COLLECTION OF INSECT PATHOGENS

INSECT PATHOLOGY MANUAL

Section II

CONTENTS

INTRODUCTION

Accurate identification of entomopathogens is an essential tool for research workers who are involved in the preparation of effective bio-pesticides as well as insect ecologists who wish to be aware of the full range of natural enemies in the field. Field workers can often find potentially useful new strains of pathogen. This guide is designed to help field workers and research workers to identify the most important species of fungi normally encountered during field studies. It will also be of use to them in identifying other types of pathogen.

1. COLLECTION OF FUNGI

DEAD INSECTS

- Dead insects in the field may indicate the presence of disease.
- Look for insects hanging from plants, under trees and bushes

Collection

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

- Leave the containers open for three to four (3-4) days so that the cadavers dry out.
- DO NOT dry the cadavers artificially.
- DO NOT leave cadavers in the sun.

Storage

- These air dried specimens can be stored at room temperature for a few weeks.
- For longer periods, store specimens in a refrigerator (5°C)
- NEVER store infected specimens in alcohol.

Identification

It is easier to identify insects when the specimens are fresh, especially if the pathogen is non-spore forming.

However, cadavers in the field may be rapidly removed by ants or other scavengers. 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 may 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

1 Collect live insects in the field.

2 Keep the insects in cages and feed them.

3 Observe the insects.

You may see insects behaving abnormally; these are signs that disease may be present:

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

Insects may also show taxic responses, climbing up high on plants, expose themselves to the sun, or hiding.

4 Collect any dead insects (cadavers) daily. If the body is soft and black you may have a bacterial or viral infection. If the body is harder, this may indicate a fungal infection. Incubate the cadaver in a humid chamber (such as a petri dish contains moistered tissue paper) to see if you get external sporulation

5 Record your results carefully; quantitative estimates of the incidence of disease in insect populations are rare.

2. MICROSCOPY TECHNIQUES

When you are identifying fungi it is useful to have some knowledge of basic micro-technique. You will need a microscope, micro-slides, cover slips, forceps, needle and lactophenol blue.

N.B. The cotton blue in lactophenol from laboratory suppliers is too concentrated to useimmediately. You must dilute it approximately ten fold using lactophenol.

A basic microscope with x100 and x400 magnification is adequate when used with mycological stains such as lactophenol cotton blue or acid fuchsin. It helps to have binocular eyepieces, phase contrast and oil immersion objectives (x1000), but these are expensive and are not essential for preliminary identification.

TEMPORARY PREPARATIONS FROM CULTURE OR FIELD COLLECTED SPECIMENS

1 Place a small piece of material in a small dissecting dish.

2 Tease off a tiny piece of both mycelium (if possible) and spores using a sterile needle.

3 Place in a small pool of stain (if used, otherwise use water) on a clean, grease-free microscope slide.

4 Lower a clean cover slip carefully onto the drop.

N.B. Be careful not to get too many air bubbles in the slide; you can remove bubbles later by carefully heating (rapidly pass the preparation through a flame); or press lightly with the tip of a needle to spread the specimen.

BE CAREFUL not to inhale the poisonous fumes from lactophenol.

ALWAYS heat slides in a ventilated place and if possible use a fume hood.

5 Observe using a microscope. Conidia should readily be seen at x 400. For a more positive identification use low power to search for the cells which produce conidia (with associated conidia).

PERMANENT FUNGAL SLIDE PREPARATIONS

1 Add a small amount of material (so that the cover-slip lies flat) to a drop of dilute stain.

2 Expel all air bubbles then paint several coats of nail varnish onto the outside edge of the cover-slip and adjacent part of slide (make sure the first few layers are very thin to prevent running underneath the cover slip).

3 Allow to dry.

4 Store upright in a box

3. ISOLATION OF 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 dry.

INSECTS WITH FRESH EXTERNAL SPORULATION

1 Take spores with a fine, sterile needle.

2 Mount spores on slides as described previously. Streak spores onto several different agar media in Petri dishes with antibiotics: tap water agar, potato carrot agar, malt extract agar, (see Section 7)

3 Incubate at 20-28°C.

4 Examine all cultures daily with a stereoscopic microscope.

NEWLY DEAD INSECTS WITH NO 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.

BE CAREFUL !

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

INSECTS WHICH HAVE BEEN DEAD FOR A LONG TIME

1 Surface sterilize 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 onto several different agar media with antibiotics: tap water agar, potato carrot agar, malt extract agar, (see Section 7)

5 Incubate at 20-28°C.

6 Examine all cultures daily using a stereoscopic microscope.

N.B. If you find prominent hyphae but little internal desiccation it is probably an Entomophthorales infection. These are very difficult to grow on agar media.

