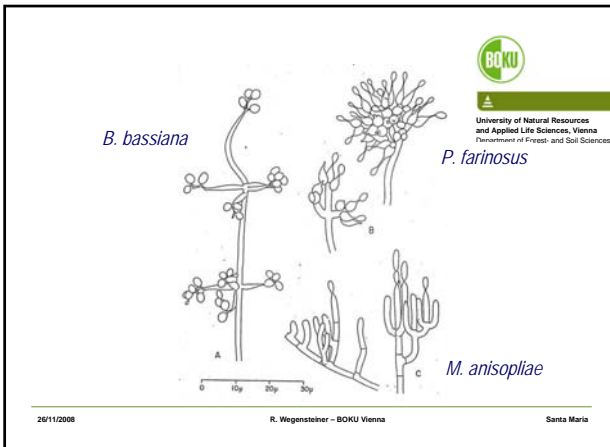


### Bacterial infections result in

- Larvae move slowly, without coordination.
- Larvae are "sluggish".
- Larvae may show changes in colour.

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- ### Entomopathogenic Fungi
- Zygomycota
    - *Entomophthora* spp., *Entomophaga* spp.
  - Ascomycota
    - *Cordyceps* spp.
  - Deuteromycota
    - *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* (*Isaria*) spp.
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## Entomopathogenic Fungi

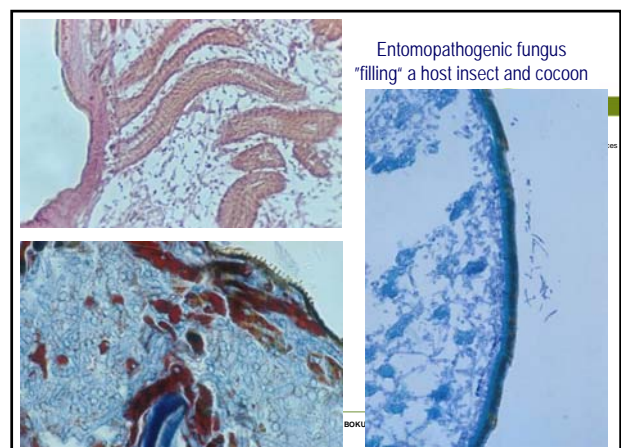
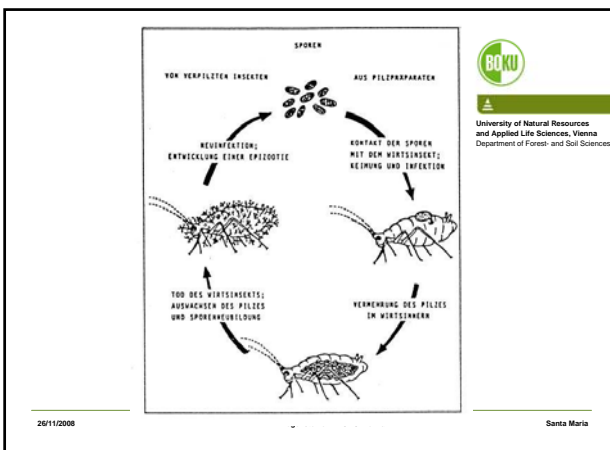
Pathogenic phase:

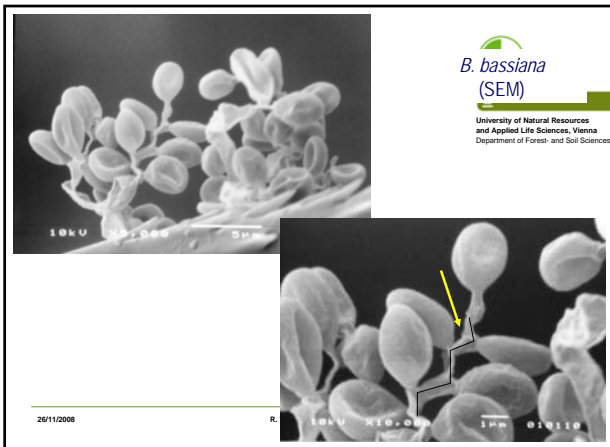
- spore inoculation on cuticle surface
- infection via integument
- development in the whole insect (blastospores)

insect dies ! → saprophytic phase:

- colonization of the whole host
- penetration of cuticle and production of conidiospores

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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.

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### Entomopathogenic Microsporidia

- spore (endo- and exosporium = “environmental spores”)
- 1 or 2 nuclei
- polaroplast (membrane, forming “umbrella-like” layers)
- polar filament (= “polar tube” after extrusion)  
↳ H<sub>2</sub>O uptake + transformation of trehalose into glucose increases the volume within the spore ⇒ explosive extrusion of polar filament and squeeze out of the germ.

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Diagramme of a microsporidian spore (*Nosema locustae*)

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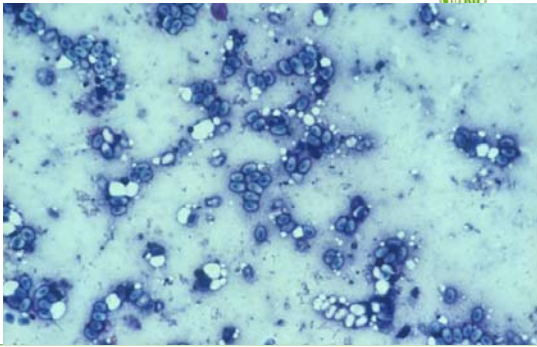
### *Edhazardia aedis* – exfilamentation through peritrophic membrane of *Aedes aegypti*

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### *Nosema apis* in *Apis mellifera*

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### *Nosema typographi* in *Ips typographus*



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### Microsporidian infections result in

- Larvae move slowly, without coordination.
- Infected larvae are "slack".
- Larvae may show changes in colour.

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### Pathogens are sensitive to

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

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### Infection

- peroral
- percutaneous
- perstigmatal
- peranal
- pertraumatic
- by vectors

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## Transmission of pathogens

- horizontal:  
pathogen transfer between individuals of the same generation
- vertical:  
pathogen transfer from parents to progeny
- by vectors:  
pathogen transfer by non targets to parents and/or progeny


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## Horizontal transmission

- Oral ingestion of (infectious) virus occlusion bodies or spores of bacteria or microsporidia (found in food or liquid)
  - sufficient quantity of spores !
  - appropriate conditions in the alimentary tract (pH, enzymes) !
- 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.

