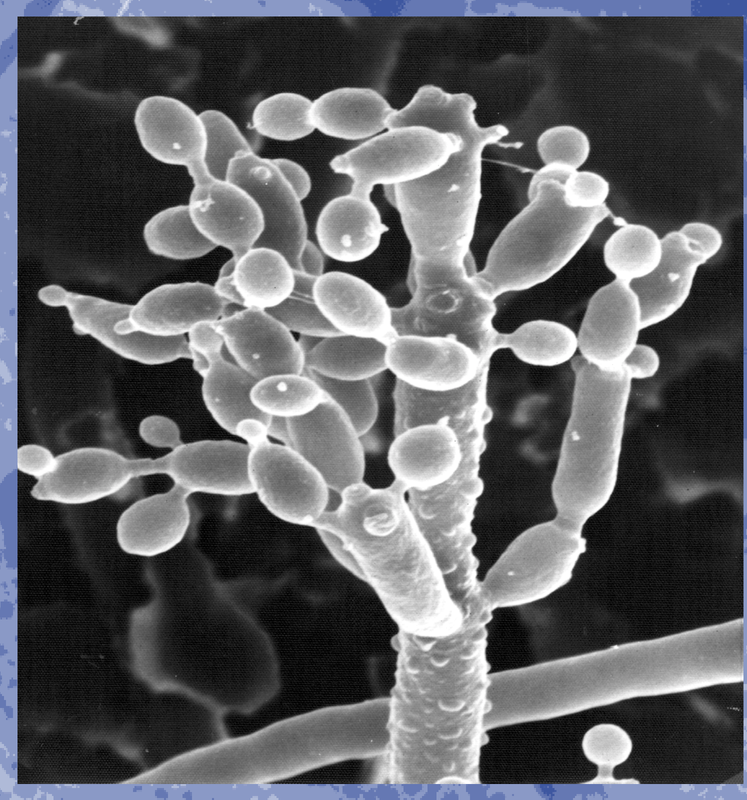
Contents

A SAFRINET MANUAL FOR MYCOLOGY

Collecting and Preserving Fungi



Compiled by the
Biosystematics Division, ARC–PPRI, South Africa

Sponsored by SDC, Switzerland

Contents

Collecting and Preserving Fungi

A Manual for Mycology

by

SAFRINET, the Southern African (SADC) LOOP of BioNET-INTERNATIONAL

Compiled by the

National Collection of Fungi
Biosystematics Division
ARC – Plant Protection Research Institute
Pretoria, South Africa

Edited by A.P. Baxter & E. van der Linde

Sponsored by

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Preface

This is a guide to a course on basic mycology for technical assistants of the SADC countries of the SAFRINET-loop of BioNET-INTERNATIONAL, presented by the staff of the National Collection of Fungi, Pretoria.

The manual, as well as the associated lectures and practical sessions, is intended to provide technical assistants with experience in basic practical mycology. To assist the user in avoiding common pitfalls, it offers information based on personal experience and reliable sources. All the steps from collecting specimens to maintaining a reference collection are covered, including topics such as record-keeping, the processing of material, choice and preparation of growth media, basic isolation methods, preserving specimens in a reference collection, and herbarium procedures. Background information on classifying and naming fungi is included, as well as the most important characters of the major groups of economically important fungi.

A list of useful references is also provided as a guide to some of the more specialised information in the literature.

Acknowledgements

- Sincere thanks are due to Ms C. Craemer for coordinating the preparation and production of this manual and to Dr G.L. Prinsloo for guidance and advice. The generous funding by the sponsor, The Swiss Agency for Development and Cooperation (SDC), is greatly appreciated.

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1. Introduction

1.1 What is a fungus?

People usually associate fungi with moulds and mildews, mushrooms and toadstools, rust and smut diseases of plants, and perhaps even ringworm or athlete's foot. In general, little is known about this large and varied group of living organisms. Fungi are also often seen as belonging to a mysterious underworld, and many people are unsure of where fungi fit into the diversity of living things. Most fungi are adaptable opportunists, even exploiting other fungi, causing stress or even death. Some fungi, e.g. some mushrooms, are extremely poisonous, and we tend to concentrate on this darker side, often forgetting the positive impact that fungi have on our lives.

Fungi are mostly microscopic organisms that reproduce by means of spores. Only those with cell walls containing chitin are classified as true fungi. True fungi are cellular, their cells being firm-walled like those of plants and usually arranged end-to-end in minute filaments known as hyphae. Hyphae typically extend at the tip, enabling them to penetrate a food source, and so form a network that translocates nutrients in solution to where they are needed by the fungus. This network of hyphae is called the mycelium. Even mushrooms are usually in a hyphal form, so their presence is often not evident.

All fungi share certain features. Like animals, they require an organic food source (are heterotrophic). They acquire their energy by using organic plant and animal matter as food. Unlike most animals, however, they release digestive enzymes into their food to obtain the required nutrients (are osmotrophic). Fungi may either live as saprotrophs on dead plants or animals, or as parasites on living plants or animals. Many fungi are, however, parasitic or saprotrophic, according to circumstances, and are then termed facultative parasites.

Many fungi are pathogens of plants that are grown for food, shelter or clothing. A smaller number are agents of diseases in animals, including man. Many saprotrophic fungi attack and degrade raw or manufactured materials of various kinds, such as foodstuffs, timber, textiles and leather. The economic loss

of such destruction, and the cost of preventative treatment, can be enormous.

On the positive side, many saprotrophic fungi are useful to man. The use of yeasts in baking and brewing is well known. Minor sources of food include edible mushrooms and truffles. Various fungi are used to give distinctive flavours to ripening cheeses. Several extremely useful antibiotics are metabolic products of fungi, e.g. penicillin.

1.2
Importance of fungi

☛ Economic importance

Fungi mediate essential processes within ecosystems such as nutrient cycling. Through their role in decomposition, fungi regulate soil fertility, making available, in mineral form, nitrogen, phosphorous, sulphur and carbon. They are the only organisms known to extensively degrade lignin, a major component of wood. Although humans cannot digest cellulose and lignin, many fungi, through their assimilation of these substances, produce food in the form of edible mushrooms. In some instances, however, fungi may spoil important products (ranging from fruit to diesel fuel), often causing enormous financial losses.Through mycorrhizal associations, fungi facilitate mineral uptake in plants: more than 85 % of vascular plants may depend on this. This is especially important in poor soils, forests and high-rainfall areas where nutrients would otherwise be leached from the soil.

☛ Ecological importance

As pathogens and parasites, fungi directly affect host growth and reproduction. They also frequently influence host interactions with other organisms, altering community structure and dynamics. An extreme example is potato blight that befell Ireland some 150 years ago. The effects of this not only disrupted the economy, but influenced world history by driving more than a million famine-stricken people to other countries.

1.3
International conservation status

Whenever conservation status or land use changes, resident fungi are endangered. Owing to their sensitivity to environmental pollution, the most threatened groups are lichens and mycorrhizas.

Very few nature conservation bodies worldwide have strategies for conserving fungi. Although indexes of fungi on plant hosts are a widely used form of inventory, these are often biased towards agronomic and forest crops and detection is frequently based on the presence of fruiting structures or disease symptoms, and not on all the fungi actually present.

2. Classification

How does one begin to study fungi without access to books and with no knowledge of fungal structure? Let us imagine that such a person is given a basket full of mushrooms that requires sorting into groups. Each mushroom displays certain obvious features such as shape, size, colour and smoothness. The person will probably begin by using these external characters to sort the specimens into piles, concentration on features that differentiate groups and ignoring minor variations. Some characters will be found to be more useful than others. Although the word 'species' may form part of the person's vocabulary, the exact meaning of the term could well be vague. Nevertheless, he or she will feel pleased at having sorted the mushrooms into 'species'.

The above scenario illustrates some of the historical steps in the taxonomy of fungi:

- ☞ First, the realisation that some characters are less variable than others.
- ☞ Further, that it is best to use established characters for the purpose of differentiation.

Eventually, the collector will realise that external appearances could be deceptive and that microscopic characters may be more informative.

2.1 Kingdoms

All living organisms can be divided into major groups, known as 'Kingdoms'.
These Kingdoms can be identified/distinguished by using a simple KEY:

1a.	**Cells with no nucleus or nuclear membrane e.g. bacteria:**	**MONERA**
1b.	**Cells with nucleus and nuclear membrane:**	**2**
2a.	**Organisms mostly one-celled; flagellar hairs present:**	**3**
2b.	**Organisms multi-celled; flagellar hairs absent:**	**4**
3a.	**Feeding (trophic) phase without cell walls, often amoeboid; walls with various constituents:**	**PROTOZOA**
3b.	**Trophic phase with cell walls; walls often with cellulose, not chitin:**	**CHROMISTA**
4a.	**Make own organic compounds (autotrophic: photosynthetic); reserve food = starch; cell walls contain cellulose:**	**PLANTAE**
4b.	**Need organic compounds made by others (heterotrophic); reserve food = glycogen:**	**5**
5a.	**Absorb their food (osmotrophic); cell walls contain chitin:**	**FUNGI / EUMYCOTA**
5b.	**Eat their food (phagotrophic); cell walls absent:**	**ANIMALIA**

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Traditionally, fungi are studied as non-vascular (without conducting tissue) cryptogamic (without true flowers or seeds) plants that reproduce by means of spores, and lack the green pigment of plants (chlorophyll). Originally fungi were classified in the plant Kingdom, but they were subsequently placed in a Kingdom of their own named Fungi.

Fungi are divided into two main groups according to the structure of their non-reproductive or feeding (trophic) phase:

- **Myxomycota** (slime moulds) — amoebic or plasmodial, and
- **Eumycota** ('true' fungi) — mycelial or unicellular.

☛ Eumycota

The true fungi (Eumycota) can be divided into five divisions based mainly on the method of reproduction. This can be summarised as follows:

- **Mastigomycota**: flagellate fungi (Greek: mastix = whip, lash).
- **Zygomycota**: zygosporic fungi (Greek: zugoutos = yoked or joined).
- **Ascomycota**: sac fungi (Greek: askos = bag, wineskin).
- **Basidiomycota**: basidium-forming fungi (Greek: basidion = little base or pedestal).
- **Deuteromycota**: secondary fungi (Greek: deuteros = second).

Each division is divided into smaller groups with successively lower ranks, the main ones being as follows:

- **Classes** (with names ending in -mycetes).
- **Orders** (with names ending in -ales).
- **Families** (with names ending in -aceae).
- **Genera** (no standard ending).
- **Species** (no standard ending).

Classification is not merely a system of grouping and ranking individuals in species, species in genera, genera in families, etc., and giving these groupings names. In addition to this, and more importantly, each name or taxon represents a series of generalisations that summarise what is known about that group. These groups may comprise individuals (species) or groups of species (genera), etc. Then the taxa are arranged in a hierarchy of consecutive ranks that are intended to reflect degrees of relationship between their members.

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2.2 Importance of systematics

Systematic studies are important because they make it easier for us to name and classify organisms. Names are the only link between various disciplines, and the only means of intelligible communication. Work carried out on material that has not been correctly sorted and named is worthless. If the wrong name is reported, or if mixtures of species are used, unrepeatable or conflicting results will be obtained.

2.3 Nomenclature

Nomenclature deals with the laws and principles governing the correct application of scientific names to taxa. It also deals with the grouping of taxa into consecutive categories of definite rank, according to a particular system of rules.

Unless there is some universal method of naming the species and other categories of fungi that one is able to recognise, nothing but confusion can be expected. Such an agreement is contained in the International Code of Botanical Nomenclature, the botanist's statute book (see reference list). This means that giving a name to a fungus is subject to the laws set out in this Code, which must be strictly adhered to.

