NOTES ON INSECT PATHOGENS

INSECT PATHOLOGY MANUAL

Section I

CONTENTS

INTRODUCTION

This section is intended to provide some background information on insect pathogens for the entomologist who is interested, but may have no specialist training, in pathology. Few entomologists are trained in pathology and it is because of this that insect pathology remains, for the most part, an under-studied field. Pathogens are often important in regulating natural insect populations. If we do not understand the role of pathogens it may be difficult, or even impossible, to fully understand insect population dynamics.

Insect pathogens may offer possibilities for manipulation of pest populations, either by introducing them as classical biocontrol agents, or by augmentation of naturally occurring populations, and the subsequent use of the pathogen as a biological pesticide. These notes provide a basic introduction to the characteristics of insect pathogens. Although they will not help in identifying pathogens to genus or species, they may provide some valuable clues as to the cause of death of insects, and also provide further sources of information. In Appendix III you will find a list of useful references for further reading

1. GROUPS OF PATHOGENS

The most important groups of entomopathogens* are:

- VIRUSES
- BACTERIA
- FUNGI
- PROTOZOA
- NEMATODES

To study or identify non-occluded viruses you will need access to an electron microscope. For bacteria and occluded viruses, use a compound microscope with an oil-immersion objective lens. Fungi and protozoa can be observed under a compound light microscope. Nematodes can usually be seen with the naked eye.

N.B. * denotes an entry in the Glossary - Appendix

VIRUSES

- BACULOVIRUSES:

Nuclear Polyhydrosis Viruses

Granulosis Viruses

Group C viruses

- ENTOMOPOX VIRUSES

Description:
Viruses are sub microscopic, intracellular*, obligate pathogens*. They can neither move nor metabolise. They consist of a template nucleic acid with or without a protein coat, which is also called an inclusion* or occlusion* body.

Reproduction:
Viruses multiply by independent synthesis of their component parts. These parts assemble to produce progeny virus within the host cell.

Infection:
Viruses normally enter the host through the mouth. The protein coat of the virus dissolves in the gut, releasing the virus particles (virions*). The virions invade, then multiply in the cells of the gut wall. Replication on a massive scale then takes place in the fat body, haemocytes* and hypodermis*.

Death of host:
Death usually occurs in three to ten days.

Survival:
After death the body ruptures and releases millions of occlusion bodies*. Occlusion bodies protect the virus which can persist for years in the right conditions.

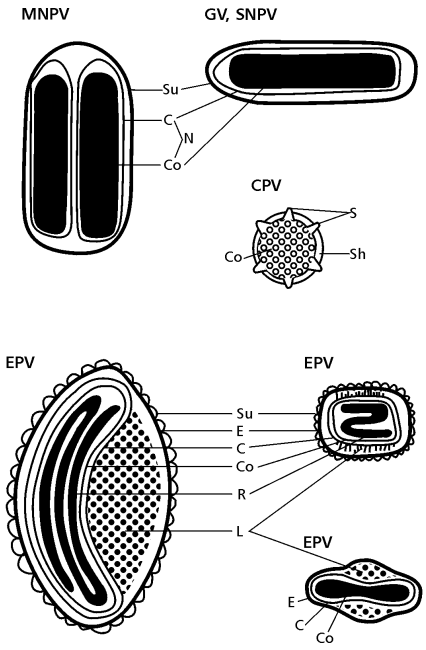
Host range:
The host range is narrow, but viruses are known from a wide range of hosts.

Culture:
Viruses can only be cultured in the live insect host or in tissue culture.

Groups:
Baculovirus, entomopox virus, picornavirus, cytoplasmic polyhedrosis virus.