4. CULTURE OF FUNGI

ALWAYS clean and sterilize your workplace and equipment with alcohol.

TO GROW FUNGI ON AGAR SLOPES (USE SLOPES FOR STORAGE AND TRANSPORT OF CULTURES)

1 Prepare agar slopes (see Section 3 for instructions).

2 Sterilise a wire loop over a flame (Section 3) and cool using drops of water on the side of the bottle.

3 Take spores from the stock culture and put them on to the agar in the bottle.

4 Leave the fungus to grow until you can see spores forming.

5 Close the bottle hermetically.

6 For short term storage keep the closed bottles in the refrigerator at 5-10°C.

7 For longer term storage cover the agar with sterile liquid paraffin.

TO GROW FUNGI IN BOTTLES

Use 300 ml medical flats or 700 ml square-sided whisky bottles for medium scale production of spores.

1 Prepare agar in bottles (Section 3).

2 Sterilise a wire loop over a flame (until red hot) and cool using drops of water on the side of, the flat.

3 Take spores from the stock culture and spread them on the agar in the flat.

4 DO NOT seal the bottles hermetically until sporulation occurs.

N.B. It is not essential to use agar prepared with antibiotic in these bottles.

5. STORAGE OF FUNGI

Fungi can be stored either as inoculum, ready to be grown further (see Storage of fungal strains), or as spores ready to spray (see Stocking spores).

FUNGAL STRAINS

It is very important to maintain fungal strains to ensure their virulence. In the short term you should keep fungi on agar slopes at 4°C and at -20°C where possible.

In the long term it is essential to deposit strains in a recognised depository such as IMI, CBS or ARSEF (see Appendix IV). At the depository they will be given an accession number and permanent storage at -20°C in liquid nitrogen or be lyophilised. Fungal strains must be properly identified.

Maintaining virulence is of the utmost importance. Fungi can lose their virulence if repeatedly grown (passaged* or sub-cultured*) on artificial media. Avoid the loss of virulence by using cultures from a stock of inoculum, or by reisolating from infected insects

You must store the original infected insect. Isolate the fungus by growing it on agar in a Petri dish, until it is free from contaminants. Next make a large batch of slopes (see Section 3). This is your culture stock.

1 Fungi grown on nutrient weak agar slopes (potato carrot agar, fifth strength potato dextrose agar; see Appendix I) in Petri dishes or in large bottles can be stored for several months on a shelf or in the refrigerator (5°C). Observe at regular intervals to check for contaminants.

2 If you grow fungi on potato carrot agar in 30ml universal bottles you must tightly screw down the tops of the bottles and you can store the bottles in the refrigerator (5°C) for several months.

3 To preserve the fungi for longer periods, fill slopes with sterile liquid paraffin. Seal tightly and store in the refrigerator (5°C) for up to three (3) years.

N.B. AIways keep three (3) slopes.

4 Fungi isolated from infected insects can be stored in the freezer on the dry insect body for about three (3) years.

5 Keep the original infected insect dry in a tightly sealed 30 ml universal bottle with some silica gel.

STOCKING SPORES

Spores are the most resistant stage of the life cycle of fungi.

Beauveria bassiana spores have over 80% viability for up to 10 days at 40°C. If the temperature and the relative humidity are high viability may expire within a week.

Metarhizium anisopliae and *Beauveria bassiana* can withstand temperatures up to 50°C for less than 10 minutes. Resting spores can withstand up to 100°C for up to 30 minutes.

Optimum conditions

Non formulated or crude formulations of conidial preparations can be used for field trials but they must be properly stored.

1 Do a germination test (see below) before a field trial - this is essential.

2 Do another germination test immediately after the field trial to check the germination rate.

3 Store conidia under cool and dry conditions.

In order to survive and grow, conidia must not be exposed to light - UV light is detrimental to conidia as it affects the chromosomes.

High temperatures can kill conidia.

Storage

When storing conidia it is essential to remember that different strains must be stored in different ways. *Metarhizium anisopliae* can be stored successfully for four (4) years at -20°C and at 4°C for more than 16 months and for 2 months at 25°C.

Infectivity, germination, growth and sporulation are all most effective at temperatures of 20 to 30°C.

Humidity is essential for germination and for sporulation with a relative humidity of >90% being ideal for most species.

The more experience you gain in manipulating fungi, the more you will realise that results are unpredictable - never assume that a given procedure will give the same result on another occasion.