☛ Binomials

All scientific names are written in a particular way. Every organism has a name consisting of two parts: a genus name and a species name. This combination is called a **binomial**. The genus name is always written first, beginning with a capital letter, followed by the species name, beginning with a small letter. The scientific name is always written in a letter type other than that of the rest of the text, e.g. in italics or underlined. In a formal citation or in taxonomic work, the name of the author of the species is placed after the binomial, e.g. *Thelephora cinerascens* Schweinitz. When a species is described as new but later discovered to be the same as another species that has already been described, the new name is termed a synonym.

3. Features of major groups

3.1 Ascomycota

The Ascomycota is the largest group in the fungi. Ascomycota reproduce by means of sexual spores (borne in a sac-like structure known as an ascus) that are termed ascospores. These may or may not be carried in a fruit-body. Many are important pathogens, wood stainers or decay organisms.

The Ascomycota are found in soil, litter, water, dead wood, as parasites on other fungi and many are plant pathogens found on leaves, stems and/or fruit.

☞ **Isolation:**

Isolations can be made directly from infested material by transferring spores or whole fruit-bodies to a suitable medium. They usually grow easily on malt extract agar (MEA) or oatmeal agar (OA). Many pathogenic fungi, however, cannot be cultivated on artificial media.

☞ **Examples:**

Guignardia (black spot on *Citrus*) forms black spots on the skin of fruit, without affecting the inside. This, however, is cosmetically unacceptable, especially for export purposes. Many other Ascomycetes may form cankers on the stems of trees, leaf spots, leaf blight, etc.

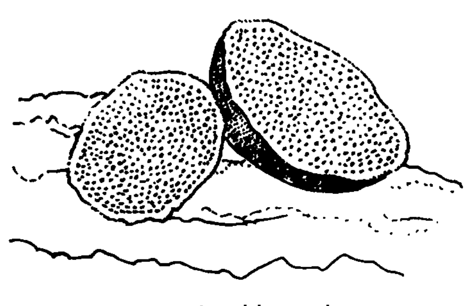
3.2 Basidiomycota

The Basidiomycota form sexual spores externally on a microscopic structure known as a basidium. Basidia are mostly carried on or in fruit-bodies, and these vary widely in size, form and complexity. The Basidiomycota are found on living trees or dead wood (e.g. jelly or bracket fungi), in soil, or as mycorrhizal partners on the roots of trees (e.g. many mushrooms and puff-balls). Some are important pathogens associated either with the leafy parts of plants such as the rusts or with flowering structures such as the smuts.

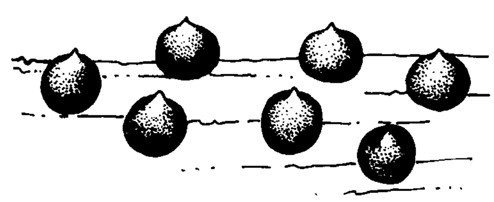
☞ **Isolation:**

Malt extract agar (MEA) is suitable for most kinds of Basidiomycota that can be cultured. Spores often germinate easily: suspend a piece of a mushroom cap above an agar plate to allow spores to be deposited onto the agar.

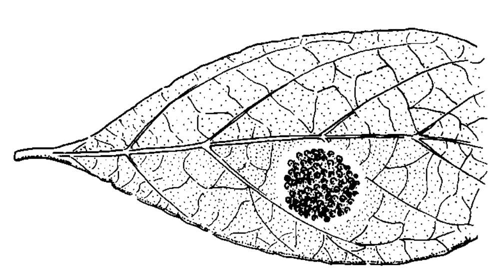
Examples of various Ascomycota



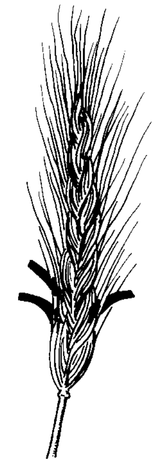
saprotrophic on dung



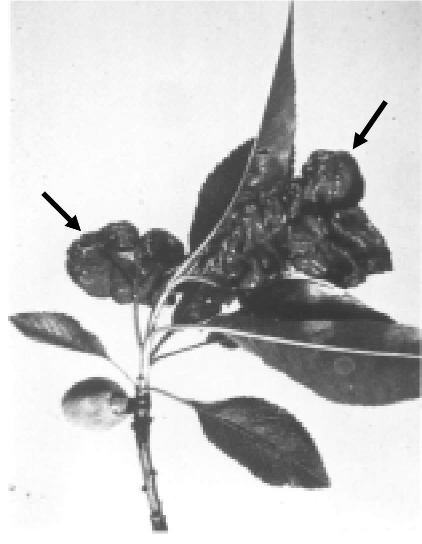
saprotrophic on dead wood



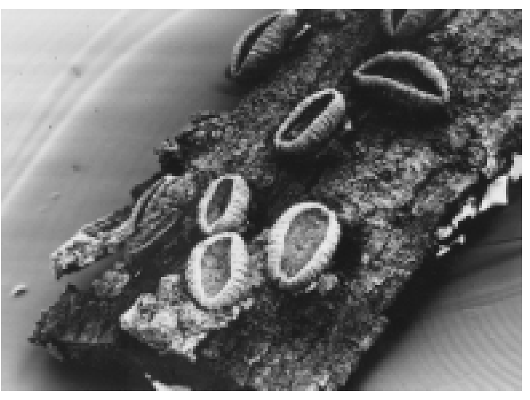
pathogenic on host plant



pathogenic on host plant



pathogenic on host plant



saprotrophic on dead wood
(scanning electron micrograph)

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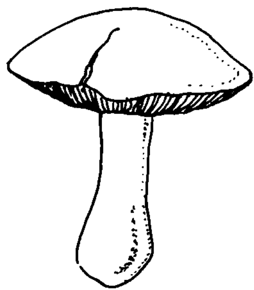
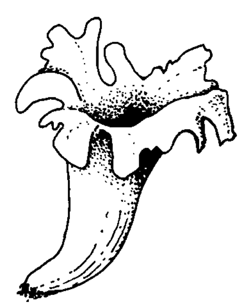
☞ **Example:**

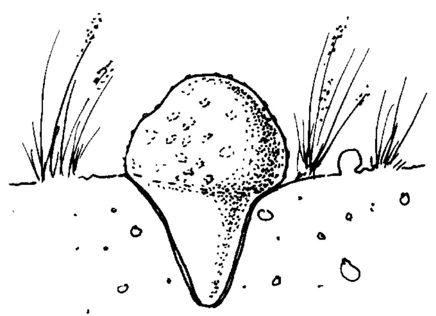
Species of *Ganoderma* are bracket fungi that grow on the stems and roots of living trees. The mycelium penetrates throughout the wood, spreading through the tree and by the time the visible fruit-body forms, the tree is already dying. Mushrooms and bracket fungi are probably the best known Basidiomycota. Many cause wood-staining, rotting of mine timbers and various plant diseases.

Examples of various Basidiomycota

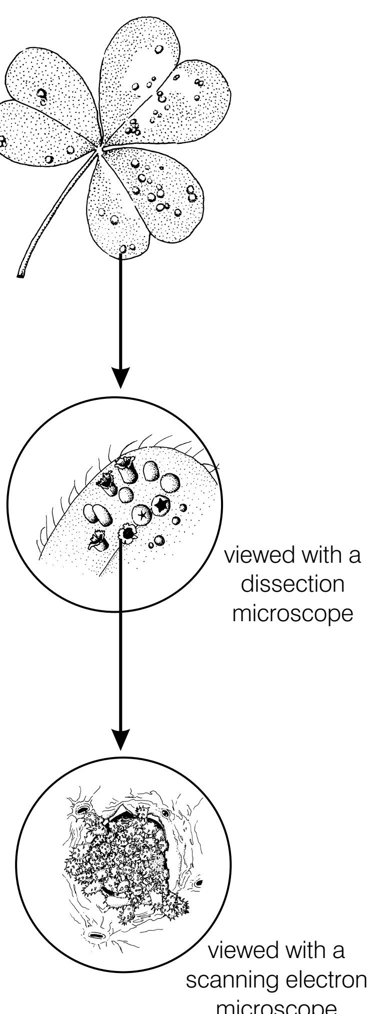


mushrooms: saprotrophic, often in association with plant roots





puff-ball: saprotrophic, often in association with plant roots



rust fungus: pathogenic on host plant

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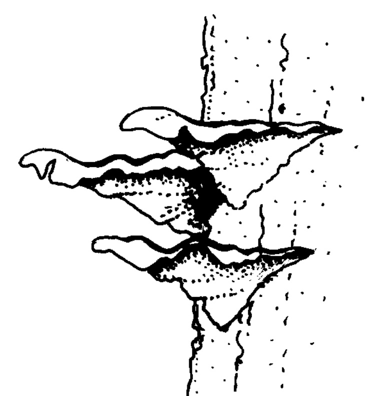

bracket fungus: saprotrophic on dead wood or pathogenic on host plant

dying tree: caused by bracket fungus

3.3
Fungi Imperfecti / Deuteromycota

The Deuteromycota have septate mycelium and form asexual reproductive spores known as conidia. Conidia are produced on separate hyphae called conidiophores. They usually form a mat of hyphae covering parts of leaf surfaces, fruit or other substrates. This group is also known as the Hyphomycetes. Some Deuteromycota may form small fruit-bodies in which conidia are formed. This group is known as the Coelomycetes.

☞ **Isolation:**

Isolation of these fungi is not difficult: malt extract agar (MEA) and potato carrot agar (PCA) can be used. Suitable selective baits or media can be used for isolation of soil-borne plant pathogens.

☞ **Examples:**

Some Deuteromycota cause important post-harvest infections, while others are pathogens that may cause die-back, leaf spot, root rot or wilting. They are secondary or asexually reproducing forms, mainly of Ascomycota and Basidiomycota. A common Coelomycete is

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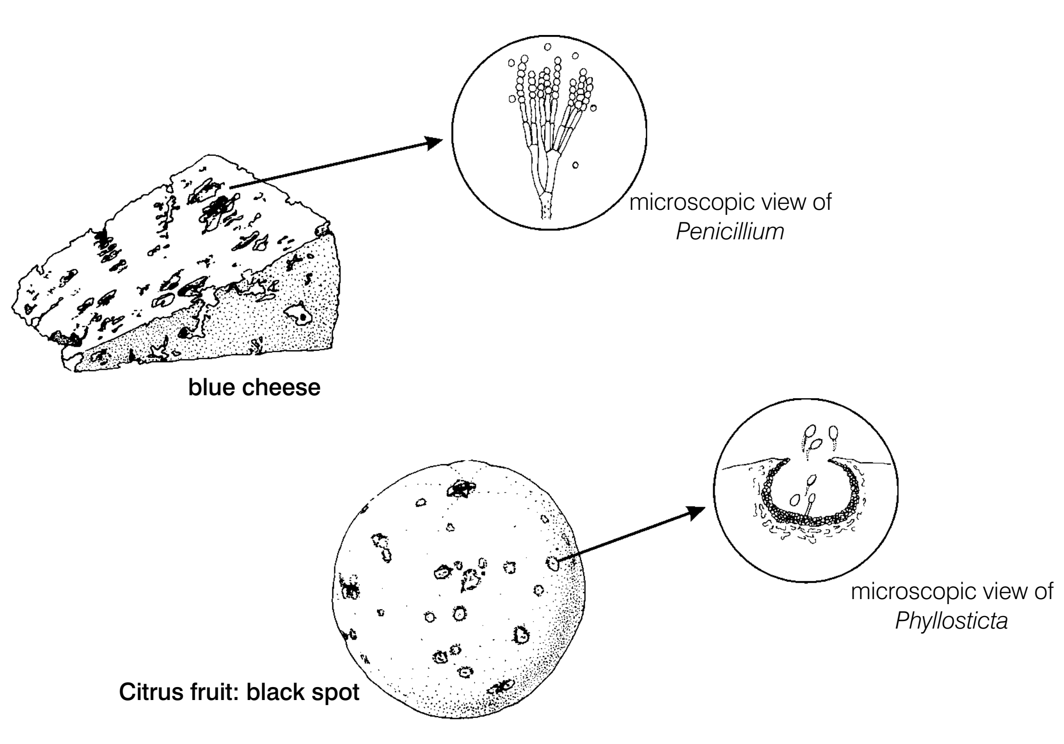
Phoma, species of which may cause die-back of stems, leaf spot and other plant-pathogenic symptoms.