Figure 1

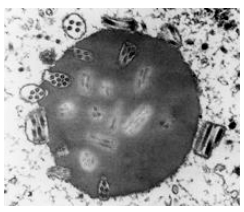
DIAGRAMMATIC REPRESENTATION OF TYPES OF VIRIONS IN OCCLUDED INSECT VIRUSES



C capsid; ***Co*** core; ***CPV*** citoplasmic polyhedrosis virus; ***E*** envelope; ***EPV*** entomopoxviruses; ***GV*** granulosis virus;***L*** lateral body; ***MNPV*** multiple nucleocapsodes per envelope nuclear polyhedrosis virus; *N* nucleocapsid; ***R*** colled rodlike structure; ***S*** splkes; ***Sh*** shell; ***SNPV*** single nucleocapsid per envelope nuclear polyhedrosis virus; ***Su*** surface units

Biocontrol agents:
At present, the main group of viruses used as biological control agents are the baculoviruses (see below)

BACULOVIRUSES



Description:
Baculoviridae are the safest insect viruses to use as pathogens, since no similar viruses are known to infect vertebrates or plants. They have double-stranded DNA and are protected by a protein coat which improves their persistence.

Infection:
Infection occurs after susceptible insect larvae eat

food contaminated with virus. The virus then attacks the haemolymph, fatty tissue and mid gut. The insect becomes paralysed.

Virulence:
Highly virulent; the presence of very few particles can initiate infections and hosts die within 3-10 days.

Susceptibility:
The gut of the host insect must be alkaline so that the occlusion body can dissolve.

Locusts:
No baculovirus has been recorded from locusts with the exception of an unconfirmed report of cross infection from a ***Spodoptera*** sp. (Lepidoptera) host.

Groups:
Three groups of baculoviruses are described below: nuclear polyhedrosis viruses, granulosis viruses and group C baculoviruses.

BACULOVIRUSES: Nuclear polyhedrosis viruses (NPV)

Description:
About 280 species known. Rounded cubic or hexagonal polyhedra. 0.5-1.5 microns (µm) in size. Singly or multiply envelopped (see Figure 1).

Infection:
Infection occurs in the adipose* tissue of the hypodermis, in the tracheae and the middle intestine.

Host:
Approximately 120 species of Lepidoptera and Hymenoptera (particularly saw-flies). Each virus is highly specific to its host.

Survival:
Nuclear polyhedrosis viruses form particles inside a crystalline protein structure (occlusion body*). This allows the virus to survive outside the host for years out of sunlight.

Biocontrol agents:
The following NPVs have all been produced on a commercial or semi-commercial scale: ***Autographa californica*** NPV, ***Lymantria dispar*** NPV, ***Malacasoma disstria*** NPV, ***Mamestra brassicae*** NPV, ***Neodiprion sertifer*** NPV, ***Spodoptera*** NPV and ***Heliothis*** NPV

BACULOVIRUSES: Granulosis viruses (GV)

Description:
About 65 species Oval or ovoid granules.

Infection:
Granulosis viruses attack the adipose* tissue.

Host:
Lepidoptera larvae.

Survival:
Granulosis viruses form particles inside a crystalline protein structure (occlusion body*). This allows the virus to survive outside the host. Can survive for years out of sunlight.

Biocontrol agents:
Include ***Cydia pomonella*** GV (codling moth), ***Phthorimaea operculella*** GV (potato tuber moth).

BACULOVIRUSES: Group C Baculoviruses

Description:
Double stranded DNA. Viruses with non-included virions. Only visible using an electron microscope. 22-30 nm in size. These viruses are unusual since they have no protective protein coat to help them to survive.

Infection:
These viruses attack the haemolymph*, fat body, mid-gut. Insects become paralysed.

Host:
These viruses are restricted to Arthropoda. Larvae and adults of Coleoptera, Hymenoptera and mites.

Biocontrol agents:
Baculovirus oryctes:
Used for the control of rhinoceros beetles, *Oryctes* spp. This virus is excreted from the living diseased insect as virions*. These are passed on to other adults during mating. Some spread occurs from contamination of adult breeding and larval feeding sites, but the virus does not survive long in the environment.

ENTOMOPOX VIRUSES

Description:
Entomopox viruses have inclusion bodies* (i.e. they are occluded* viruses) which are important for identification. Spherical or ovoid particles. 5-20 µm in size.

Infection:
These viruses must be ingested by the host. They then attack the fat body.

Virulence:
They kill hosts more slowly than baculoviruses.

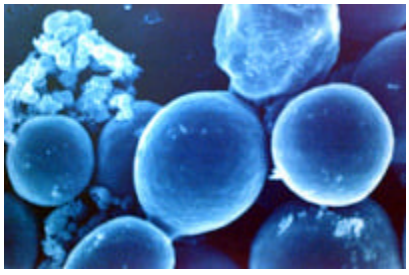
Host:
Lepidoptera larvae, Diptera, Coleoptera, Orthoptera.