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## Vertical transmission

in most cases maternally mediated ("trans ovum")

- pathogens are transferred to egg stage:
  - within the ovary (stage ? germination ?)
  - 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.

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## 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.


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## 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

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## Infections

- single infection
- ↓
- multiple infections
  - specificity (host range) ?
  - contact ?
  - action ?
  - interactions ?
    - ↳ indifferent, additive, synergistic, antagonistic.

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## Tolerance and resistance



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- **age tolerance of insects:**  
L1-larvae are ± more sensitive than older larval instars !
- **resistance of insects:**  
using always the same strain → sensitive individuals will be killed, tolerant or resistant individuals will survive !

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## “Advantages” of microbials



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- Specificity to the target organism (or a very limited number of hosts)
- Little or no direct impact upon parasitoids and predators
- Harmless to vertebrates and plants
- No toxic residues
- Possibility of long-term control
- .....

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## “Disadvantages” of microbials



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- Specificity only to target organism – costs ! - especially for obligate pathogens (niche markets ?)
- Strict timing of application for maximal effect
- Relatively long period of lethal infection
- Inactivation by environmental factors

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## Microbial control



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- **Release/application methods:**
  - inoculative release = autodissemination
  - inundative release = mass application (biopesticide concept)
- **Use/management of organisms (or virus):**
  - classical biological control
  - neo-classical biological control
  - conservation biological control

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## Dispersal of pathogens ?



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The capacity to “disperse” is a key factor !

- **Passive dispersal of inoculum:**  
inoculated living individuals 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 by infected hosts (“summit disease”)

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## Identification of pathogens



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- **Externally visible symptoms**  
colour, habits, ... ?
- **Internally visible symptoms (needs dissection) –**  
morphological studies (use of light microscope, use of electron microscope)
- **Molecular characteristics**  
molecular studies

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## Morphological Diagnosis



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- macroscopic inspection + documentation of symptoms
- microscopic inspection + documentation of symptoms resp. of pathogens:
  - native (in H<sub>2</sub>O or insect Ringer),
  - after Methanol fixation and staining with Giemsa's dye.

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## Literature



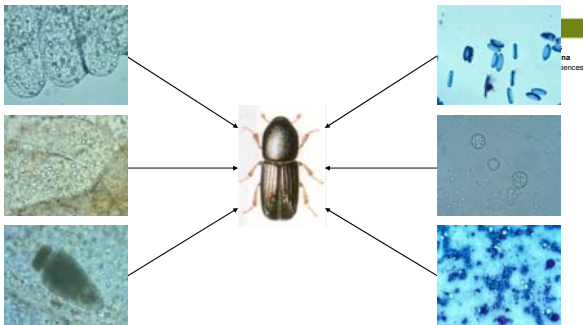
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## Identification of Pathogens in *Ips typographus*