Many *Fusarium* species are known to cause wilting and root rot and can also be toxigenic. These pathogens can be soil-borne or seed-borne. Species of *Penicillium* and *Aspergillus* may cause post-harvest rot of fruit and vegetables. Symptoms of infections are often the visible growth of the fungus on substrates such as bread, cheese and fruit.

Examples of various Deuteromycota



post-harvest rot of oranges

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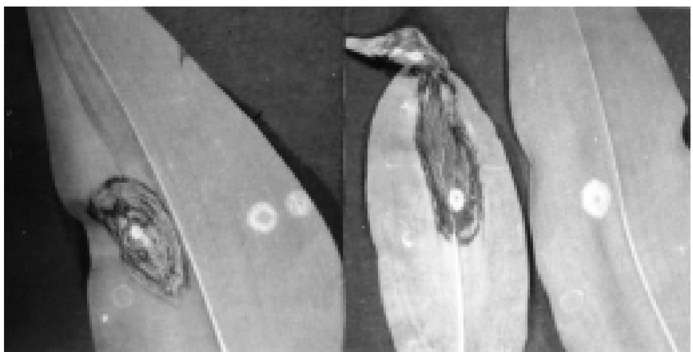

leaf-blight symptoms

fruiting bodies on dead twig

3.4 Oomycota (Orders: Peronosporales, Pythiales)

The advanced Oomycota are highly specialised and are important plant pathogens. Some, such as the downy mildews (*Peronospora*, *Sclerospora*) and the white blister rusts (*Albugo*), will only grow on living plants (obligate parasites). Others can be grown in artificial culture.

☞ **Isolation:**

The Oomycota may be isolated from infected plants (above and below ground level), and also from soil and water. Those species that can be grown artificially are usually poor competitors, meaning that they are easily overgrown by other fungi. Special requirements for *Pythium* and *Phytophthora* include the use of a suitable, selective bait (e.g. host seedlings, grass blades and hemp seeds) and selective media with antibiotics, including fungicides.

☞ **Examples:**

Phytophthora and *Pythium* species are well-known root pathogens on a wide range of cultivated and wild-growing plants.

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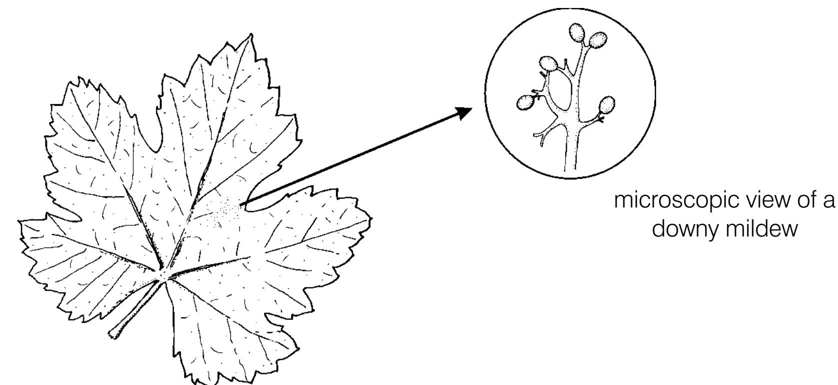


obligate parasite: downy mildew on grape leaves

Example of an Oomycota

3.5
Zygomycota (Order: Mucorales)

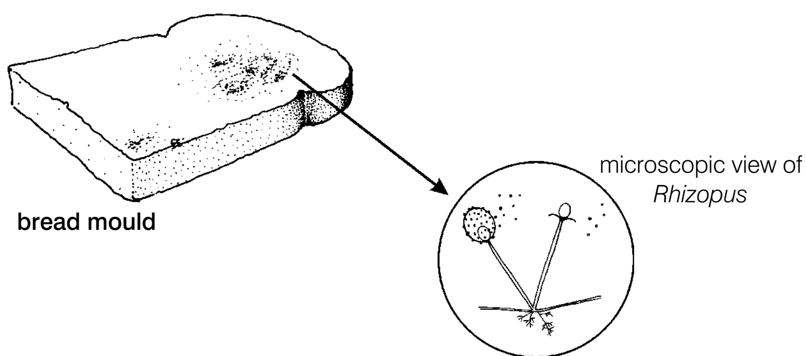
Members of the Mucorales form large numbers of spores in balloon-like structures known as sporangia. They commonly occur in soil, on dung and as the cause of post-harvest diseases in a number of important fruits and vegetables. In the laboratory they tend to become contaminants, e.g. *Rhizopus.*

☞ **Isolation:**

A nutrient-poor medium is recommended for culturing Mucorales, e.g. potato carrot agar with added antibiotics. For investigating soilborne fungi, the soil must be spread thinly on plates and then incubated. Isolations can be made directly from fruit or other infected substrates.

☞ **Example:**

Rhizopus stolonifer commonly invades bread and various fruits in storage.



bread mould

Example of a Mucorales

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4. Collecting and processing of field material

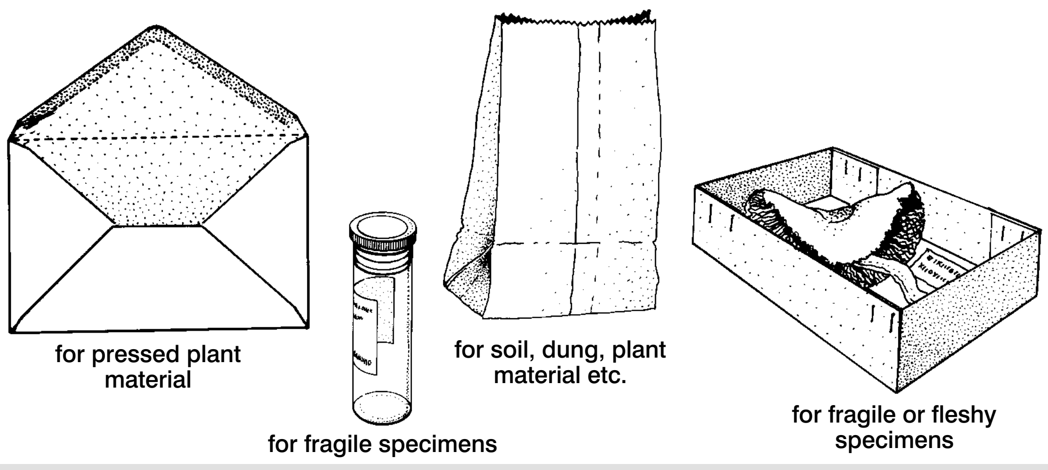
4.1 Equipment

Elementary equipment required when collecting fungi include:

- ☞ **Pruning shears or secateurs** (to cut off branches, leaves, etc. A sharp knife may also be used).
- ☞ **Saw** (to remove thicker branches).
- ☞ **Trowel** (to collect soil samples).
- ☞ **Handlens** (to examine specimens for fruiting structures).
- ☞ **Paper bags or envelopes** (for storing most samples; fragile specimens like mushrooms should rather be placed in suitable boxes to prevent damage).
- ☞ A **plant press** (for drying plant material).

Every sample should be accompanied by a label on which detailed information is recorded (as indicated in Section 4.2). A record book can be used for notes, sketches and other details.

- ☞ It is **very important to take a FIRST-AID KIT** along when going on a collecting trip.



Some containers for different types of samples

4.2 Labelling and record-keeping

All samples or specimens must be accompanied by the following information:

- ☞ locality;
- ☞ date;
- ☞ name of collector;
- ☞ substratum or host;
- ☞ obvious symptoms of host;
- ☞ collection number (if one has a personal recording system).

It is advisable to use a numbering system, especially when doing a survey or when collecting is regularly undertaken. Use a system that is self-explanatory: for instance, for the first specimen collected in 1997, the number 97/01 could be used. A register must be kept that contains relevant information concerning the material.

It is very important to collect sufficient representative material of a sample or specimen. Excess material can be discarded later. In the case of plant pathogens, collect healthy as well as infected material. This is to show the normal appearance of the plant material, as well as what the symptoms are, e.g. circular halos and tar spots. Keep these samples separate.

A label with the following information must accompany each sample or specimen:

Example of information that should accompany a plant pathogen:

Plant Protection Research Institute, Pretoria
Trip/Survey:

Specimen no.:
Fungus name:
Host/Substrate:
Symptoms:
Lacality:
Grid reference:
Collector: **Date collected:**
Determined by: **Verified by:**
Additional notes:

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4.3 Succession of fungi

Ecological succession in a sample is the progressive change in the composition of the fungal species (mycobiota) that takes place over a period of time. In other words, the species composition in a soil sample just after it has been collected will be different from that when the soil sample is plated out after it has been in storage for some time.

Succession is very important when sampling coprophilous fungi (from dung), for instance, because the stage of decay determines the mycobiota. When investigating pathogens on plants too, one should ensure that the actual disease-causing agent is collected.

4.4 Different techniques for different specimens

There are various ways in which samples can be collected and processed. Each group of fungi has its own requirements and should be handled in a different way. Different groups of fungi are collected at different times of the year, e.g. rust and smut symptoms are evident during autumn, while mushrooms are collected during or soon after the rainy season.

For the identification of Ascomycetes and Basidiomycetes particular care should be taken to collect fertile fruit-bodies as these fungi often do not sporulate again after having been collected. A handlens may be used to ascertain whether spores are present. The Deuteromycetes (Coelomycetes and Hyphomycetes) will often develop further in humid chambers, allowing pure cultures to be made.

Some fungi can only be seen with a microscope. To spot them in the field is nearly impossible even if a handlens is used. Their presence is often betrayed by the symptoms they cause, such as spots, rots, discolouration of plant parts, or woolly growths on some substrates.

The identity of the host is always very important because many fungi are host-specific.

Large and fleshy specimens

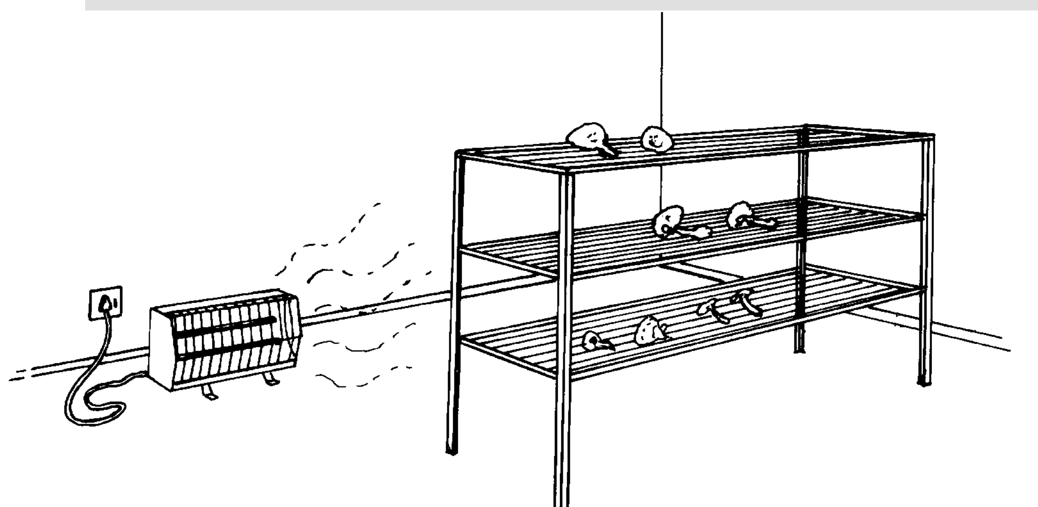
Large and fleshy specimens such as mushrooms, puff-balls and most bracket fungi must be handled with care. Special care must be taken to keep them dry, otherwise they may become overgrown with moulds. Never keep damp

material in plastic bags. Large and fleshy specimens can be cut into slices before being dried. Fragile specimens should be carefully packed in paper bags or preferably in suitable small boxes when collected and transported to the laboratory. Specimens are often infested by insects which can cause serious damage.