Survival:
Little is known.

Locusts:
Entomopox viruses have been recorded from locusts and grasshoppers.

BACTERIAS

Description:



Bacteria are microscopic prokaryotes*. There is no well-defined nucleus or organelles*. They have a structurally distinct cell wall.

Groups:
Spore forming and non-spore forming bacteria.

Reproduction:
Bacteria grow independently in the insect haemocoel*.

Infection:
Bacteria must be ingested. They rupture the gut wall, then invade the haemocoel.

Survival:
Some bacteria form spores which are moderately resistant.

Host range:
Lepidoptera, Coleoptera and Diptera.

Culture:
They can easily be grown in Petri dishes or in liquid culture but specific conditions are needed for spore formation in artificial media.

Biocontrol agents:
The main bacterial control agents of insects are species from the genus ***Bacillus***. Other groups exist but typically only cause disease in weak, injured or stressed insects.

Bacillus spp.

Description:
These are aerobic, unicellular, usually bacilliform*, spore forming bacteria.

Infection:
Infection occurs after ingestion of bacterial cells or spores. They mainly affect phytophagous* larval stages. Bacteria invade and reproduce in the haemocoel*, inducing lethal septicaemia.

Culture:
Easily produced by fermentation.

Symptoms:
The bacterial toxin attacks the gut lining and causes muscle paralysis in the alimentary tract and the mouth parts. Feeding stops. Insects may regurgitate and have diarrhoea.

Death of host:
Between 30 minutes and 24 hours. Death may be caused by starvation.

Susceptibility:
Susceptibility depends on the gut of the host being alkaline.

Locusts:
No strains of *Bacillus* are known to affect locusts, although there is a report of an NPV infection.

Biocontrol agents:
There are several species of ***Bacillus*** which are used in biological control:

Bacillus thuringiensis:
During sporulation the bacteria produce a large proteinaceous crystal which is bipyramidal in shape and also a thick walled endospore*. The crystal is an endotoxin* which dissolves inside the host in alkaline gut fluids and releases toxic polypeptides*. Mainly lepidoptera are attacked, but strains specific to mosquitoes and Coleoptera are also known. It has been developed as a commercial product, widely available throughout the world.

Bacillus sphaericus:
Certain strains produce a proteinaceous toxin which poisons mosquito larvae. Host death may also be induced by the spores alone.

Bacillus popilliae:
Produces no toxins in the infection cycle. Japanese beetle **(*Popillia japonica*)** larvae ingest spores in the soil which germinate and produce vegetative cells which fill the gut in three to five days. Some cells penetrate the gut wall, then grow and sporulate in the haemolymph. 14-21 days after initial infection the insect body is swollen and creamy white (milky disease). After death, the spores are released into the soil and establish persistent infection sites.

PHOTO: Michael Klein, USDA: ARS: Horticultural Insects Research Lab, OARDC, Wooster, OH

Healthy beetle larva (left)
larva infected with ***B. popilliae*** (right).

FUNGI

- ENTOMOPHTHORALES
- DEUTEROMYCETES

Description:
Fungi are eukaryotes*, with a well-defined nucleus and organelles, characterised by chitinised* cells. The cells are formed into filaments or hyphae* together forming a mycelium*.

Groups:
Entomopathogenic fungi are found in several subdivisions. The fungi in the Entomophthorales (e.g. ***Entomophaga grylli***) have complex life cycles involving a sexual stage and resting spores, whereas Deuteromycete fungi (e.g. ***Metarhizium*** and ***Beauveria***) have a simple life cycle with no known sexual stage.

Reproduction:
Fungi reproduce by spores which are formed sexually or asexually.

Infection:
Spores germinate on the host cuticle and penetrate using enzymes and mechanical pressure. Inside the haemocoel the fungus multiplies rapidly by budding or hyphal fission*. The resulting yeast-like cells (blastospores*) spread through the body.

Death of host:
By extensive mycelial colonisation* causing asphyxiation or starvation, or by toxins released in the

yeast phase. The cadaver desiccates as the hyphae use host nutrients and water to develop.