6. DEFINITIONS OF COMMON TERMS

For terms not defined in this glossary, refer to the "Dictionary of Fungi" 7th edition (CAB International) or other standard text books. Terms used to describe colours can be found in *A mycological colour chart* by R.W. Rayner, IMI,

BLASTOSPORE •Self-contained yeast-like mycelial fragment, more regular in shape than hyphal bodies, but not clearly distinguishable from them. These are usually produced by budding in submerged liquid cultures.

CHLAMYDOSPORE • An asexual resting spore originating through the modification of a hyphal segment with an inner secondary wall. Usually impregnated with hydrophobic* material.

CONIDIOPHORE • A simple or branched hypha bearing conidiogenous* cells from which conidia are produced.

CONIDIUM (plural: conidia) • An asexual, non-motile* spore; usually aerially produced in entomopathogenic fungi. Conidia have thicker walls and are more environmentally persistent than blastospores. They are occasionally produced in submerged liquid cultures, but retain characteristics of aerially produced forms and are distinguished from blastospores by their method of production. **Macroconidia**: the larger and generally more diagnostic conidia of a fungus that has microconidia in addition (e.g. *Fusarium*) **Microconidia**: the smaller conidia of a fungus that also has macroconidia.

COREMIUM AND SYNNEMA (plural: coremia and synnemata) • Compacted, erect group of conidiophores*, bearing conidia.

HYPHA (plural: hyphae) • A filamentous fungal cell, either discrete or more usually continuous with other hyphae. Divided by cross walls in the "higher" fungi (most entomopathogens) but these are rare or absent in the "lower" fungi (e.g. Entomophthorales)

HYPHAL BODY • An irregular fragment of mycelium, produced in submerged culture or the haemocoel of the insect during the early stages of infection. Less regular in shape than blastospores but not easily distinguished from them.

MYCELIUM • A mass of hyphae, thallus* of a fungus.

PHIALIDE • A conidiogenous cell which develops one or more open ends from which a basipetal succession of conidia develops without an increase in the length of the phialide itself (Basipetal: the youngest conidium is the closest to the phialide).

SPORE • A generic word for any single or multi-celled propagule produced sexually or asexually

7. DESCRIPTIONS OF FUNGI

The following descriptions of fungi are divided into three groups:

1 The commonly encountered entomopathogenic genera and species with powdery conidia with lipophilic cell walls. These include species of *Metarhizium*, *Beauveria*, *Nomuraea* and *Paecilomyces*.

2 Other entomopathogens which are frequently encountered but have less potential or development as ULV mycopesticide formulations. These include *Verticillium lecanii* and *Entomophaga grylli*

3 Saprophytic species not apparently useful for control purposes, that frequently cause confusion and contaminate spore cultures. These include *Aspergillus spp.* strains of which are known to be toxic or carcinogenic to man, other mammals and birds. Other genera that are commonly mistaken for pathogens include: *Fusarium* and *Penicillium*.

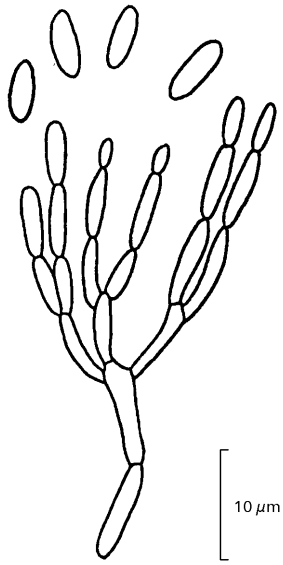
Also inlcuded is a key for preliminary field identification of fungi. This is particularly useful for Acrididae in West Africa, but may also be of use elsewhere. Some identification is possible without a microscope (see Part A).

Acknowledgments

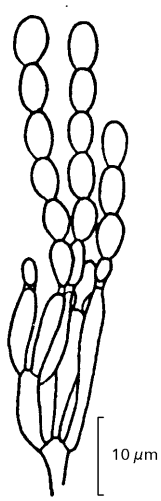
The International Mycological Institute series of Descriptions of Pathogenic Fungi and Bacteria, published by CAB International.

Metarhizium spp.

Metarhizium anisopliae



Metarhizium flavoride



On insect

Forms a green crust – like velvet, found on both inner and outer walls of the insect cuctile.

On agar

Both *M. anisopliae* and *M. flavoviride* grow readily on Molisch's, potato dextrose agar (PDA) and malt extract agars (see Appendix I), radial growth is slow.

M. anisopliae: Colonies on PDA have a white mycelial margin with clumps of conidiophores which become coloured with the development of the spores, varying from olivaceous buff to yellow/green to olivaceous/dark herbage green. Sometimes pale luteous to citrine in the centre with yellow pigment diffusing into the medium.