Notes on the size, shape, colour, texture, etc. of specimens should be written down, and colour photographs should be taken if possible. Most fungi with fleshy fruit-bodies shrink considerably during drying, and features such as colour are frequently lost. For mushrooms, a spore print should be made to aid identification. This is achieved by cutting off the stems of fresh mushrooms and placing one cap on a white and another cap on black paper. These are then placed flat in a polythene bag or covered with a glass or plastic container. The spores fall onto the paper and the colour of the spores is easily seen.

Plant material such as fruit, large flowers, seeds or thick leaves should also be treated with care. Collect diseased and healthy specimens. They can be cut in half or into sections before being dried, or the infected portions may be excised and glued onto cardboard.

All fleshy specimens should be dried slowly at a mild temperature. This can be done by placing them on a grid near a heater, or in a mild convection oven. To ensure that they do not curl up, a grid can be placed over them.



When the specimens are completely dry, they should be carefully packed into boxes together with their labels if meant for deposition in a herbarium. An insect repellent such as naphthalene should also be placed in the boxes.

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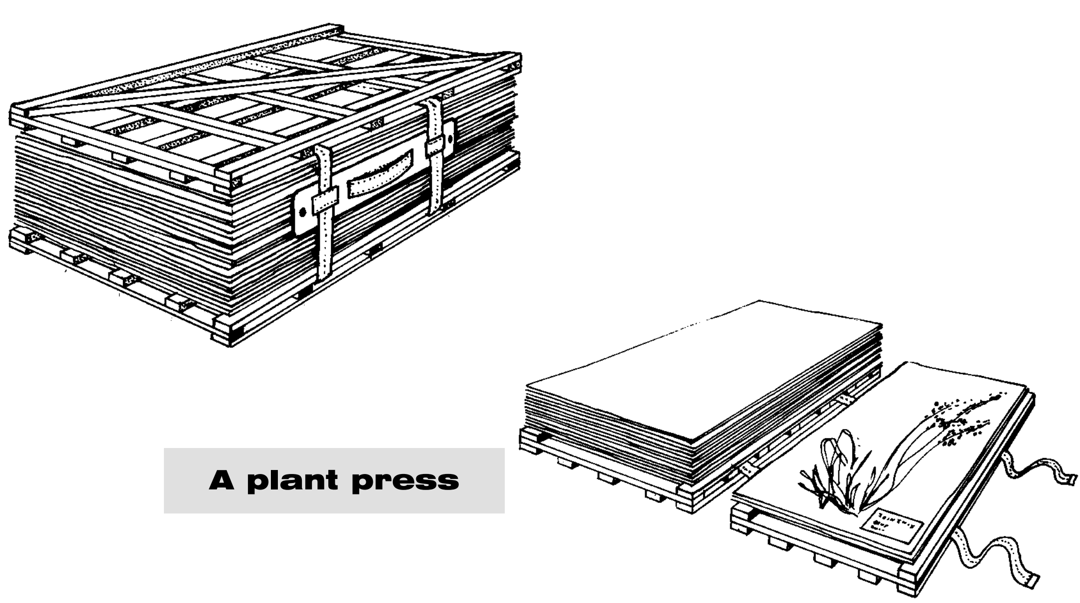
Herbaceous plant material

Herbaceous plant material freshly collected from the field should be placed in a plant press to dry. This prevents the leaves from curling up and breaking when handled, and makes it easier to store these specimens in envelopes for deposition in a herbarium.



A plant press

If a plant press is not available, plant material can also be dried between the pages of a telephone directory, or between sheets of any other absorbent paper. A weight can then be placed on top. Enclose a label with all the necessary information with each specimen. If the plant press is almost full, the absorbent sheets dividing the specimens must be replaced regularly with dry sheets until the specimens are dry.

Roots

Roots should be disturbed as little as possible, and can be collected with some of the soil surrounding them. They should be stored in paper bags to dry. This type of material must be processed as soon as possible.

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Soil

Soil samples must be collected in **paper bags** to ensure that they dry quickly. If the presence of plant pathogens is suspected, some samples should also be collected in plastic bags, to retain their moisture. This is one of the few exceptions when plastic containers should be used for samples. A representative sample must be taken: e.g. take five or more samples at various spots at a particular locality and mix them in one bag.

When investigating soil specifically for pythiaceous fungi (*Pythium* and *Phytophthora*), samples must be kept at their original moisture level. This entails packing them in plastic bags, insulating them in cool-boxes and using a quick, reliable method of transport, e.g. a courier service.

Animal feeds

These are usually composed of a mixture of ingredients consisting of different-sized particles and with various textures. If possible, each ingredient of a feed sample should be placed in a separate container. Samples must be kept dry, **and be processed as soon as possible**.

Airborne spores

These may be trapped by using specially designed spore traps, following a specific procedure. If a spore trap is not available, however, spores can be collected on agar plates exposed to the open air. The lid of a Petri dish should be placed close by so that the dish can be closed quickly after a given period of time. **Plates should be exposed in different places and at different heights in order to collect a representative sample**. Use nutrient-poor media such as water agar (WA) and potato carrot agar (PCA), with or without antibiotics (see Chapter 8). The agar plates must be examined at regular intervals after about the second day because spores of different species have different germination times.

Aquatic fungi

Aquatic fungi can be isolated from floating leaves, sticks, fruits and seeds **in any body of water**. They should be collected in strong plastic bags; foam can be collected in a jar. Specimens should be surrounded by ice packs, or placed in a Thermos flask, or smeared onto an agar plate containing antibiotics. Foam can be kept for up to a month when stored at 13 °C. It can be preserved in a fixative such as formalin-acetic alcohol, allowing the conidia to be identified afterwards by a specialist.

Dung fungi

Dung fungi are very interesting although they are not economically important. A wide spectrum of fungi belonging to different groups may be found in the

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same sample. Because **succession** takes place, different fungi may be found at various intervals over a period of time. Collect dung in paper bags to facilitate drying.

Fungi growing on other fungi

Also called fungicolous fungi, specimens of these kinds are treated in the same way as the fungus on which they are growing.

Fungi on insects and spiders

These fungi are fragile and must be handled with care. Specimens should be secured in small boxes or other containers and kept dry.

4.5. Dispatching specimens

If specimens are sent away for study or identification, they will usually require posting, and certain precautions must be taken to ensure that they arrive intact at their destination.

Material must be packed securely. Each specimen or sample must be labelled individually — the easiest way is to give it a reference number, making sure the number cannot be removed. The necessary data for each specimen, with its corresponding reference number, should be listed on a sheet of paper and forwarded under separate cover.

- ☞ Cultures for dispatch must be grown on a medium that is more rigid than usual (2.5–3.0 % agar) until sporulation: ensure that the correct species is sent. They are grown in test-tubes or McCartney bottles (small screw-top bottles) on agar slants. Petri dishes, even when properly sealed with parafilm or clingwrap, are prone to drying out or become contaminated.
- ☞ Each test-tube or bottle should be wrapped separately in packaging material (e.g. 'bubble plastic'). The dispatching box must be sturdy and should be filled with packing material that provides a cushioning effect. A *polystyrene mould* made especially for test-tubes is ideal. The mouth of a test-tube should be covered with parafilm, over the cotton wool stopper. Do not send glassware in envelopes: it will break. When sending dried herbarium material, it should be packed firmly into boxes in a similar way.

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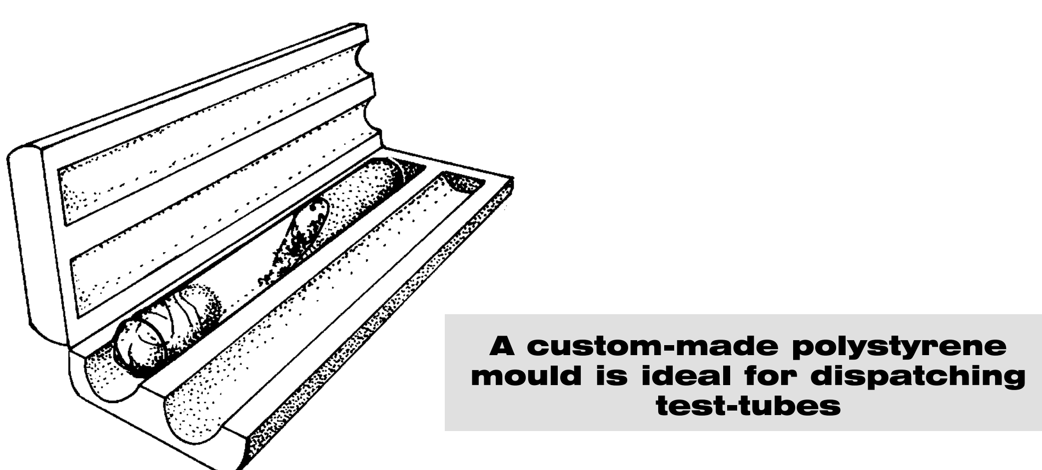

A custom-made polystyrene mould is ideal for dispatching test-tubes

☞ Material should be packed in the middle of the box, and the space between the material and the walls should be filled with packing material such as styrofoam chips, wood-wool or paper shreds. If too tightly packed, the advantage of this double-packing will be lost and every shock that the box is subjected to will be transferred directly to the specimens. If loosely packed, most of the shock will be absorbed by the packing material, but if too loosely packed, the material could move around and be damaged, and the outer box could lose the structural support given by the packing material.

The box should be well-taped at all seams to withstand handling during postage. The addressee's and sender's addresses should be written or typed in clear, bold lettering and should be firmly attached to the parcel. At least one warning label should be affixed to the parcel, stating for instance 'Fungi: very fragile' or 'Glass: handle with care'.

☞ Parcels with valuable specimens should be registered, and those destined for other countries should be sent by airmail.

☞ Material that is not **completely dry** (such as mushrooms and plant material) must be packed in porous containers or preferably in paper bags, and wrapped in absorbent paper, otherwise it will become mouldy. It should preferably be sent by courier.

☞ **Mounted specimens or material in envelopes** should be protected by placing them between the folds of paper (double flimsies or

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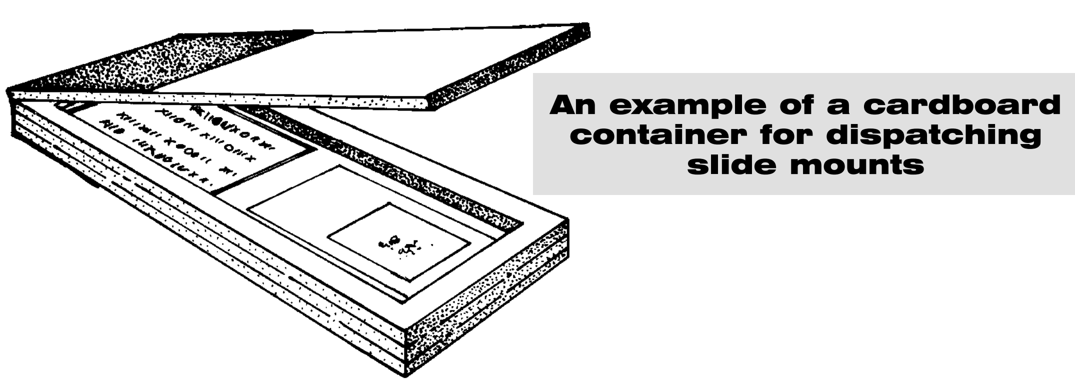
newspaper). The material should be secured by bundling it together between light but strong boards, or wrapping it together in paper.

☞ **Microscope slides** are best sent in specially made cardboard containers. Otherwise the slides can be stacked back-to-back, with cardboard spacers separating the pairs of slides, using clean slides (without coverslips) for bracing on either side. These are then carefully taped together to make a suitable unit.