Survival:
Hyphae break through the cuticle after death. Spores may be liberated passively or actively to continue the infection cycle.

ENTOMOPHTHORALES

Reproduction:
Entomophthoralean fungi have complex life cycles involving non-sexual conidia, and sexual resting spores.

Culture:
Entomophthorales fungi must be cultured in complex media and some cannot be cultured in artificial media.. Some produce mycelium* but will not sporulate.

Biocontrol agents:
***Entomophaga praxibulli* :**
1has been introduced to the USA for control of grasshoppers.

Grasshopper killed by a complex of ***Entomophaga*** species.

***Entomophthora plutellae* :**
used against *Plutellai* in Thailand.

Zoophthora radicans:
has been used as a classical biocontrol agent against aphids in Australia.

Leafhopper infected by ***Zoophthora radicans.***

DEUTEROMYCETES

Reproduction:
The deuteromycete fungi have no known sexual stage. Although they do have mechanisms for the exchange of genetic material, most reproduction is asexual, by the production of asexual spores called conidia.

Culture:
Many deuteromycetes are easily cultured in simple artificial media.

Biocontrol agents:
Metarhizium: *M. anisopliae*,
and the sub-group *M. flavoviride* have been produced on a large scale on rice substrate in several tropical countries.

Beauveria: *B. bassiana* and *B. brogniarti*
have been produced on a large scale in China, Europe and America.

Verticillium lecanii:
has been developed as a biopesticide for use in glass-houses in Europe.

Table 1

MAJOR GROUPS OF ENTHOMOPATHOGENIC FUNGI

SUB-DIVISIONS	ORDERS	FAMILIES	EXAMPLE
Mastigomycotina	Oomycetes	Lagenidiaceae	*Lagenidium*
	Chytridiomycetes	Blastocladiaceae	*Coelomomyces*
Zygomycotina	Entomophthorales	Entomophthoraceae	*Entomophaga*
			and many other genera
	Mucorales	Mucoroceae	*Sporodiniella*
Ascomycotina	Clavicipitales	Clavicipitaceae	*Cordyceps*
	Hypocreales	Hypocreaceae	*Cordycepioideus*
	Laboulbeniales	Laboulbenioceae	many genera
	Pleosporales	Podonectriaceae	*Podonectria*
Basidiomycotina	Septobasidiales	Septobasidiaceae	*Septobasidium*
Deuteromycotina	Hyphomycetes		*Verticillium*
no formal classification			*Aspergillus*
			Beauveria
			Metarhizium
	Coelomycetes		*Sorosporella*

From Ainsworth *et al.* 1983

PROTOZOA

Description:
These are unicellular organisms classified in several phyla.

Groups:
Ciliophora, Sarcomastigophora, Apicomplexa, Microspora.

Reproduction:
Reproduction is asexual and occurs in the gut or fat body cells by multiple or binary fission. The fusion of two gametes* forms a zygote* which divides repeatedly.

Host range:
Wide range in insects, mammals and man but especially obligate pathogens of arthropods.

Death of host:
Very slow. Not highly pathogenic but they do reduce the rate of development and fecundity.

Infection:
Chronic rather than lethal caused by ingesting spores followed by penetration of digestive tract. Spores have a polar capsule which, after ingestion and germination develops into a tube that can penetrate gut cell walls. Protozoa kill the host only at very high levels.

Survival:
The liberated spores are highly resistant. Transmission of spores is also possible through the eggs.

Culture:
In live insects only.

Biocontrol agents:
Microspora:
The most important protozoans for biological control.

Nosema:
There are some species which can be used in biocontrol. *Nosema locustae* infects a wide range of grasshoppers.

Vairimorpha:
Vairimorpha necatrix is a broad spectrum agent which is infectious to many Lepidoptera.

Malamoeba:
Species of *Malamoeba* are mainly known as a contaminant of laboratory cultures of locusts.

NEMATODES

- MERMITHIDAE
- SECERNENTIA

Description:
Nematodes are unsegmented, worm-like organisms with a tough outer cuticle. Nematodes are larger than other entomopathogenic organisms and can usually be seen with the naked eye. They can act as vectors* for entomopathogenic bacteria.