M. flavoviride: A white mycelial margin is formed, with older regions becoming coloured as sporodochial groups of conidiophores develop and become confluent. The surface is powdery and finally crustose as chains of conidia are released. Varying from pale to yellow/green to pale olivaceous buff. Sometimes sectoring.

M .anisopliae:
Conidiophores:
4-13.5 x 1.4-2.6 µm.

Phialides:
6.3-13.5 x 1.8-3.6 µm.

Conidia:
Usually 5-8 µm long by 1.5-3.5 µm wide var. *anisopliae* or 10-14 µm long by 1.8-4.5 µm wide in var. *major* (var. *major* is mainly confined to the Coleoptera: Scarabaeidae).

Differs from *M. flavoviride* by the shape of the conidia which are ellipsoid in the latter.

M. flavoviride:
Vegetative hyphae:
2-3 µm Conidia:

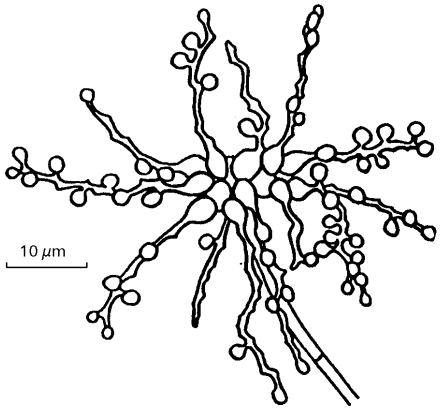
6.5-11 x 3.5-5 µm produced in long chains.

Differing from *M. anisopliae* by the broadly ellipsoidal, compared with the narrow cylindrical conidia of *M. anisopliae*.

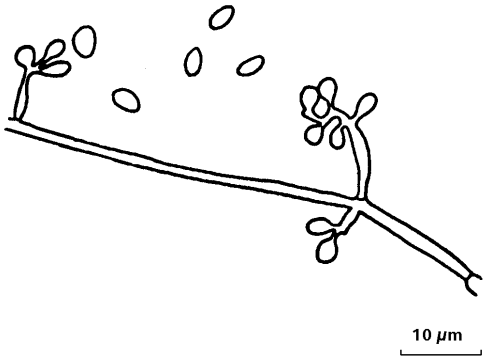
IMI Descriptions of, Pathogenic Fungi and Bacteria, No's 609 and 662

Beauveria spp.

Beaveria bassiana



Beaveria bassiana



On insect

White to pale yellow, velvety to powdery, rarely forming synnemata.

On agar

Grows readily on malt extract and Molisch's agars (see Section 3).

Colonies are generally white at the edge becoming cream to pale yellow, occasionally reddish.

Conidiophores:
abundant, rising from vegetative hyphae, 1-2 µm wide, bearing groups of clustered conidiogenous cells 3-6 x 3-5 µm which may branch to give rise to further conidiogenous cells, globular to flask shaped with a well developed rachis (stalk) up to 20 µm long by 1 µm wide, geniculate with denticles up to 1µm long.

Conidia:
2-3 x 2-2.5 µm.

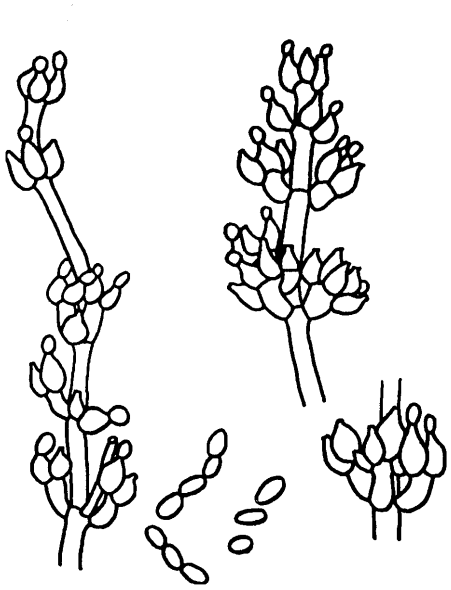
Chlamydospores:
absent.

B. bassania differs from *B. brongniartii* by the more clustered conidiogenous cells and the globose conidia.

IMI descriptions of Pathogenic Fungi and Bacteria, No. 602

Nomurea riley

Conidia and conidiophores



On insect

Most frequently seen on Lepidoptera larvae, it produces a thin white mycelial felt from which an even compacted stand of conidiophores arise to give a fine dusty velvety growth covering almost the entire body in a pale green dust of conidia.