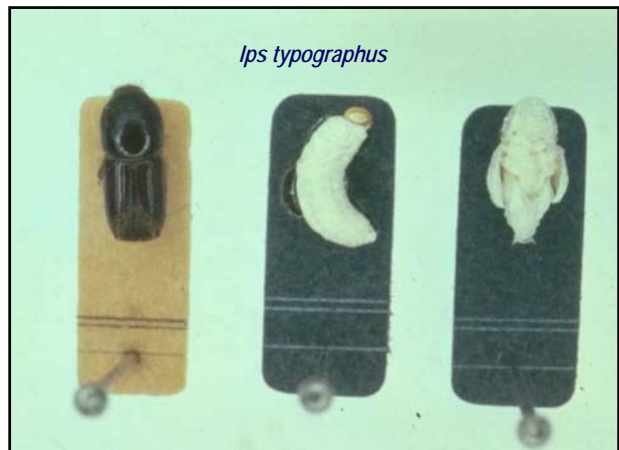


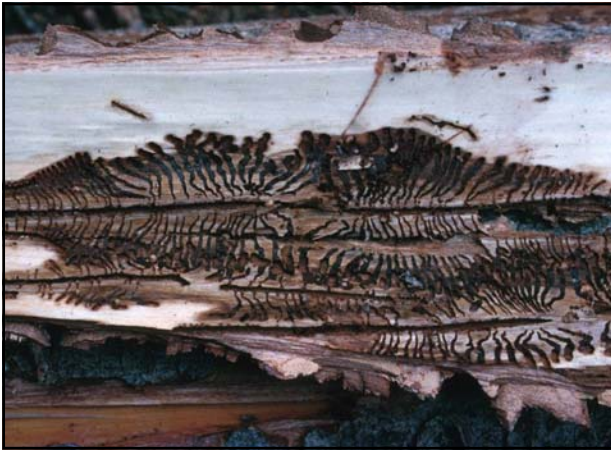
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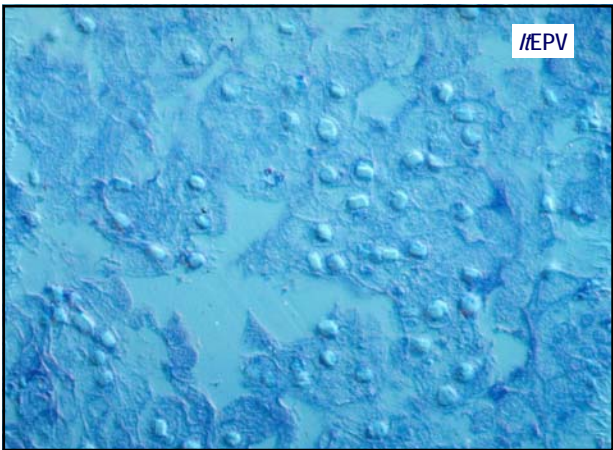
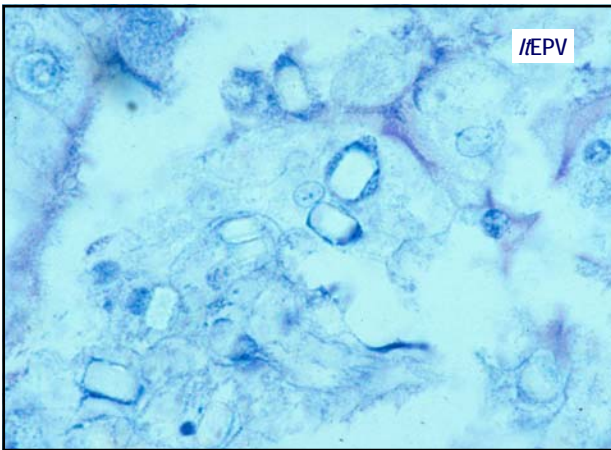
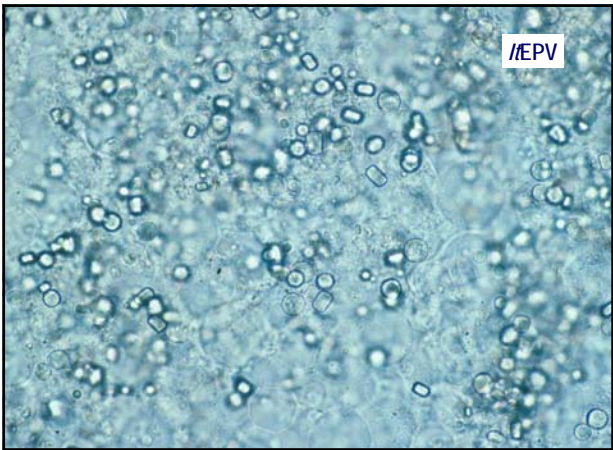
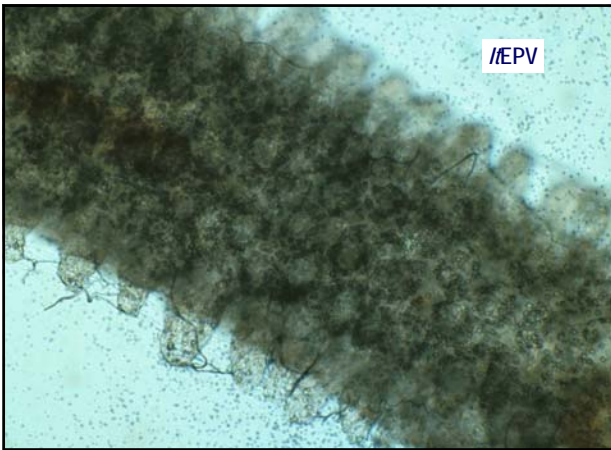
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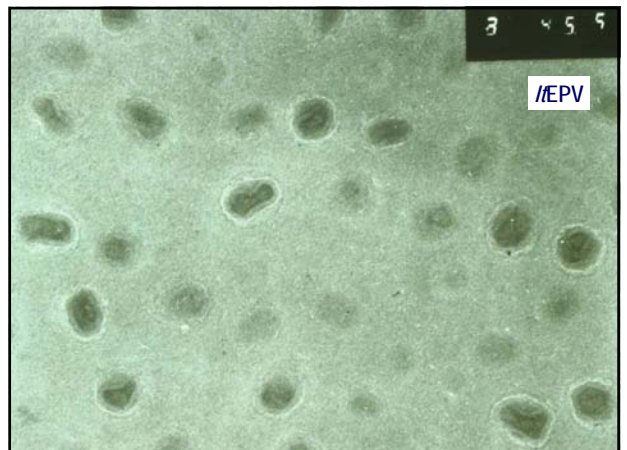
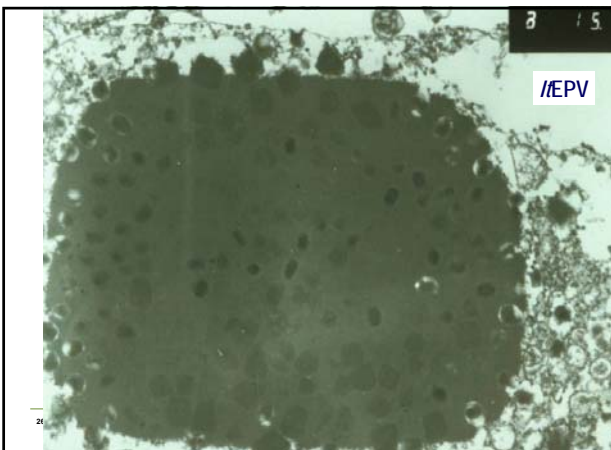
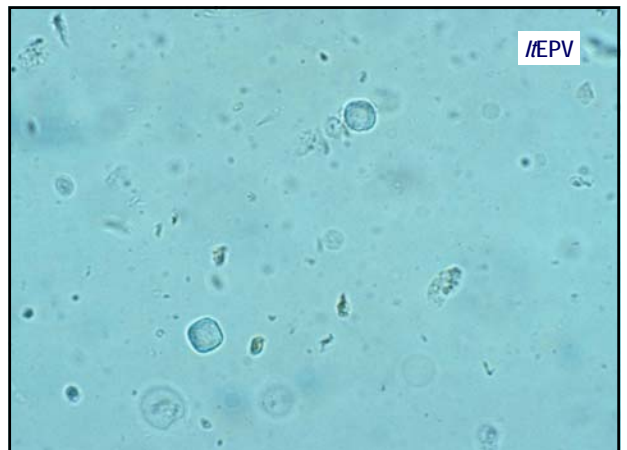
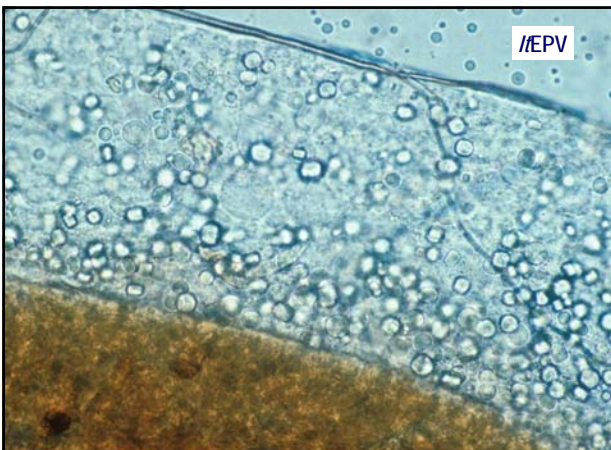
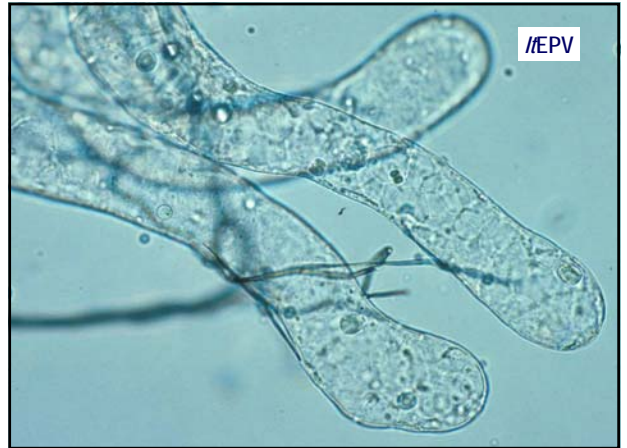
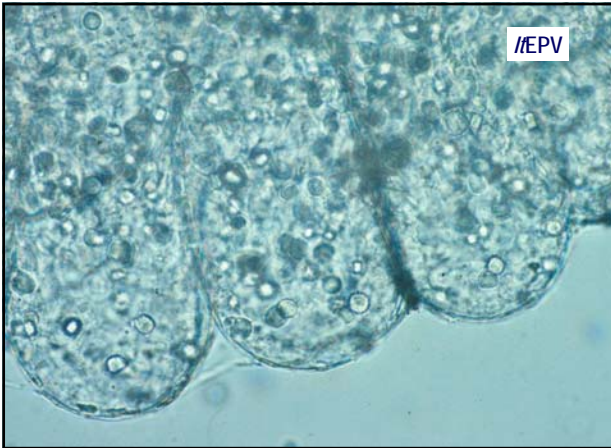
## *Ips typographus*