Groups:
There are eight or nine orders associated with insects. Most biocontrol agents are among the Mermithidae and Senecentia.

Reproduction:
Adults are bisexual. They develop through four larval moults before reaching maturity.

Survival:
Generally grow in the host and develop in the environment, in both aquatic and terrestrial habitats.

MERMITHIDAE

Subclass Penetrantia, family Mermithidae; a large group of obligate parasites.

Infection:
These invade through the cuticle at the second larval stage and develop to the fourth larval stage filling the host body cavity. This shows as a swollen abdomen.

Death of host:
Larvae emerge after rupturing the host cuticle.

Survival:
Mature in surrounding soil and water

Culture:
In live insects or in macerated liver.

Host range:
Romanomermis culicivorax can kill early instar mosquito larvae. Other mermithids sometimes attack locusts and grasshoppers.

SECERNENTIA

These are free living saprophytes and do not kill insects. Exceptions are from the *genera Steinernema (=Neoaplectana)* and *Heterorhabditis*, known as rhabditid nematodes.

Infection:
Free-living third instar rhabditid larvae are attracted chemically to susceptible hosts. They penetrate through the mouth or anus, enter the haemocoel* via the gut wall. Some may enter directly through the cuticle. They release highly virulent bacteria (*Xenorhabdus* spp.) into the haemocoel.

Death of host:
From septicaemia in 2-10 days.

Survival:
Nematodes feed and develop on host tissues and bacteria. Third stage infective larvae containing bacterial inoculum emerge from the cadaver 10 days after penetration. The larvae need free water to disperse in.

Culture:
In live insects or in macerated liver

2. COLLECTION OF PATHOGENS

LOOK FOR DEAD INSECTS

Collection:
Collect dead insects in sterile glass or plastic containers with screw tops, paper bags or envelopes.

Treatment:
Leave them open for three to four (3-4) days so that the cadavers dry out.

DO NOT dry artificially.

DO NOT leave in the sun.

Storage:
These air dried specimens can be stored for several days.

Identification:
Easier with fresh insects especially, if the pathogen is non-spore forming.

NEVER store infected specimens in alcohol. If necessary, store specimens in a refrigerator at a maximum temperature of 5°C. In the field, ants may rapidly remove cadavers. So, although finding cadavers is the best way of collecting pathogens, if none can be found in the field, you must collect live insects. A small proportion of these may be infected. Disease incubation in live insects can take up to three (3) weeks, so insects should be kept in cages. Stress (crowding, high humidity) may lead to the appearance of disease.

LIVE INSECTS

Collection:
Collect live insects in the field.

Treatment:
Keep the insects alive and feed them.

Follow up:
Observe the insects. You may see insects behaving abnormally.

Signs that disease may be present:

- not feeding,
- poor coordination,
- jerky movements,
- excessive grooming,
- loss of orientation.

Insects may also climb up high on the plant, expose themselves or hide.

3. PRELIMINARY IDENTIFICATION (external symptoms)

To determine the cause of death of an insect, first look at the insect. External symptoms may tell you which kind of pathogen is responsible for death.

VIRUSES

Infection by viruses mainly occurs in the larval stage. Larvae become pale and flaccid*. Dark in colour after death.

Baculovirus infections: body contents become liquid. Larvae may hang by their prolegs. May ooze white fluid. Infected larvae are sometimes smaller than healthy larvae.

BACTERIA

Larvae remain normal colour. After death darken to brownish-black. Often flaccid. Do not liquefy.

FUNGI

All stages can be affected. After death the cadaver is desiccated*, never flaccid. In soft-bodied insects the body is covered with fungal mycelium*. In hard-bodied insects bands of hyphae* grow between the integuments and mycelial strands grow from the orifices* fixing the insect to the substrate*. Spores may form on the outside of the insect, but if the environment is very dry the fungus may not show on the outside of the insect.

PROTOZOA

Larvae are poorly developed. May become lethargic and collapse. Death is usually from other causes than infection by protozoa.

NEMATODES

Can often be seen through the insect cuticle. In rhabditids the body changes colour after death from cream to grey ***(Steinernema)*** or reddish ***(Heterorhabditis)***.