☞ The container or box should be double-wrapped in thick brown paper.



An example of a cardboard container for dispatching slide mounts

5. Microscopic study of fungi: Getting started

5.1

Examination of material

No fungi can be scientifically and correctly identified without proper microscopical examination. This entails the making of microscope preparations.

5.2

Equipment

Microscope slides, cover slips, dissecting tools (forceps, needles and scalpels), stains and mountants, dissection microscope, research microscope.

5.3

Study of fungi

Specimens are studied in one of two ways: those that cannot be grown under artificial conditions are **studied directly**, and those that must or can be cultured or isolated are **studied indirectly**.

☛ Indirect study

Many pathogens in plant material, soil and water must first be isolated and obtained in pure culture (as described in Chapter 6) before they can be studied. These fungi are usually not visible to the unaided eye, although the disease symptoms often are.

☛ Direct study

Specimens that are studied directly include mushrooms and the larger fungi, which represent the final stage in the reproductive cycle. Fungi such as rusts, smuts, mildews and other obligate parasites that cannot be cultured, are also studied in this way. A microscope preparation can be made directly from the material, using stains or a lactophenol mountant, to examine the microscopic morphology of the specimen. Some hard or dry specimens may have to be rehydrated before examination. This can be achieved by soaking them in 5 % potassium hydroxide (KOH).

Specimens or selected pieces of specimens can be incubated in a humid

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chamber (Section 6.1). It is important to work under sterile conditions to ensure that competitive saprotrophic fungi do not contaminate the chamber and destroy the specimen.

Still smaller pieces containing the desired structures can be immersed in 5 % KOH and rehydrated. These can then be washed and stained using routine methods, or studied directly. Competitive saprotrophs will not grow, because KOH will kill any fungi present on the surface.

5.4 Making a basic microscope preparation

- ☞ Place the microscope slide next to your dissection microscope and place a small drop of stain or mountant in the middle of the slide. If the material is dark, use colourless lactophenol; if it is colourless use a stain; if you need to examine a special structure, use an appropriate stain.
- ☞ Take a small piece of the infected material or remove some of the sporulating structures using a fine needle and transfer them to the liquid.
- ☞ Take a clean cover slip and hold it on one side, then let it slip onto the liquid from one side without trapping air bubbles underneath. If the material is very oily (resulting in the formation of air bubbles), first place a few drops of 70 % alcohol on the slide, then the material to be studied and then add the stain or mountant.
- ☞ Press the cover slip down gently. There must be just enough mountant or stain to suspend the cover slip.

Hint: Always aim to make the preparation as thin as possible: this ensures a better depth of field and the preparation will seal properly.

5.5 Temporary versus permanent slides

One must remember that each method has its advantages and disadvantages. Some methods produce excellent results but the preparation does not last for longer than an hour, e.g. when using 3.5 % erythrosine (see recipes in Section 5.8). Other preparations will last as long as the slide remains intact, e.g. when using lacto-fuchsin (see recipes in Section 5.8), and these can be sealed and stored.

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5.6

Sealing of slides

Microscope preparations can be sealed with melted dental wax, paraffin wax or a commercially available sealant. Ensure that there are no air bubbles in the mountant. Bubbles can be prevented by using alcohol as described above, or eliminated by warming the slide slightly (do not let the mountant boil).

5.7

Different types of microscope preparations

Squash mounts

Remove young, sporulating fruit-bodies with a fine needle and place them in a mounting medium. Cover with a cover slip and press down gently. These slides will be usable for up to an hour. When using lacto-fuchsin (see recipes in Section 5.8) the slides can be sealed and stored. Different mounting media do, however, give different information because the stains affect different structures differently.

Sections

Sections are useful to determine the internal structure of hyphal aggregates, and also of individual fruit-bodies in relation to their substrate.

- ☞ **Hand sections** require a dissection microscope and making them is a delicate process.
- ☞ **Microtome sections** are made using special equipment. Microtomes produce sections of a required thickness and using them results in a series of sections in sequence. In this way, a three-dimensional model of a fruit-body can reconstructed. The material must be fixed and embedded in a special resin, and then cut using a specific microtome. There are different kinds of microtomes, each with its own application: sliding microtomes for wood; rotary microtomes for wax and resin-embedded material, and freeze microtomes for frozen material. Resin-embedded material cut with an ultra-microtome gives high-quality sections that can be used in transmission electron microscopy (TEM).

5.8

Recipes for mountants and stains

☛ Lactophenol

The basic recipe for this colourless mountant follows. A number of stains may be

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added to it, e.g. fuchsin, cotton blue or fast green. Because of the lactic acid and glycerol base, this produces only semi-permanent slides.

Phenol crystals (N.B. very toxic) — 20.0 g
Lactic acid — 20.0 g
Glycerol — 40.0 g
Water —20.0 g

Method:
1. **Heat the phenol crystals carefully in water until dissolved.**
2. **Add the lactic acid, glycerol and stain (if applicable). Store in a dark container to prevent oxidation.**

Stains (0.05 g) can be added.

☛ Erythrosine

Slides made using this stain are temporary, lasting up to an hour. It stains cytoplasm, giving good contrast between cytoplasm, septa and cell walls. Erythrosine is especially useful when studying conidiogenesis in the Coelomycetes.

Method:
1. **Prepare a 10 % ammonia solution (NH_4OH).**
2. **Dissolve 3.0 % erythrosine in the above.**

6. Isolating fungi for study

Micro-fungi in particular are usually studied in pure culture. They are generally isolated from infected material after it has been incubated under conditions that encourage fungal growth.

Inoculations and isolations should preferably be done in a laminar-flow cabinet (when dealing with wet-spored fungi) or in a 'bio-hazard' cabinet (when working with dry-spored fungi). These cabinets should be used when working with potentially dangerous organisms.

6.1 Isolation methods

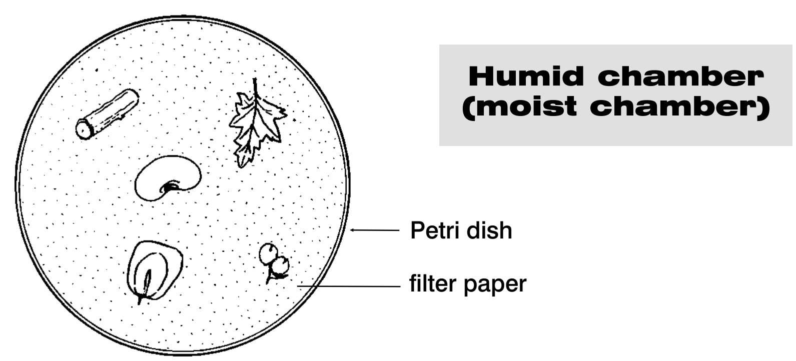
Isolations can be made from material as follows:

Direct isolations

If there is obvious fungal growth on material, isolations can be made without first incubating it. The developing fungus is removed from the substratum and plated out onto a suitable agar medium.

Isolations from humid chambers

Humid chambers (also called moist chambers) are used to incubate material before making isolations. Fungi on organic substrates need mainly moisture and a suitable temperature to encourage them to grow. To make a humid chamber, a few sheets of filter paper are placed in a Petri dish and moistened with distilled water. The Petri dish is then wrapped in foil and sterilised in an autoclave and allowed to cool down before use. Water agar plates can also



Humid chamber (moist chamber)

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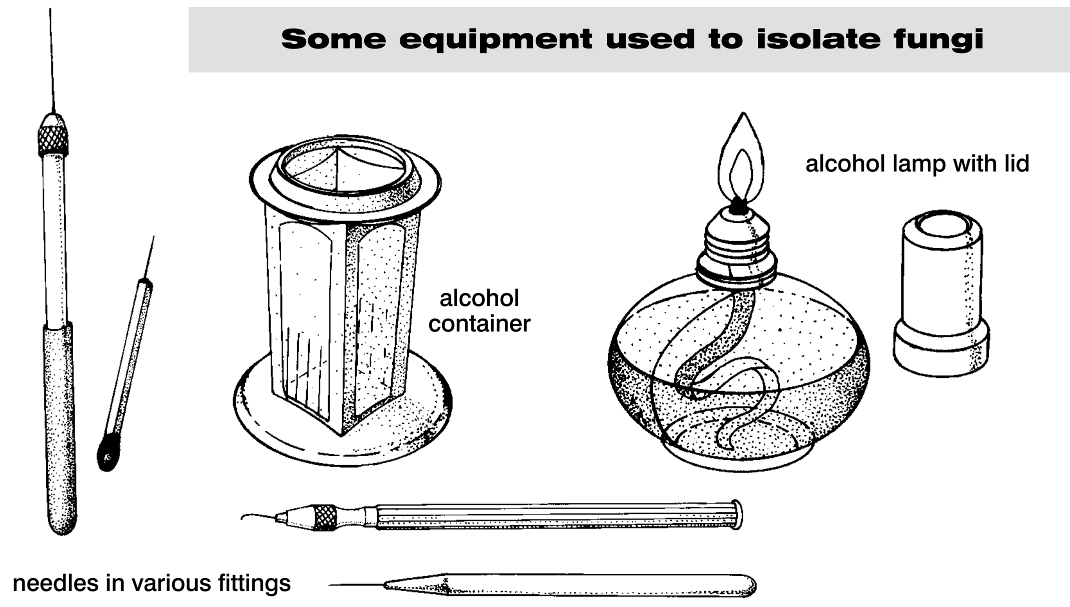
be used. Material placed in a humid chamber should be left for 2–5 days for the fungi to develop.

6.2
Equipment for isolating fungi

Dissecting needles (no. 10 sewing needles or thin insect pins can be used), fine forceps (watchmakers' forceps are best), blades or sharp knives, a spirit- or gas burner or similar source of flame, as well as 70 % alcohol. A dissecting microscope is required in most cases.

Some equipment used to isolate fungi



6.3
Isolation procedure

☞ To sterilise the inoculation needle, dip it in alcohol and pass it through a flame, or heat it in the flame until red-hot and cool it down in 70 % alcohol. Handle it carefully. Heating may eventually damage the tip of the needle, rendering it useless for fine work: special heat-resistant wire can be used instead, e.g. the rigid wires suspending the element in a light bulb.

☞ A dissection microscope is used to locate a suitable group of spores. The tip of a sterilised needle is then pressed into a sterile agar plate to wet it and then gently pressed against the spores. The surface tension of the spores will be broken by the moisture on the needle and they will stick to it.

☞ The spores are then transferred to a sterile agar plate.

When inoculating onto **agar media** in a Petri dish, the lid should be lifted up slightly so that the gap is just wide enough to allow inoculation. Do not remove the lid completely, and do not place it on the work surface.

The mouths of **bottles** must be flamed before inoculating the agar, and again before replacing the lid.

The mouths of **test-tubes** have to be flamed before and after inoculating the agar; do not place the stopper on the work surface.

Hint: When isolating fungi, remember that only a tiny piece of fungal material should be transferred, not big clumps of mycelium or lumps of agar. This lessens the possibility of contamination.

The following information must be written on the plates, test-tubes or bottles:

☞ Type of medium (e.g. WA, PCA, MEA+N),

☞ date inoculated, and

☞ the reference number of the material.

A felt-tipped pen with permanent ink should be used. Information regarding the host or substrate, locality, collector, etc. must be kept separately in a book or file.