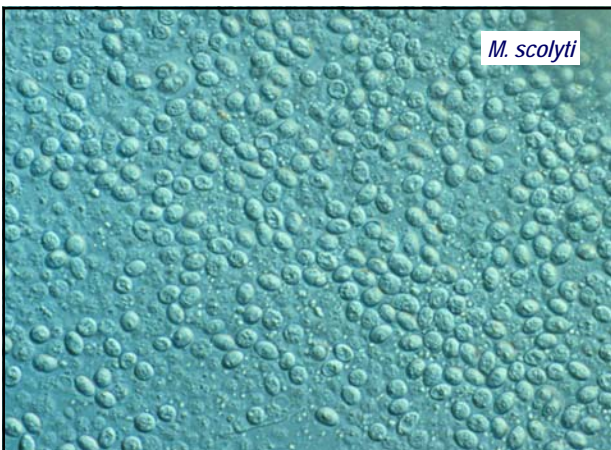
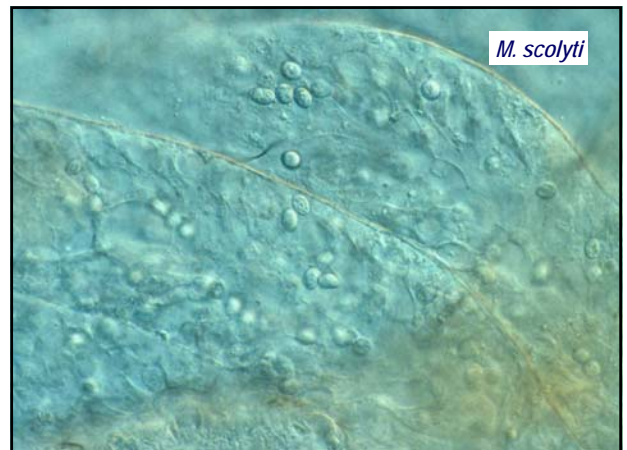
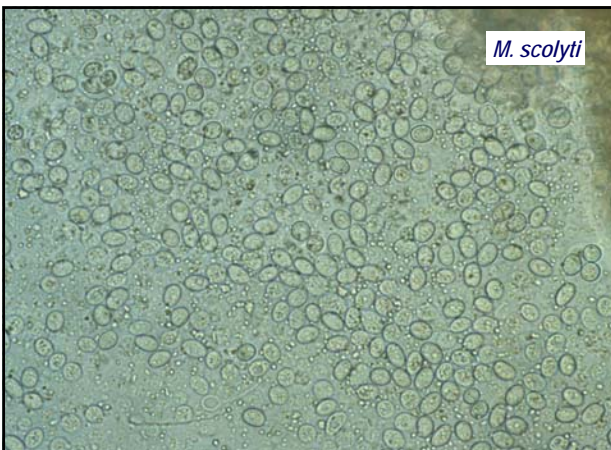
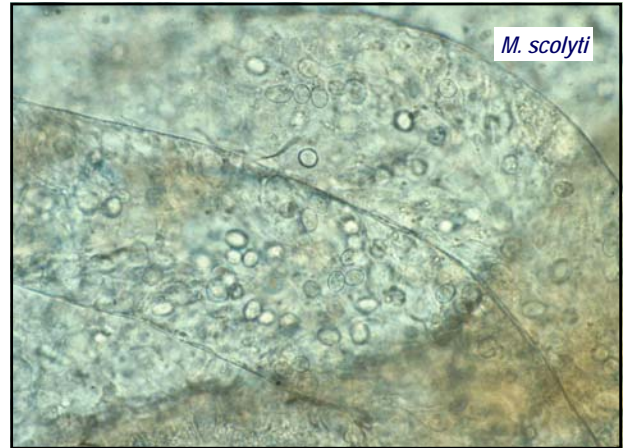
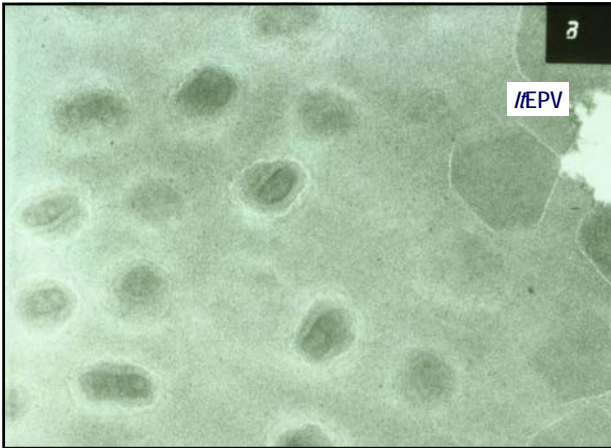