4. PRELIMINARY ISOLATION

VIRUSES

1 Place living diseased insect in a culture tube with sterile distilled water.

2 After several days inclusion bodies gather in a white layer at the bottom of the tube.

3 Centrifuge to remove any insect or bacterial cells. This partially purified virus can be used to inoculate healthy insects to confirm that it is a pathogen.

BACTERIA

1 Sterilise the host externally by dipping in 70-95% ethanol for several seconds.

2 Transfer to a 50% solution of sodium hypo chlorite for 3-4 minutes.

3 Rinse in three (3) changes of sterile distilled water.

4 Dissect the insect using sterile techniques.

5 Streak the body contents using a wire loop on to nutrient agar.

6 Incubate at 30°C for 24 hours.

FUNGI

What to do with newly dead insects with no visible external growth:

1 Incubate for several days at high humidity.

2 Observe for sporulation.

3 Mount spore structures on a slide in water, or use a specific fungal stain e.g. cotton blue in lactophenol (see Section 7).

BE CAREFUL !

If the insect was not properly dried or has been dead for too long other contaminating saprophytes may hide the growth of the pathogen which killed the insect.

What to do with insects with fresh external sporulation:

1 Take spores with a fine, sterile needle.

2 Streak spores onto several different agar media with antibiotics: tap water agar, potato carrot agar, malt extract agar, (see Section 7)

3 Incubate at 20-25°C.

4 Examine all cultures daily with a stereoscopic microscope.

What to do with insects which have been dead for a long time:

1 Surface sterilise the insect in sodium hypochlorite for several minutes.

2 Rinse in three (3) changes of sterile, distilled water.

3 Dissect internal tissues (usually replaced by fungal hyphae).

4 Streak spores on to several different agar media with antibiotics: tap water agar, potato carrot agar, malt extract agar (see Section 7).

5 Incubate at 20-25°C.

6 Examine all cultures daily with a stereoscopic microscope.

How to remove germinating spores:

1 Cut round the agar.

2 Transfer the block of spores to fresh media.

N.B. If you find prominent hyphae but not much internal desiccation this is probably an Entomophthorales infection. These are very difficult to grow on agar media.

PROTOZOA

Examine the fat body, Malpighian tubules, gut epithelium or haemolymph using a phase contrast or bright field microscope. Mature spores should be easy to see. Use Giemsa stain if necessary (see Section 7). It is easy to confuse spores of fungi with protozoan spores. HOWEVER, fungal spores will not usually be found in the internal organs.

NEMATODES

1 Take individual nematodes from the insect cadaver, or from a suspension of the internal contents.

2 Use a stereoscopic microscope and improvised needles: bamboo splinters, sharpened feather quills, toothbrush bristles, eyebrow hairs.

3 Place the nematode in a drop of sterile water on a slide.

4 Heat over a low flame for four to six (4-6) seconds.

5 Add formalin as a fixative.

6 Transfer nematode to a pre-heated mountant: lactophenol, glycerol in a cavity slide.

5. FURTHER IDENTIFICATION

VIRUSES

Occluded insect viruses are the most commonly found viruses.

1 Use a bright field or phase contrast microscope.

2 You should see the characteristic shining white (mono-refringent*) inclusion bodies*.

3 Use stains such as Giemsa and Feulgen-Schiff (see Section 7) to confirm presence of inclusion bodies.

4 Further identification of non-occluded* viruses must be done using electron microscopy and serology techniques.

BACTERIA

1 Before the insect decomposes, examine a drop of haemolymph under a phase contrast microscope.

2 If the bacteria are saprophytic* you will see bacterial cells of different shapes and sizes.

3 If there are concentrations of bacilli-form cells this indicates the possible presence of an entomopathogenic* bacterium.

N.B. Insect gut normally contains saprophytic bacteria. You must take care not to mistake these for disease-causing bacteria.

Bacillus thuringiensis:
Good growth on nutrient agar, catalase* positive, crystal parasporal body present.

Bacillus popilliae:
Little or no growth on nutrient agar, catalase* negative, crystal parasporal body usually present.

Bacillus sphaericus:
Spores are almost spherical. No crystalline parasporal body.

FUNGI

Entomopathogenic fungi sporulate on the outside of the host insect under moist conditions and on the inside of the host when the environment is too dry.