On agar

Colonies are very slow growing. Velvety, with dense production of dusty pale leek green conidia, sometimes forming crusts, becoming darker in age, pale turtle green deepening to malachite green. Occasional isolates especially in age become fluffy and more spreading with sparse or unevenly distributed conidia.

Vegetative hyphae:
2-3 µm diam.

Conidial structures complex:
up to 160 µm long and 2-3 µm diam.

Phialides:
Short and rounded, cylindrical to almost globose, with very swollen base tapering abruptly to a narrow neck 4-6 (3-4) x 2.5-3.5 µm.

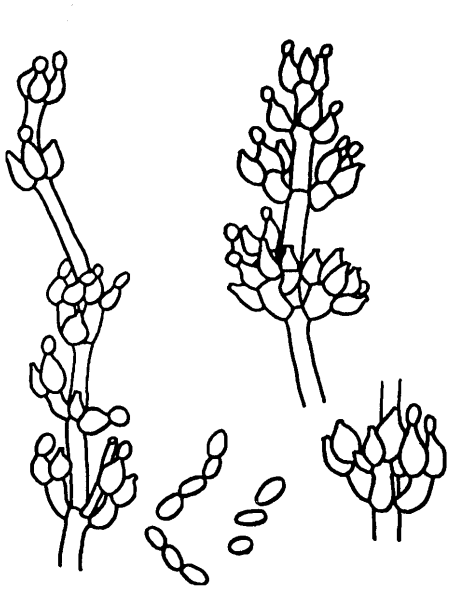
Conidia:
Aseptate, in chains, smooth, hyaline, elliptical to sometimes cylindrical 3-4 (4.5) x 2-2.5 (3) µm.

The conidiophore branches are short and swollen almost as broad as long 3.5-5 (6) x 2.5-3.5 µm.

IMI Descriptions of Pathogenic Fungi and Bacteria, No. 612

Paecilomyces fumosoroseus

Conidia and conidiophores, x1400;



On insect

Produces simple mononematous conidiophores or distinct but loose synnemata. The synnemata are erect, up to 3 cm long and maybe branched, appearing dusty with conidia.

On agar

On malt agar (MA) and potato dextrose agar (PDA, see Appendix I) growth is moderately rapid at room temperature (25°C) 4-8 cm in 14 days, with a basal felt with regular or irregular raised floccose overgrowth, or maybe thinner, dusty and granular, and producing definite coremia which are powdery when first isolated. White at first, remaining so or changing to shades of pink which may become tinged grey with age.

Vegetative hyphae:
smooth walled, hyaline, 1-5 - 3.5 µm diam.

Conidial structures:
tending to be complex consisting of erect conidiophores arising from the basal felt or from aerial hyphae.

Conidiophores:
produced singly or together to form synnemata, up to 100 µm long x 1.5-2 (3) µm diam. Smooth walled, hyaline, bearing verticils of branches, in turn bearing whorls of 3-6 phialides, occasional phialides produced at the same level as the branches and in the same verticil. Sometimes the verticillate pattern is broken and single branches are produced irregularly on the conidiophore.

Phialides:
are 5-7 x 2.5 (3) µm, with a swollen base which tapers to a long thin neck about 0.5 µm diam.

Conidia:
cylindrical to fusiform* with rounded ends, smooth, hyaline, borne in chains, 2-4 x 1-2 µm, occasionally up to 5 µm long.

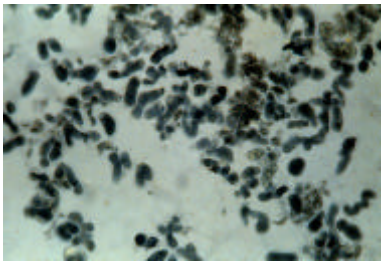
On insects the conidiogenous apparatus tends to be more compacted with the branches and phialides inflated, slightly shorter and more rounded, 3.5-6 x 1-2.5. Conidia as in culture.

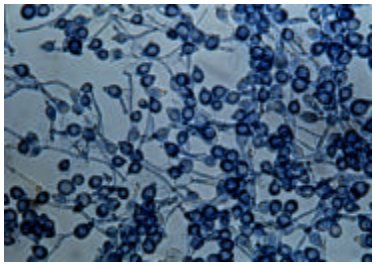
There are many other similar species of *Paecilomyces* which attack insects.