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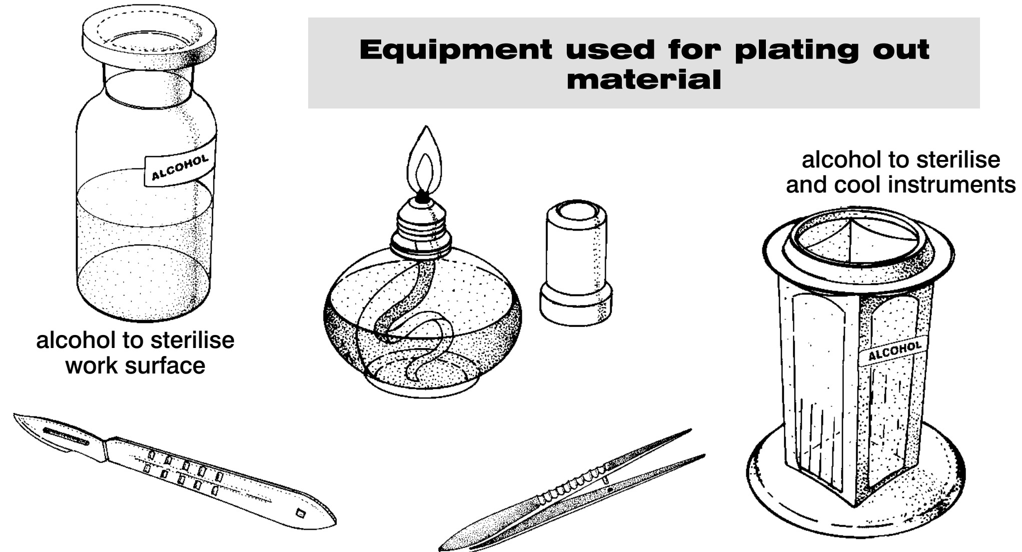
7. Plating out material

Samples are plated out onto agar plates when isolations cannot be made directly from infected material. Different techniques are used for different kinds of material (as discussed below), but the basic procedures are the same for all material. Never place too much material in a Petri dish. Always open the Petri dish lid as little as possible and close it as soon as possible. In most cases, nutrient-poor agar media are used such as water agar (WA) or potato carrot agar (PCA) (see also Chapter 8). Antibiotics are usually added to the media to prevent bacterial growth: it is always advisable, however, to also use a few Petri dishes without antibiotics. Plant material should be surface-sterilised before it is plated out for examination for specific pathogens. Some that have not been surface-sterilised must be plated out as a control.

Most material can be incubated at 22–24 °C. Illumination with a twelve-hour light/dark cycle is preferable and UV-light is essential for the sporulation of many fungi.

7.1 Equipment

The following equipment is needed when plating out material: Ethyl alcohol (70 %), cotton wool, scalpel with a sharp blade, forceps, alcohol burner and suitable agar media.



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7.2 Surface disinfestation

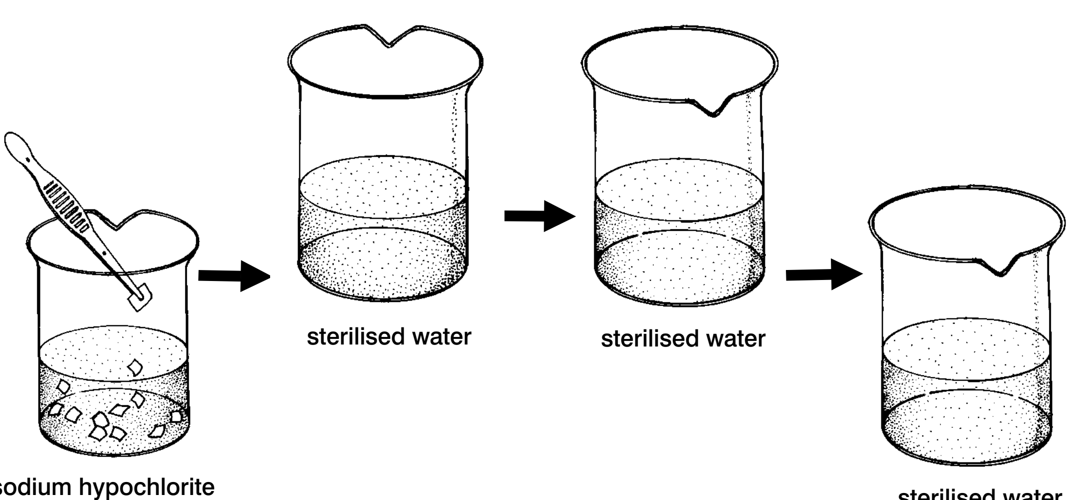
To promote the growth of poorly competitive fungi (especially those in lesions in plant tissues and in seeds or roots), contaminants are removed from the surface of the material. Pieces of plant material for plating out should include part of the infected material or visible lesions, as well as some of the adjacent, healthy tissue. Wood must be cut into manageable pieces before plating out, or shavings can be used. Large specimens must also be cut into smaller pieces.

The most common method of surface disinfestation is to immerse the material for 1 minute in 3.5 % sodium hypochlorite (commercial household bleach such as Javel = **3.5 % sodium hypochlorite**). This may be diluted, in which case the material must be immersed for a longer time. **Hydrogen peroxide** may also be used, especially for leaves. Material is usually left for 5 minutes in a 1 % solution.

It is then washed three times in sterilised, distilled water. Use three beakers and transfer the material with forceps. It can be dried on clean tissue-paper and then plated out.

Dry material, such as wood, usually absorbs liquid disinfectants. This prevents the fungi from growing out. Rather than using the above method, quickly dip each piece in alcohol and flame it, rapidly extinguishing the flame.

Surface disinfestation of material



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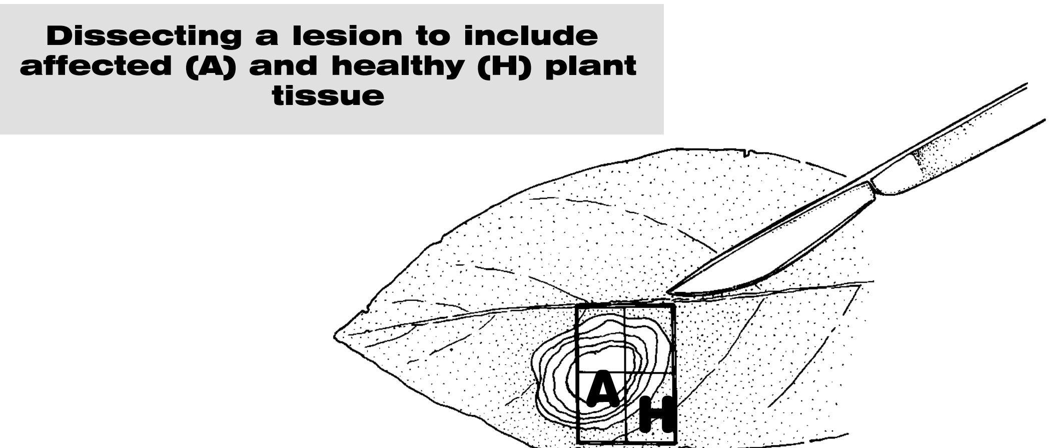
Dissecting a lesion to include affected (A) and healthy (H) plant tissue



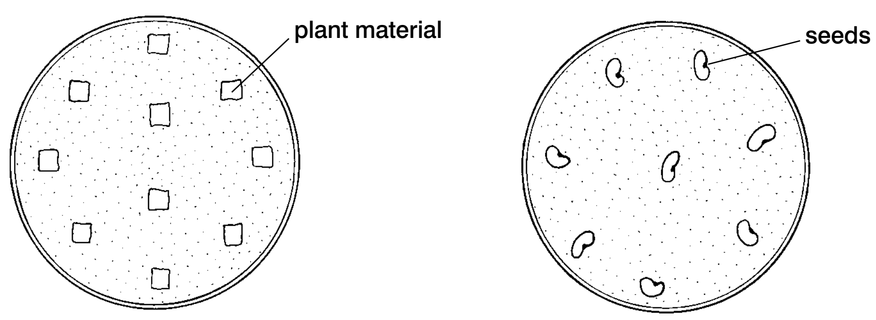
7.3

Different techniques for different kinds of material

Plant material

Plant material to be plated out must contain both **healthy** and **affected** parts. Never put too much material in a Petri dish because pathogens may easily be overgrown by other fungi.

After surface-disinfestation, **seeds** can be plated out onto nutrient-poor agar media such as water agar (WA) and potato carrot agar (PCA) containing antibiotics (see Chapter 8). Depending on the size of the seeds, up to ten can be placed in a circle on the agar. Rather use too few than too many. According to international standards, 400 seeds per sample should be plated out to be representative.

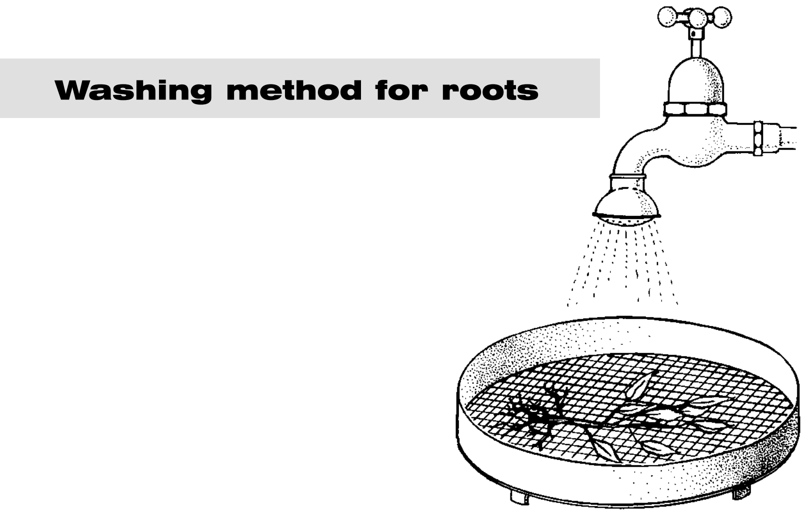


Petri dishes containing different kinds of material

Roots

Roots must be processed as soon as possible after collection. They can be cleaned by placing them into a sieve and washing them under a tap provided with a shower nozzle that provides a fine but strong spray. Washing should be continued for three hours or even longer. The clean roots should be placed on clean tissue or filter paper to dry.

Roots can be surface-disinfested as described above. They must then be plated out onto a nutrient-poor agar medium such as potato carrot agar containing antibiotics (PCA+N) (see Chapter 8) to prevent the growth of bacteria.



Washing method for roots

Soil

Soil should be processed as soon as possible after collection because of ecological succession of fungi in soil samples. Weigh ten or more parts of e.g. 2 g each and sprinkle each part thinly and evenly onto the surface of an agar plate. Use a nutrient-poor medium such as potato carrot agar containing antibiotics (PCA+N) (see Chapter 8). Remember that many nematodes may be found in soil, so be sure to examine plates within a day or two after plating out, before they ruin them.

It may be necessary to make dilution plates if the soil is heavily infected. In this case the weighed soil sample is e.g. mixed with 10 ml of a 1 % water agar solution at room temperature. Only 1 ml of this soil suspension per Petri dish is plated out and incubated. This way, the sample has been diluted ten times. The fungi on the plates can be identified and the colonies counted as soon as they sporulate.

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Animal feeds

These are usually mixtures of straw, seeds, grass, etc. and are usually tested for a specific fungus. Make sure what is required: if a sample has to be tested for *Fusarium,* for example, it should be plated out onto a selective *Fusarium* medium. Otherwise, plate it out on nutrient-poor media containing antibiotics, e.g. water agar (WA+N) or potato carrot agar (PCA+N) (see Chapter 8). These plates should be examined daily because they become overgrown very easily. Such samples are usually of mixed origin, so they are difficult or impossible to surface-sterilise. Larger pieces and seeds can be sorted out, surface-sterilised and plated out separately.

Aquatic fungi

To obtain aquatic fungi from a water sample, water can be filtered using millipore filters (8 μm pore size) and incubated. Baits, such as submerged plant material, can be placed in stagnant water, ditches or slow-running streams, collected after a period of time and then incubated.

Airborne spores

Airborne spores are useful in the prediction of epidemics. Petri dishes with suitable agar can be opened at different heights and kept open for up to 20 min., depending on the wind. Do not attempt this in rainy weather. Special spore-trapping devices are available that continuously sample the air to record an entire day's spore release. The spores trapped in some devices can be incubated and grown out. A spore sampler powered by solar energy is useful.