Deuteromycete fungus: indicated by the presence of masses of powdery spores.

Beauveria spp.:
white spores.

Metarhizium spp.:
green spores.

Prominent hyphal structures, often brightly coloured: ascomycete (sexual stage) or an asexual genera e.g. ***Hirsutella*.**

ENTOMOPHTHORALES

stout hyaline hyphae* surrounded by halos of white spore deposits.

N.B. You must also use the microscope to confirm identification.

PROTOZOA

It is essential to differentiate between saprophytic gut protozoans (flagellates, amoebae) which are not pathogenic, and microsporal infections which are pathogenic.

To do this, use the Giemsa stain (see Section 7) to pick out polar filaments extruding from the spore - these are Microsporidia.

Watch out for the type of locomotor organelle and the size and structure of the spores.

NEMATODES

Mermithids:
long, whitish, several times longer than the host.

Rhabiditid nematodes:
small and accompanied by ***Xenorhabdus*** bacteria**.**

Heterorhabditis spp.:
insect turns red.

6. CULTURE OF INSECT PATHOGENS

VIRUSES

Viruses cannot be cultured on artificial media. They only grow in a living insect host. Use an extract of the internal contents of an infected insect and introduce the virus particles into the mouth of the potential host by:

1 using a hypodermic syringe or,

2 contaminating the food supply.

BACTERIA

Use the same procedure as with viruses, or culture the bacteria on nutrient agar.

FUNGI

Deuteromycotina and Ascomycotina fungi are easily cultured. Use agars and/or boiled grains (rice, wheat, barley) as culture media. Other fungi may need special media , such as meal worm agar. Entomophthorales are difficult to grow in culture. DO NOT sub-culture fungi continuously. Infect some healthy insects after three (3) or four (4) tranfers and reisolate.

PROTOZOA

Culture of protozoa is unlikely to succeed in artificial media. Continuous transfer from host to host, is a better method to use.

NEMATODES

Rhabditids feed on bacteria, so it is possible to culture these nematodes on standard agar media (nutrient agar) seeded with the symbiotic* bacterium concerned. They can be cultured in higher animal tissues (e.g. liver), or in living insects

1 Take a Petri dish with two (2) filter papers 20-30 Lepidoptera larvae and the infective juvenile stages of the nematode.

2 Keep for one (1) week after death of the host.

3 Remove nematodes and put in a separate collecting dish; (damp filter paper over a water reservoir)

4 Infective second stage juveniles migrate from decaying host into the water.

5 Collect, concentrate and store the culture in small flasks at 6-9°C.

7. STORAGE OF INSECT PATHOGENS

VIRUSES

Store tissues containing the virus in the fridge or freezer. All occluded insect viruses can survive freeze drying.

BACTERIA

Store in a cool, dry place.

FUNGI

1 Freeze-dry or store in liquid nitrogen.

2 Grow fungi in glass tubes on weak media agar slants (potato carrot agar, fifth strength potato dextrose agar; see Section 7). Seal with wax or screw caps (Universal bottles). Add sterile paraffin oil to prolong survival.

Label and date the flasks clearly. Make sub-cultures every three to five (3-5) years.

3 Spores growing on cadavers will survive at 4°C in the refrigerator as long as they are kept dry. They can also be frozen.

PROTOZOA

Store resistant stages in the refrigerator.

NEMATODES

Adult or egg stage of mermithids and third stage juvenile of rhabditids can be stored in a moist environment at about 5-15°C.

8. INSTRUCTIONS FOR SENDING SPECIMENS

LABELLING

Use waterproof ink.

Clearly write:

- name of the collector;
- date of collection;
- habitat;
- geographical location.

On the package write: "Biological specimens for scientific study".

PACKING

Use crush proof paper envelopes.

DO NOT use plastic. (Plastic causes condensation which can lead to contamination of the specimens.)

DESPATCH

Send by air-mail NOT by air-freight. Air-freight is often delayed and you may have to pay import charges.

RECIPIENT

Always send to a recognised institute e.g. IITA or CAB International Mycological Institute. If possible, address the package, by name, to an individual whom you know, as some institutes charge a fee for identifications.

See Appendix V for useful addresses.