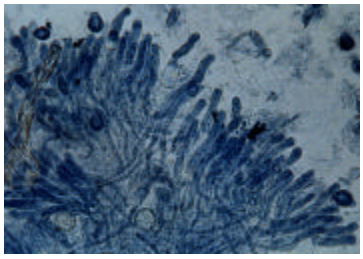
IMI Descriptions of Pathogenic Fungi and Bacteria, No. 614

Entomophaga grylli

Conidia and conidiophores, x1400;







On insect

Hyphal bodies grow rapidly within the insect giving rise to conidiophores which grow out through the thin inter-segmental and interjoint membranes of the host and produce club shaped conidiogenous cells at the surface of the host.

Conidia give a white buff or greenish furry appearance, especially at the inter-segmental membrane, leg, joints, junction of head and thorax and at the base of the antennae.

On agar

This fungus is unable to grow in agar culture.

Conidia:
Obovate to pyriform (mostly) with a broad papillate base and an evenly rounded apex, 25-45 x 20-37 µm. Single walled hyaline contents, granular with one or two large fat globules, multi- nucleate.

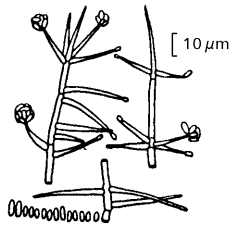
Hyphal bodies:
Unicellular, multi nucleate, varying from spherical 23-31 µm diam. to oval 32-50 x 25.5 µm.

Resting spores:
Azygospores - spherical 30-45 µm. Outer wall 4µm thick.

IMI Descriptions of Pathogenic Fungi and Bacteria, No. 606

Verticillium lecanii

Conidia and conidiophores



On host

Aerial hyphae arise from the dead host and cover the body.

On agar

Colonies at 10 days on malt agar (MA), oatmeal agar (OA) or potato dextrose agar (PDA) are white or cream, thin cottony with reverse colourless to pale or deep yellow.

Hyphae:
1-2µm wide.

Phialides:
formed either singly, or directly from mycelium or in whorls of 3 or 4 erect conidiophores much like the vegetative mycelium. Phialides delicate, of variable size depending on both the strain and the age of the culture, from 8.5-16 x 0.8-1.2 µm to 30-40 x 2-2.2 µm.

Conidia:
produced singly and aggregating in heads at the tips of the phialides, in mucilaginous matrix ellipsoidal to cylindrical with rounded ends, varying in size with the strain from 2.3-3.4 x 1-1.3 µm to 7.2-10 x 2.1-2.6 µm.

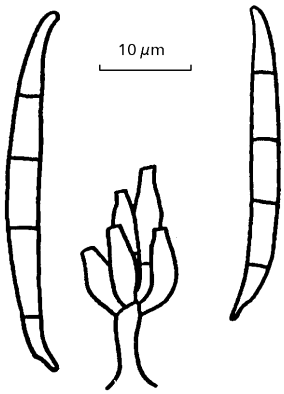
Chlamydospores:
absent.

Blastospores are produced in submerged culture.

IMI Descriptions of Pathogenic Fungi and Bacteria, No. 610

Fusarium oxysporum

Conidia and conidiophores



On agar

Mycelium delicate white or peach but usually with a purple tinge, sparse to abundant then floccose becoming felted and sometimes wrinkled in older cultures.

Fusarium spp. isolated from grasshoppers and other Orthoptera are likely to be the saprophytic or weakly parasitic species of *F.oxysporum* or *F.solani*. Three entomopathogenic species *(F.coccophilum, F.larvarum, F.juruanum)* are confined to diaspidid scale insects.

Microconidia:
borne on simple phialides arising laterally on the hyphae or from short sparsely branched conidiophores. Generally abundant, oval ellipsoid cylindrical, straight to curved 5-12 x 2.2-3.5 µm.

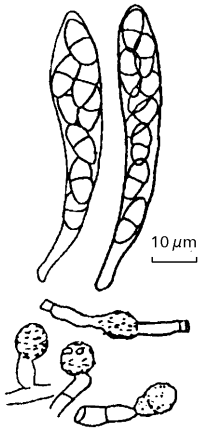
Macroconidia:
sparse in some strains, are borne on more elaborately branched conidiophores or on the surface of Tubercularia-like sporodochia. Thin walled generally 3-5µm septate fusoid-subulate and pointed at both ends, occasionally fusoid-falcate, macroconidia are found with a somewhat hooked apex and a pedicellate base: 3 septate, 27-46 x 3-5 µm; 5 septate, 35-60 x 3-5 µm; 6-7 septate, 50-66 x 3.5-5 µm. The 3 septate spore in the range 27-46 x 3-4.5 µm is most commonly found.

Chlamydospores:
both smooth and rough walled are generally abundant and form both terminally and intercalary, generally solitary but occasionally formed on pairs or chains.