Dung fungi

Dung pellets can be broken-up under aseptic conditions and incubated. This is especially important if pellets are contaminated with soil. They are best placed in a humid chamber and incubated (see Section 6.1). Dung can also be incubated on plates of nutrient-poor media such as water agar (WA) or potato carrot agar (PCA), but the plates quickly become overgrown. **These plates should be examined daily because of the rapid succession of fungi and also because they may be heavily infested with nematodes**. Isolations can usually be made directly by spore transfer using a sterilised needle.

Insect bodies and larvae

These are best surface-sterilised before being placed in a moist chamber (see Section 6.1) or on suitable agar because many saprotrophs will also grow under these conditions. **Various entomopathogens cannot grow in culture, so microscopic examination is essential**. After incubation in moist chambers, specimens are best preserved in glass containers filled with 70 % ethanol.

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8. Preparation of media

8.1 Choice of media

Most agar media are commercially available as soluble powders that contain the required nutritional ingredients. The formula is usually printed on the label of the container. This powder is mixed with distilled water and sterilised. Many media, however, can be easily made in the laboratory. A few recipes are given below.

Some media are poor in nutrients while others are rich. Nutrient-poor media are generally recommended because they do not encourage abundant growth, so slower-growing fungi will not be overgrown by others. Also, the measurements (dimensions) of some fungi on nutient-poor media are closer to those found in nature. Examples are water agar and potato carrot agar. First platings of material are usually made on nutrient-poor media.

Antibiotics can be added to media to prevent the growth of bacteria. These are especially used for first platings, and also to purify cultures contaminated with bacteria. Useful antibiotics are e.g. chloramphenicol, novobiocin and tetramycin. They are used singly or in various combinations, usually at a concentration of 5 ml/l. Antibiotics are added to the medium with a syringe and needle only after sterilisation, and when the agar has cooled down to about 45–48 °C.

Each fungus has its own **nutritional requirements.** Different fungi, and even various isolates of the same species, often have individual requirements. This is why certain agar media are better suited to the growth of some fungi than others. Specific media have been developed for certain fungi: these are known as **special** or **selective** media. Such selective media are required for the comparative study and identification of members of many genera, including *Fusarium, Aspergillus* and *Penicillium.*

8.2 Conditions for the successful preparation of media

The prevention of contamination is of utmost importance when preparing media (i.e. work aseptically).

☞ Use sterilised, cooled apparatus and media; also sterilised distilled water when needed.

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- ☞ Always use ethyl alcohol (at least 70 %) to wipe the surface on which you are going to work.
- ☞ Do not work in a draught: spores are carried in air currents.
- ☞ Agar requiring sterilisation should be placed in bottles with screw-on tops. Ehrlenmeyer flasks with cotton-wool stoppers are also suitable.
- ☞ The type of medium should be clearly written on the containers with a felt-tipped pen, in permanent ink.
- ☞ The container in which the agar is sterilised must not be filled completely, because the contents will boil over (the volume of agar increases when the medium is heated in an autoclave or pressure cooker).
- ☞ Unscrew all bottle tops a quarter of the way before putting them in an autoclave. Close them properly after sterilisation, before cooling.

Petri dishes, test-tubes and McCartney bottles should also be clearly marked with the name of the medium they contain (e.g. WA, PCA, etc.).

8.3 Sterilisation

Equipment can be sterilised by wet or dry methods:

☛ Wet sterilisation

Wet sterilisation is used for plant material and media. Items are heated to 121 °C for 15 min. at 15 p.s.i. in an autoclave or pressure cooker. All agar media are sterilised this way.

☛ Dry sterilisation

Dry sterilisation is used for glassware such as pipettes, glass Petri dishes, etc. Articles are packed in suitable containers and then heated; heating for 1–2 hours at 160 °C is sufficient to allow penetration and ensure sterility (Booth, 1971).

Note: A microwave oven cannot sterilise. Although it heats, the required pressure is not attained. Use it only to melt small amounts of agar. Remember that agar has a high boiling point and can cause serious burns if spilled.

8.4 Pouring agar into different containers

Petri dishes

Lift one side of the lid carefully, just enough to be able to pour the agar into the plate. Do not remove the lid completely, and do not place it on the work surface.

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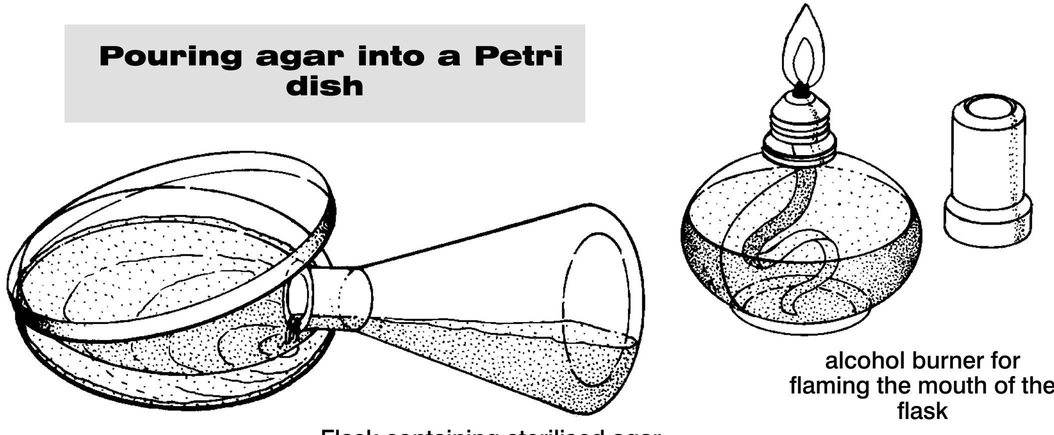


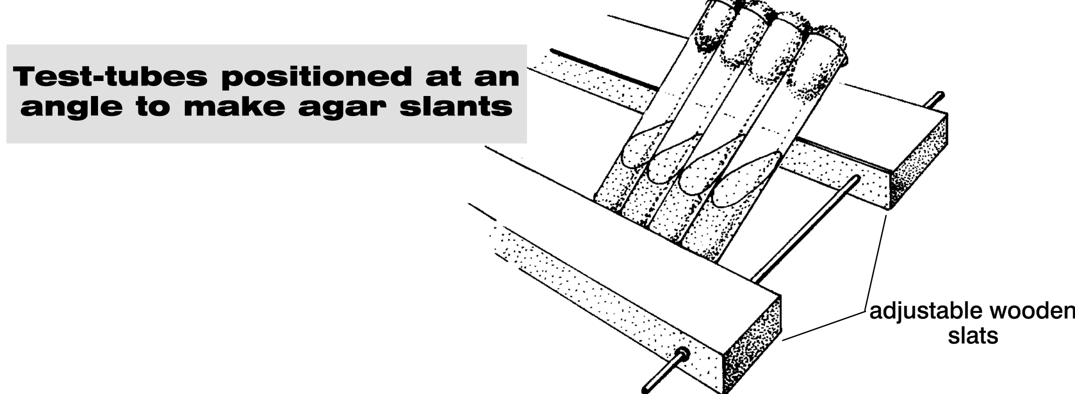
Pouring agar into a Petri dish

Plastic Petri dishes cannot be sterilised by wet or dry methods. They are bought sterile and can only be re-used when sterilised by means of radiation. If this facility is not available they must be discarded after use. Glass Petri dishes must be sterilised before agar is poured into them: they must be placed in suitable closed containers or covered with foil and then sterilised. They are sterilised wet or dry. Dry sterilisation is preferable because they remain sterile longer.

McCartney bottles

The agar and water suspension is boiled until the agar is properly dissolved. Alternatively it can be placed in the autoclave for one cycle. It is then carefully poured into the bottles after it has cooled down a little, stirring the mixture each time before pouring it.

The caps of the bottles must be unscrewed a quarter of the way. They are then put into the autoclave to be sterilised. After sterilisation the caps are screwed on properly. The bottles are then positioned at a slight angle to make agar slants and allowed to cool down.



Test-tubes positioned at an angle to make agar slants

Test-tubes

Test-tubes are filled in the same way as McCartney bottles. Non-absorbent cotton wool stoppers are preferable The tubes should be positioned upright in wire baskets with their stoppers in place. The top of the basket is covered with aluminium foil or a paper bag. After sterilisation the test-tubes are positioned at an angle and then allowed to cool down.

Note: Be careful when using flammable liquids, hot equipment and hot agar or other potentially dangerous materials needed for culture work. Sterilised agar should be allowed to cool down to about 45–48 °C before being poured. Antibiotics should be added just before the agar is poured.

8.5

Recipes for common media

Note: PDA, OMA and MEA are nutritient-rich media; PCA and WA are nutrient-poor media. Depending on the grade of agar, 10–20 g agar per litre can be used. The more agar used, the stiffer the medium.

☛ Water agar (WA)

Agar — 15 g
Water — 1 l

Method:

1. **Dissolve the agar in the water.**
2. **Sterilise for 15 min. (15 p.s.i. at 121 °C).**

☛ Malt extract agar, 2 % (MEA)

Malt extract (e.g. Difco, etc.) — 20 g
Agar — 15 g
Water — 1 l

Method:

1. **Boil the malt extract in the water until dissolved.**
2. **Add the agar.**
3. **Sterilise as above.**

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☛ Oatmeal agar (OMA)

Oats (rolled, porridge) — 30 g
Agar — 15 g
Water — 1 l

Method:

1. **Boil the oats in the water for 1 hour, stirring now and then.**
2. **Press through a sieve (and a muslin cloth if a transparent medium is needed).**
3. **Top up the water to 1 l, add the agar and boil until dissolved.**
4. **Sterilise as above.**

☛ Potato dextrose agar (PDA)

Potato — 200 g
Dextrose — 20 g
Agar — 20 g
Water — 1 l

Method:

1. **Scrub potatoes and cut into cubes: do not peel.**
2. **Weigh, and boil in 1 l water until soft.**
3. **Mash, squeeze through a sieve.**
4. **Add agar and boil until dissolved.**
5. **Add dextrose and stir until dissolved.**
5. **Make up to 1 l.**

For *Fusarium,* use sucrose (i.e. PSA) instead of dextrose, mix, and adjust the pH to 6.5 (Booth, 1971; Johnston & Booth, 1983).

☛ Potato carrot agar (PCA)

Grated or chopped potato — 20 g
Grated or chopped carrot — 20 g
Agar — 20 g
Water — 1 l

Method:

1. **Boil the vegetables in water for 1 hour.**
2. **Press through a sieve and add agar.**
3. **Boil until the agar is dissolved.**
4. **Sterilise as above.**

9. Why a reference collection?

Everything that is known about an organism is linked to its name. The unique scientific name of each fungus can be seen as the key to the information about that fungus. Names, therefore, are the tools that make it possible to successfully store or retrieve data. In fungi, the true identity of the organism can be captured by an actual sample of that organism. **To confirm the identity of any fungus on which research is published, it is necessary to preserve a reference specimen.**

Collections are maintained for:

9.1 Repeatability (verification)

Research **must be verifiable.** This is why the materials and methods used in any scientific work are clearly stated in a publication. Reference material of the fungi involved must be preserved in a public collection where it is available for study by the international scientific community.

9.2 Supporting published data

New information is constantly being gathered about fungi. This often results in changes in the way in which species and genera are grouped or separated and in turn may result in name changes. **By preserving reference specimens, the identity of an organism used in a publication can be checked and kept in line with these changes**.

If the identity of a fungus used in a published research paper is called into question and no reference specimens of the fungus were preserved, the information associated with it in the paper may be rendered worthless. If a reference specimen is available in a recognised collection, however, other scientists could verify the identification, and in so doing either confirm the original finding or associate the data with the correct name. For example, *Polyporus tomentosus* was reported to be a source of galactose oxidase but subsequent workers were unable to confirm this; fortunately the original material had been preserved and it was possible to show that the original report was in fact based on *Dactylium dendroides* that occurs parasitically on *P. tomentosus.*

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9.3 Records of geographical distribution, host range and biodiversity

Reference material is also kept to show or confirm **geographical distribution patterns and host ranges**. It acts as a permanent record of the organisms found in a particular area. At the same time, a reference collection documents the **morphological variation** in a taxonomic group. A collection may be regarded as part of a country's national heritage.