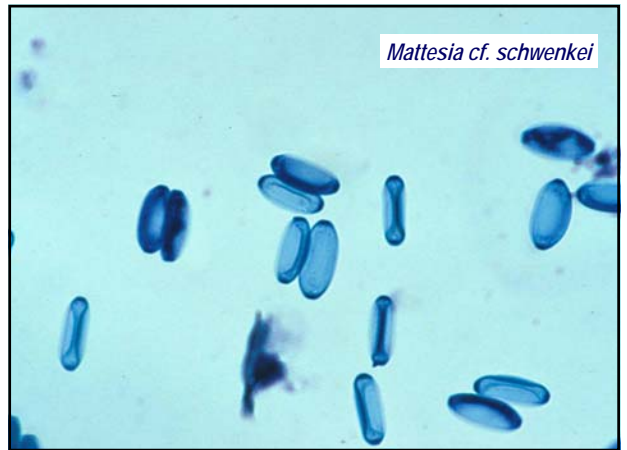
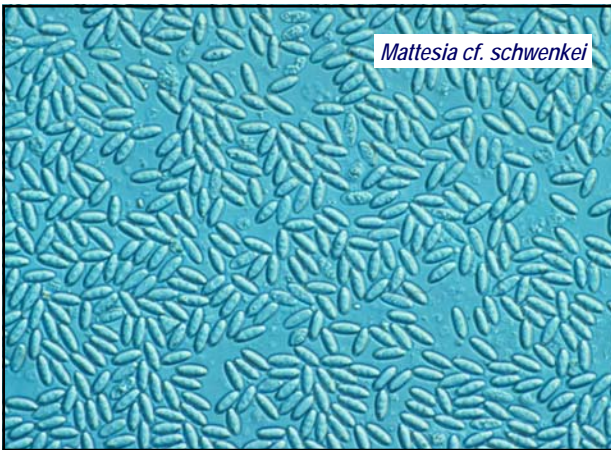
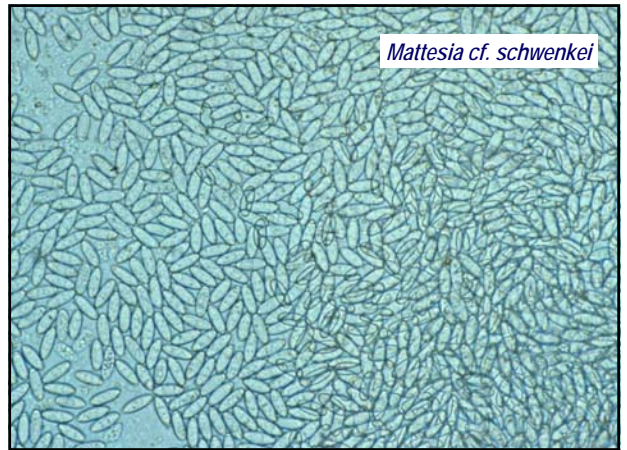
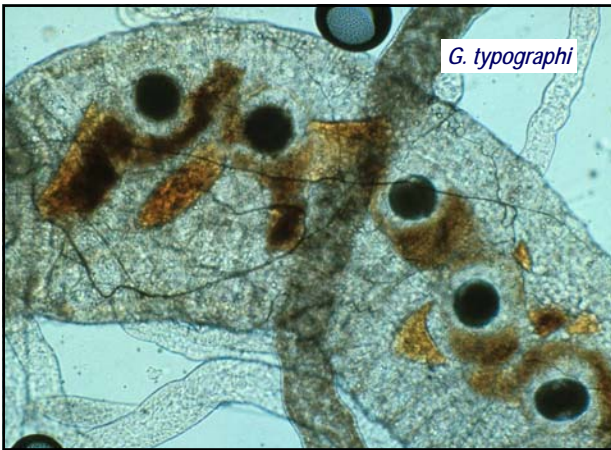
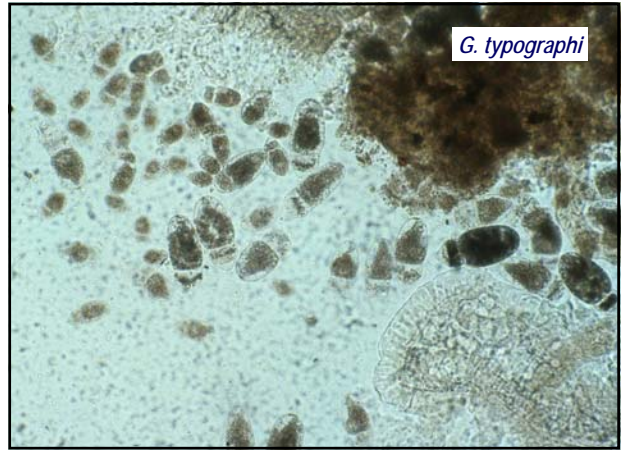