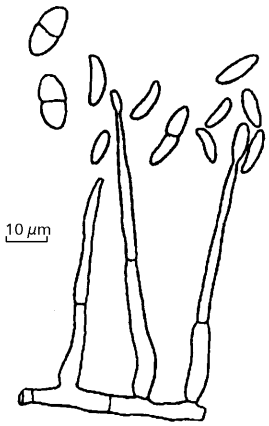
IMI Descriptions of Pathogenic Bacteria and Fungi, No.211

Fusarium solani

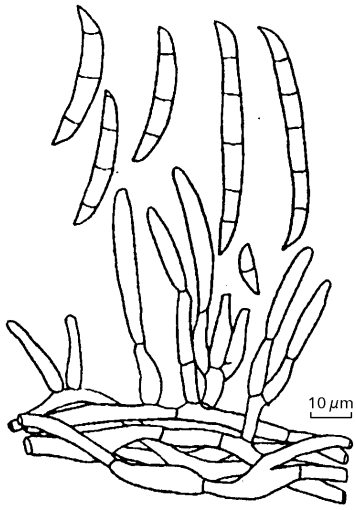
a) Asci and ascospores from host



b) Microconidia and conidiophores



c) Macroconidia and conidiophores



On agar

Growth moderately rapid, sparse floccose greyish white mycelium. Typically a bluish to bluish -brown discoloration develops in agar.

Fusarium spp. isolated from grasshoppers and other Orthoptera are likely to be the saprophytic or weakly parasitic species of *F. solani* or *F.oxysporum*. Three entompathogenic species *(F. coccophilum, F. larvarum, F. juruanum)* are confined to the diaspidid scale insects.

Microconidia:
form in aerial mycelium from elongated lateral phialides; they are hyaline, cylindrical wedge shaped or allantoid 9-16 x 2-4 µm and may become 1 septate.

Macroconidia:
develop in 4-7 days from branched and well developed conidiophores; they are cylindrical to falcate often slightly wider towards the apex and with a well marked foot cell; they measure 40-100 x 5-7.5 µm.

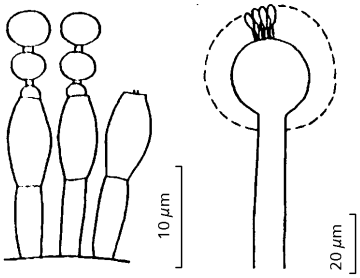
Chlamydospores:
Globose to oval, smooth to rough walled 10-11 - 8-9 µm developing intercalary or terminally.

Homothallic strains of F. solani occur frequently and produce perithecia in culture under suitable conditions.

IMI descriptions of Pathogenic Fungi and Bacteria, No. 29

Aspergillus flavus

Conidia and conidiophores



On insect

Conidial heads can be seen protruding from insect cuticle, usually on or around the abdomen or head.

On agar

Colonies usually spreading yellow green reverse colourless to dark red brown, occasionally dominated by hard sclerotia white at first becoming red brown to almost black with age.

Aspergillus spp. are heavily sporing, fast growing saprophytes and are common contaminants of laboratory cultures.

Conidial heads:
Typically radiate, splitting to several poorly defined columns; rarely exceeding 500-600 µm diam.

Conidiophores:
Heavily walled and uncoloured, coarsely roughened usually less than 1 mm in length 10-20 µm diam.

Vesicle:
Elongated when young, becoming sub-globose to globose varying from 10-65 µm diam., commonly 25-45 µm diam.

Metulae:
6-10 x 4-5.5 µm (sometimes up to 15-16 x 8-9µm diam.).

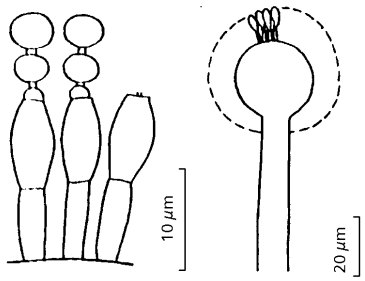
Phialides:
6.5-10 x 3-5 µm.

Conidia:
Typically globose to sub-globose, variable 3-6 µm diam. often 3.5-4.5 µm sometimes elliptical or pyriform at first and occasionally remaining so and then measuring 4.5-5.5 x 3.5-4.5 µm.

IMI Descriptions of Pathogenic Fungi and Bacteria, No. 91

Aspergillus niger

Conidia and conidiophores



On insect

Conidial heads can be seen protruding from insect cuticle usually on or around the abdomen or head.