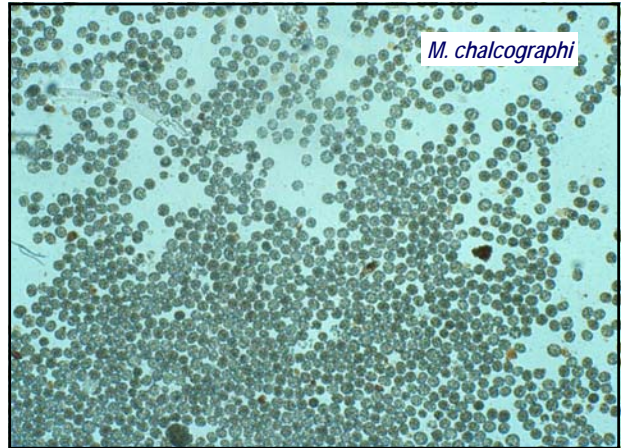
| Pathogen species                        | in organ                                |
|---|---|
| ▪ <i>Ips typographus</i> Entomopoxvirus | midgut epithelium                       |
| ▪ <i>Malamoeba scolyti</i>              | Malpighian tubules<br>midgut epithelium |
| ▪ <i>Gregarina typographi</i>           | midgut lumen                            |
| ▪ <i>Mattesia cf. schwenkei</i>         | adipose tissue                          |
| ▪ <i>Menzbieria chalcographi</i>        | adipose tissue                          |
| ▪ <i>Chytridiopsis typographi</i>       | midgut epithelium                       |
| ▪ <i>Nosema typographi</i>              | adipose tissue, gonads                  |
| ▪ <i>Unikaryon montanum</i>             | midgut epithelium,<br>gonads            |
| ▪ <i>Metschnikowia typographi</i>       | midgut epithelium                       |
| ▪ <i>Beauveria bassiana</i>             | whole insect                            |

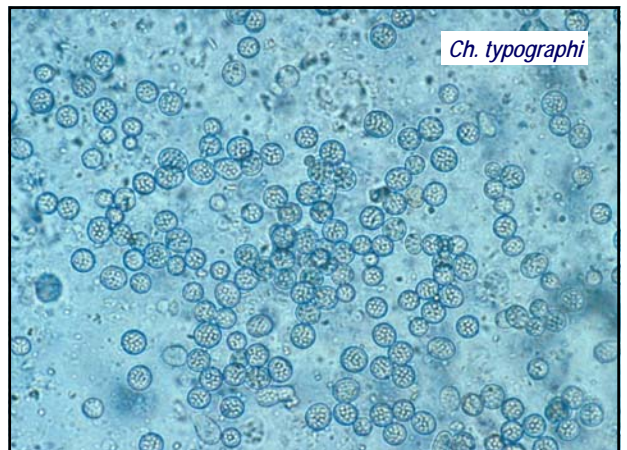
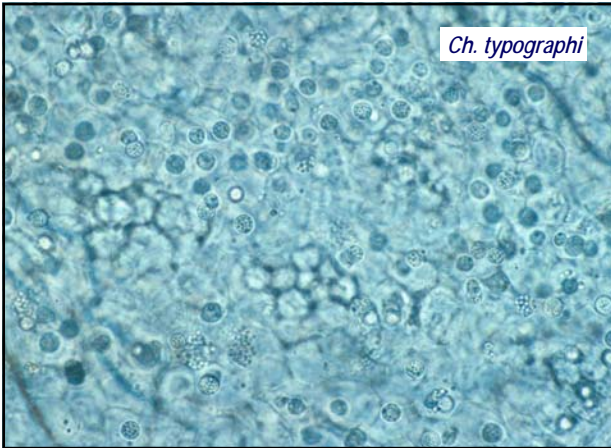


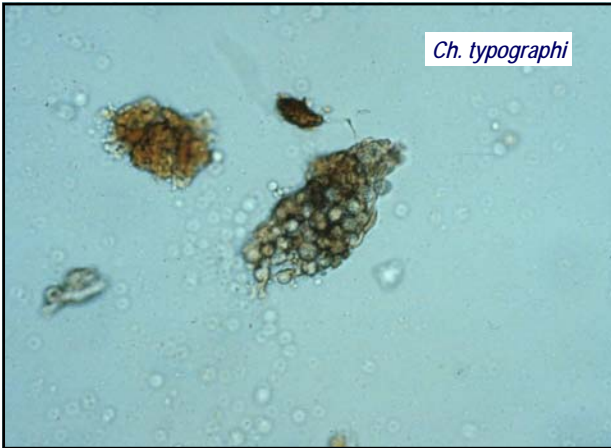




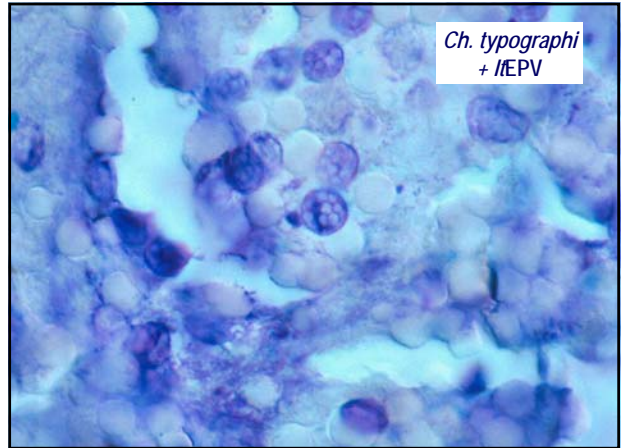




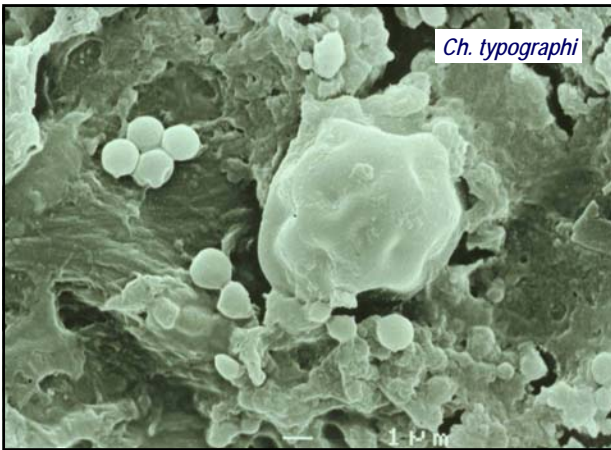




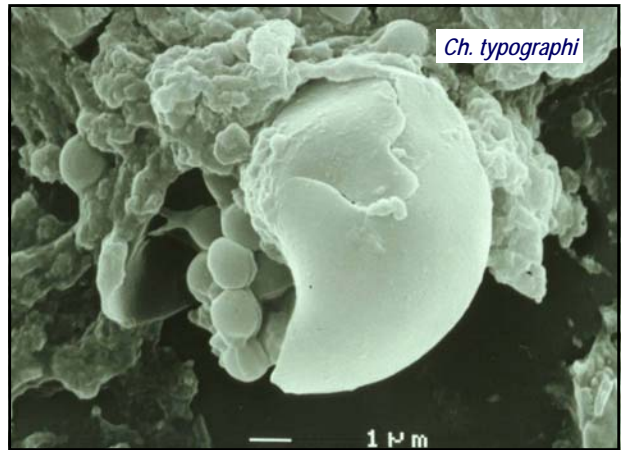
*Ch. typographi*



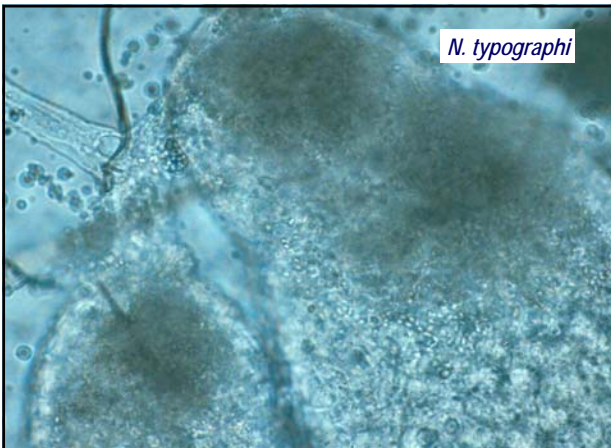
*Ch. typographi*  
+ IEPV



*Ch. typographi*



*Ch. typographi*

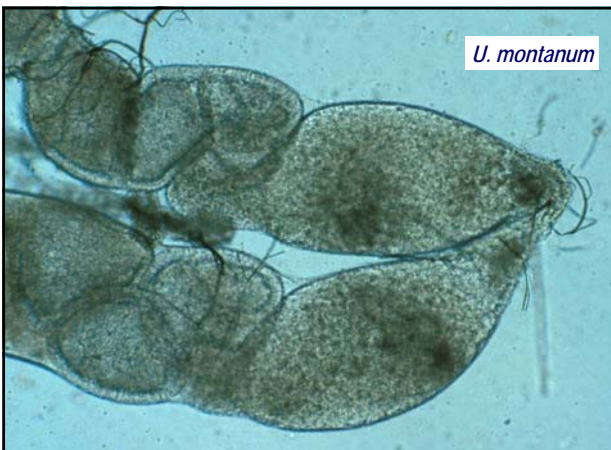
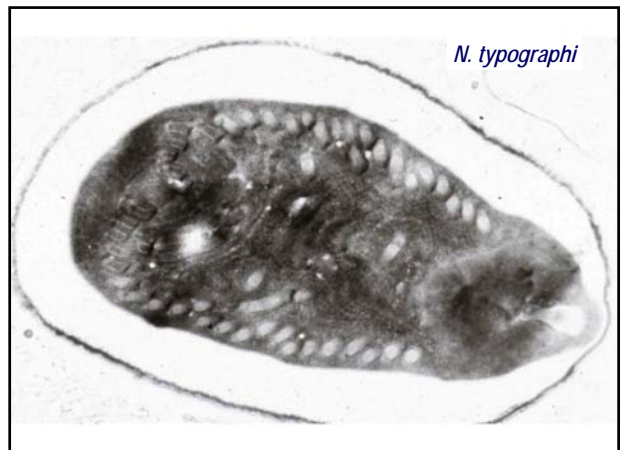
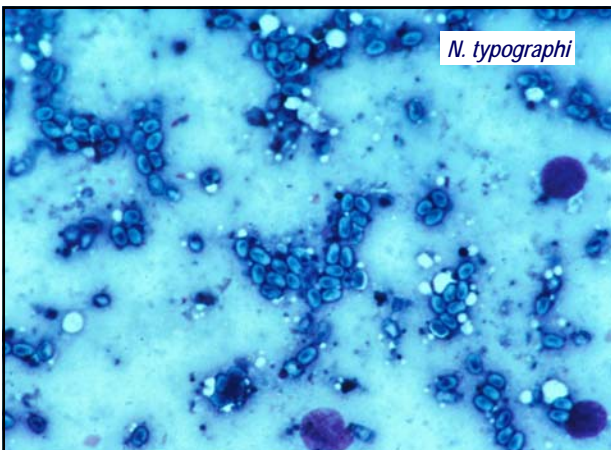
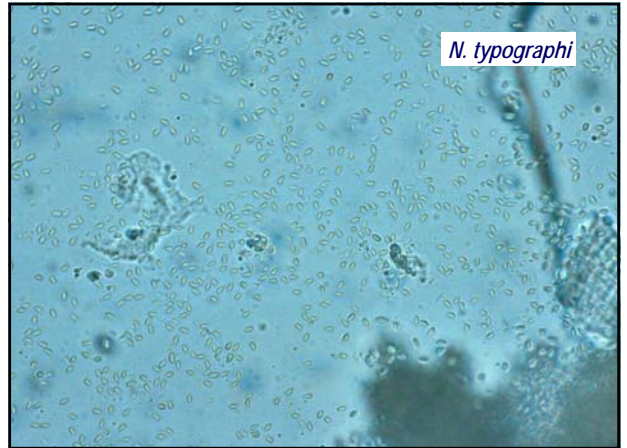
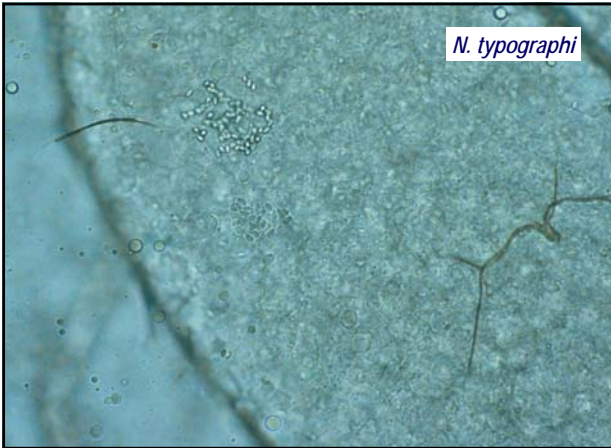