On agar

Loose white to yellowish mycelium, rapidly becoming black to dark brown on the upper surface with development of conidia. Occasionally characterised by the presence of large white sclerotia about 1 mm diam.

Aspergillus spp. are heavily sporing, fast growing saprophytes and are common contaminants of laboratory cultures.

Conidial heads:
Globose, tangled or splitting into columns.

Conidiophores:
Smooth hyaline or faintly brownish near the apex up to 3 mm x 15-20 µm diam.

Vesicles:
Globose or nearly so, up to 75 µm diam. but often quite small, fertile over entire surface.

Metulae:
Of varying lengths, sometimes septate but when mature usually 20-30 µm long though immature heads are frequently shorter (occasionally long ones are produced).

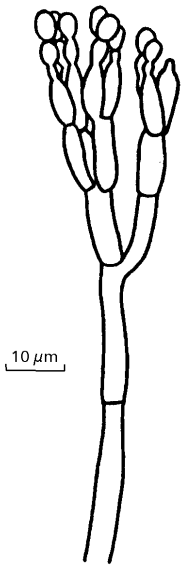
Phialides:
More uniform in length, usually 7-10 x 2-3 µm.

Conidia:
More or less globose often very rough or echinulate, mostly 4-5 µm diam. very dark.

IMI Descriptions of pathogenic Fungi and Bacteria, No. 94

Penicillium spp.

Conidia and conidiophores



On agar

Penicillium spp. are very common contaminants of laboratory cultures. Growth is rapid on most agars, colonies are usually non-spreading, often with radial wrinkles up to 2 mm deep.

Conidiophores:
arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in phialides.

Conidia:
(phialospores) hyaline or brightly coloured in mass, single celled, mostly globose or ovoid, in dry basipetal chains.

IMI Descriptions of Pathogenic Fungi and Bacteria.

Illustrated Genera of Imperfect Fungi
H.L. Barnett & Barry B. Hunter (3rd Edition)

8. KEY FOR INSECT PATHOGENS

There are two parts to this key. Part A is a field identification guide. Part B should be used with a microscope. Always confirm your tentative identification by examining spores under a microscope.

DEAD INSECTS

1 Can you see an exit hole?	**YES** - small;at the knee or in abdomen - nematode. **YES** - larger; behind head - parasitic fly e.g. *Blaesoxipha* **NO** - go to 2
2 Is the body hard?	**YES** - go to 4 **NO** - go to 15
3 Is the insect attached vertically to plant?	**YES** - go to 5 **NO** - go to 6
4 Can you see green or buff sporulation on the abdomen?	**YES** - may be *Entomophaga grylli* (Fresenius) Batko **NO** - go to 6
5 Is the inside of the body dark red and fragile?	**YES** - may be *Sorosporella* spp. **NO** - go to 7
6 Is there green or white fungus present?	**YES** - go to 8 **NO** - incubate 2-3 days, then go to 9
7 Is there green internal and external sporulation, body sometimes red?	**YES** - may be *Metarhizium* spp. **NO** - go to 10
8 Is there rapid olive green, black or yellow external sporulation?	**YES** - may be *Aspergillus* spp. **NO** - go to 11
9 Is there rapid white external sporulation?	**YES** - go to 13 **NO** - go to 12
10 Incubate 3-5 days. Is there external sporulation?	**YES** - **green** - may be *Metarhizium* spp. - **white** - may be *Beauveria* spp. **NO** - go to 13
11 Is external growth rapid, white and covering body?	**YES** - non-sporulating mycelium; no identification possible. **NO** - go to 14
12 Is external sporulation white with black sporosphores?	**YES** - *Mucor* spp. **NO** - go to B.-17

13 Is the insect large? (>1 cm)	**YES** - go to 16 **NO** - go to 17
14 Use a syringe to take some haemocoel from the insect and make a Giemsa smear	go to 18
15 Cut open the insect and smear the insides on to a slide	go to 18
16 Are particles motile, rod-shaped and just visible at x400?	**YES** – bacteria **NO** - go to 19
17 Are particles easily visible at x400 polyhedra and capsules which dissolve in weak solution of NaOH?	**YES** - inclusion viruses **NO** - go to 20
18 Are particles of various shapes 2.0-20.0 µm diameter and do not dissolve in weak solution of NaOH?	**YES** –protozoa **NO** - go to 21
19 Uniform particles staining blue with lactophenol	**YES** - fungi - go to 22 **NO** - oh dear!
20 If you can see fungal spores make a preparation from the original specimen to see the phialides.	
21 Use the identification key beginning	
22 Try to grow the fungus see **CULTURE OF FUNGUS**	