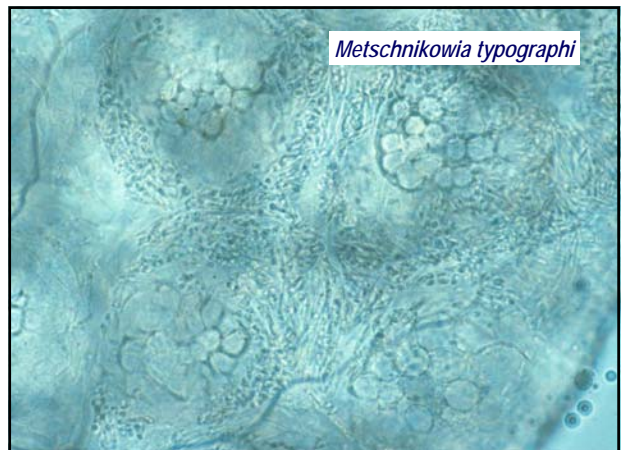
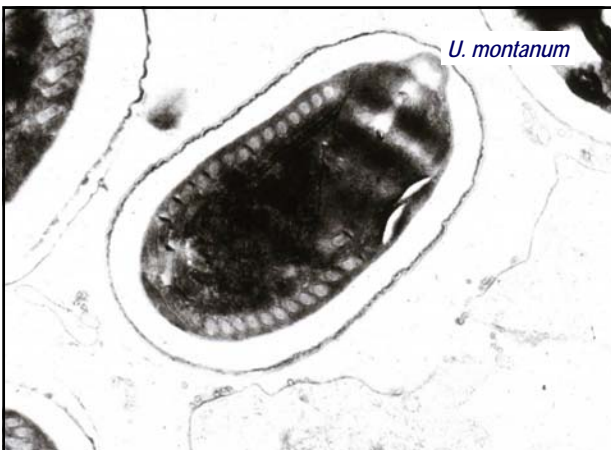
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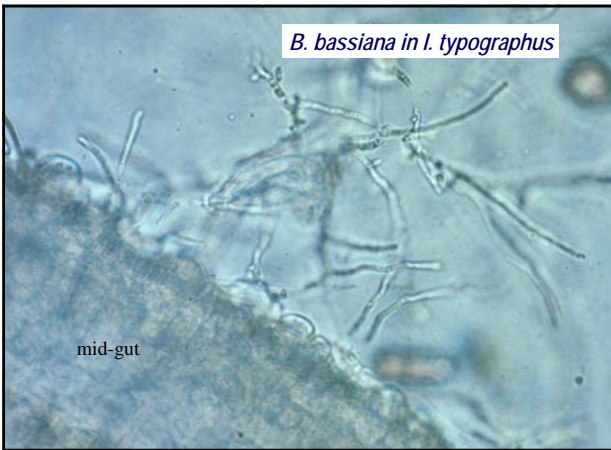
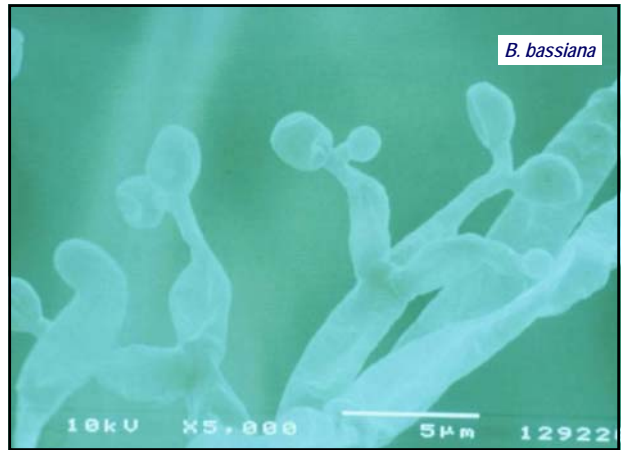
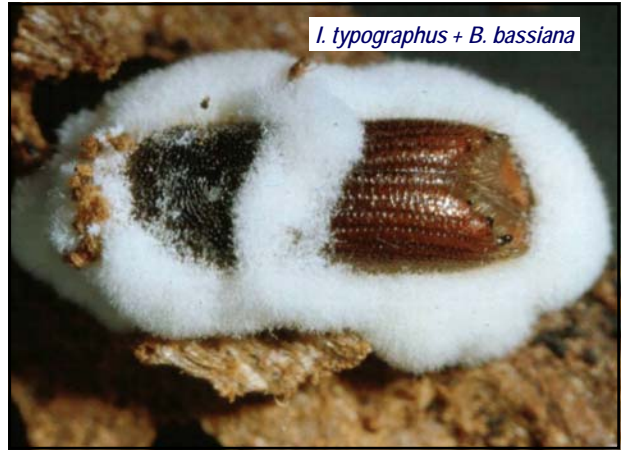
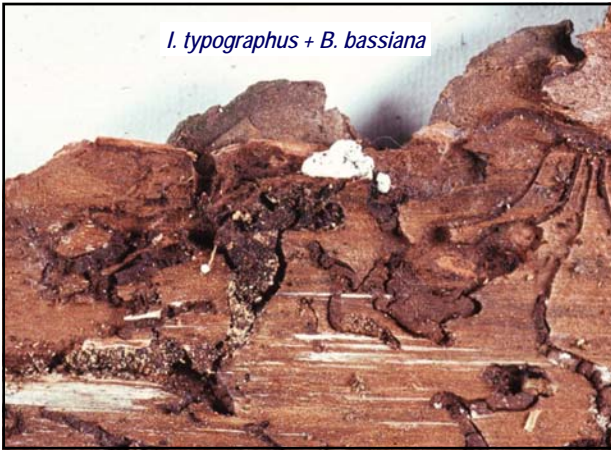


*N. typographi*











Department of Forest and Soil Sciences  
Institute of Forest Entomology, Forest Pathology and Forest Protection  
University of Natural Resources and Applied Life Sciences, BOKU  
Vienna

Dr. Rudolf Wegensteiner

Gregor Mendel-Straße 33, A-1180 Vienna, Austria  
Tel.: +43 1 3686352-30, Fax: +43 1 3686352-97  
rudolf.wegensteiner@boku.ac.at, www.boku.ac.at