10. Reference collections: Processing of material

A special area or workroom is needed where specimens can be processed for deposition in an herbarium or a reference collection. This entails the drying of cultures and other specimens, recording and labelling them, disinfesting them (of insects) and mounting them or packing them in special containers.

10.1 Equipment and other requirements

☞ **Work area:** This space must be separate from the area where the main collection is kept to prevent pests from entering the collection. Keep specimens in the workroom in an orderly fashion, properly marked, and with their field notes.

☞ **Grill or grid:** Used for drying large specimens such as mushrooms and bracket fungi over a heat source.

☞ **Heat source:** The best is a convection oven but other heat sources can be used as long as health and fire hazards are taken into account. Use an electric oven that can be set at low temperatures (50 °C) for small specimens. Otherwise an ordinary heater can be placed near the specimens (see Section 4.4).

☞ **Drying cabinet** with good ventilation (or with a vent to the outside) is required to dry specimens properly. This reduces the risk of air contamination in the laboratory and results in more controlled drying.

☞ **Airtight container** with silica-gel crystals and blue indicator crystals can be used for small specimens. Place the specimens in a container filled up to one third with silica gel, then fill it up with crystals and put the cover on. The specimens should be dry after 24–36 hours. When the indicator crystals have turned white, spread the silica gel in a flat pan and place it in an oven set to no more than 107 °C (225 °F). When the indicator crystasl have turned blue, the silica crystal are ready to be re-used after they have cooled down.

☞ **Jars for liquid storage** can be of glass or perspex. Ensure that containers have one flat side so that the specimens can be viewed without visual distortion. The containers should seal tightly.

☞ **Plant press** and sheets of absorbent paper such as blotting paper,

special botanical drying paper or old newspaper. The press can be of slatted wood or metal mesh and is kept compressed by weights or straps. If a plant press is not available, use an old telephone directory (see Section 4.4).

☞ **Water agar** (1 % tap water agar) is used to dry cultures on the back of Petri dishes. Alternatively use **wax, glue and a drying pan** of about 45 × 30 cm (or any other convenient size). The glue is made by dissolving 0.75 g agar in 200 ml water. Add 100 ml glycerine, 4 ml formalin and 4 ml commercial dishwashing liquid. This can be placed in a wash-bottle for application. **Muslin** cloth is used to cover the pan with wax and protects cultures from dust.

10.2 Processing different kinds of reference material

All reference material must:

☞ be of good quality;

☞ be insect-free;

☞ contain all the characteristic structures needed for correct identification;

☞ be sufficient in quantity and quality for future studies.

Cultures: Cultures for drying are grown on agar until they are sporulating and display all the important characters of the species. Preserve cultures of a fungus on various agar media before they stop sporulating owing to frequent subculturing. Cultures should not be contaminated, either by other fungi, bacteria or mites. Mite-infested cultures must be destroyed on detection to prevent the spread of contamination. Cultures can be dried in various ways:

☞ Cultures can be dried **on a smooth surface:** the culture is killed by placing a piece of filter paper soaked in a formalin solution with the culture in the test-tube or Petri dish for two days. A culture can also be killed by placing it, along with formalin, in a desiccator.

Afterwards the culture is removed from the dish or tube. Excess agar is trimmed off and only the superficial layer with the fungus colony is retained. Flood a smooth surface such as hardboard, ground glass or plastic with a thin layer of tap-water agar. Place the colony, surface facing upwards, on the semi-solid tap-water agar.

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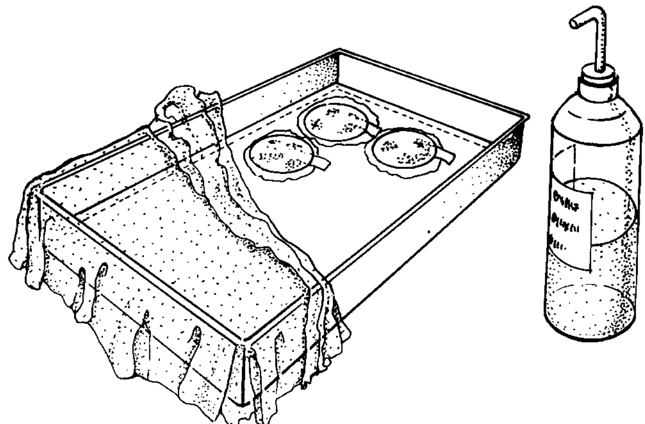
Leave the specimen at room temperature and humidity in a drying cabinet for 12–72 hours. When dry, the specimen can be easily peeled off the board. If the culture has buckled, it can be softened and straightened by placing it in a humid chamber for a few hours.

☞ Cultures can also be dried on the inside of **the lids of Petri dishes** in which they were grown. Plastic dishes must be used since cultures stick to glass when dry. Add 2.5 % glycerol to the tap-water agar: this gives a stronger base and renders the agar smoother and more pliable.

☞ Cultures can also be dried **on wax melted in a pan.** Melt wax and pour into a conveniently sized pan. Let the wax harden; the surface must be smooth.

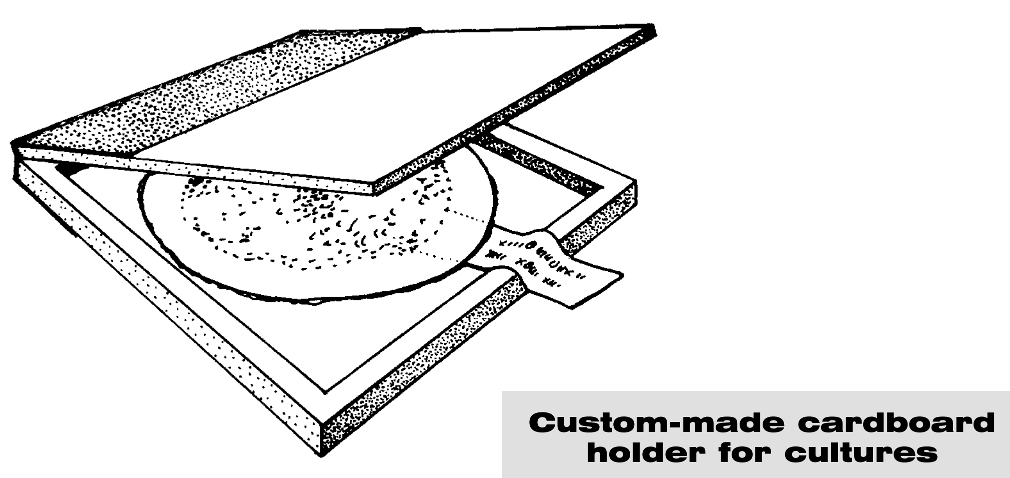
Cultures drying on wax in a pan



Place enough glue (see Section 10.1) on the surface of the wax to cover the area underneath the culture. Place the culture on top of this glue. Cover the edges of the culture carefully with the glue to prevent it from curling when dry.

Write the reference number of the culture in pencil on a small paper label of about 3.0 × 1.5 cm. Place a part of the label underneath the culture on the glue but with the number still visible on the edge. Position a grid, grill or any other suitable support across the pan and cover it with a muslin cloth to protect the culture from dust. Let the culture air-dry.

When dry, glue cultures into a flat cardboard holder. There should be enough free space above the colony so that no structures are damaged when the lid is closed. This gives a very compact form of dried reference material. If the back of the culture will require examination, it can be glued to the edge of a cardboard ring that snaps into the space in the cardboard holder.



Custom-made cardboard holder for cultures

Herbaceous plant material should be dried flat between sheets of absorbent paper. Use a botanical plant press (see Section 4.4), layers of newspaper or an old, thick book such as a telephone directory.

If material is dried flat, it:

- ☞ does not crush as easily;
- ☞ is more convenient to fit into envelopes for preservation in a reference collection; and
- ☞ is easier to examine with a dissection microscope.

Secure delicate specimens by cutting the stems or twigs in half. Specimens small enough to be placed in herbarium packets are first inserted into translucent paper envelopes.

Fruit-bodies and large specimens: Include only specimens that have been removed from the substratum without having been damaged. This means that their stem or attachment point must be intact. Dried mushrooms and bracket fungi have been successfully preserved for more than 140 years. Specimens can be air-dried, dried over a convection heater or in an oven. Large specimens can be cut in half to facilitate drying. When the climate is extremely humid, overgrowth of moulds can easily occur. In these cases it is better to dry them in an electric oven at 50 °C for 2–3 days. When completely dry, they can be placed in cardboard boxes. Large specimens can be trimmed to fit but in such a way that essential characters are still displayed. Very large specimens that cannot be trimmed or do not fit into the cardboard boxes are placed separately in cabinets to keep them dust-free. If fruit-bodies must be preserved in liquid, use 70–90 % alcohol, a formalin solution with or without glacial acetic acid, or various other solutions for preserving the colour of fungi. Formulae are

given in Hawksworth et al. (1995) and Johnston & Booth (1983). Make sure that all air bubbles are removed before sealing containers and that containers are adequately sealed and/or regularly topped up.

Fragile specimens may be wrapped in soft tissue-paper or glued or tied with linen tape onto blank index cards. They can then be placed inside containers that allow enough space around the specimens so that structures do not get damaged. They can be secured by glue, cord or linen tape. Fragile specimens should be stored in boxes in folded packets or kept in drawers in cupboards. Protective waxes (c. 5 × 5 × 0.5 cm) glued to cards or herbarium sheets are also useful for protecting material from damage caused by friction.

Microscope slides: Storing slides along with specimens facilitates subsequent examination and lessens the risk regarding the depletion of specimens. This is especially important for type or authenticated material.

- ☞ Microscope slides can be sealed with clear nail varnish to render them semi-permanent. Slides can also be prepared as described by Volkmann-Kohlmeyer and Kohlmeyer (1996).
- ☞ Store the slides inside protective cardboard boxes (see Section 4.5).

10.3

Mounting different kinds of reference material

Envelopes or paper capsules are glued onto mounting-boards. Let the glue dry. The following processed material can now be inserted into envelopes:

- ☞ specimens in translucent (glassine) packets,
- ☞ fragile specimens on cards,
- ☞ cultures glued into culture boxes, and
- ☞ microscope slides in slide boxes.

Often more than one envelope is necessary to accommodate all the material, or the various parts of one specimen. Six to eight packets, depending on size, may be glued to each mounting-board. Additional boards should contain the same information as the original, but reference must be made on each duplicate board to the existence of the others.

Specimens of the same species but from different hosts or localities (these should all have a separate unique accession number) are attached to separate mounting-boards. In other words, a mounting-board should have only material with one accession number on it.

Attach data labels to all the mounting-boards (if not pre-printed on the boards) and fill in the information.

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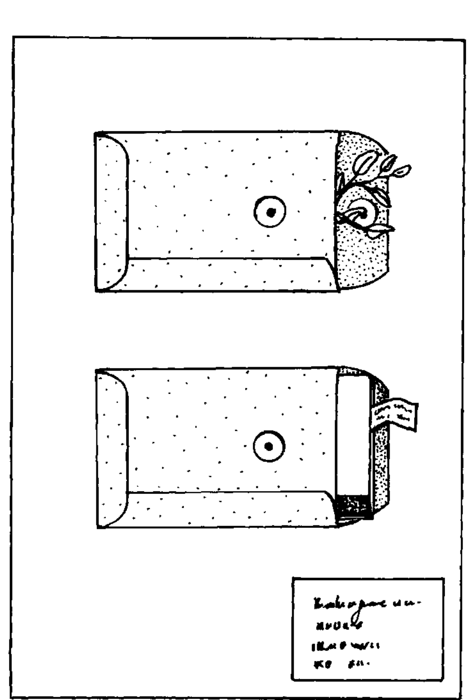
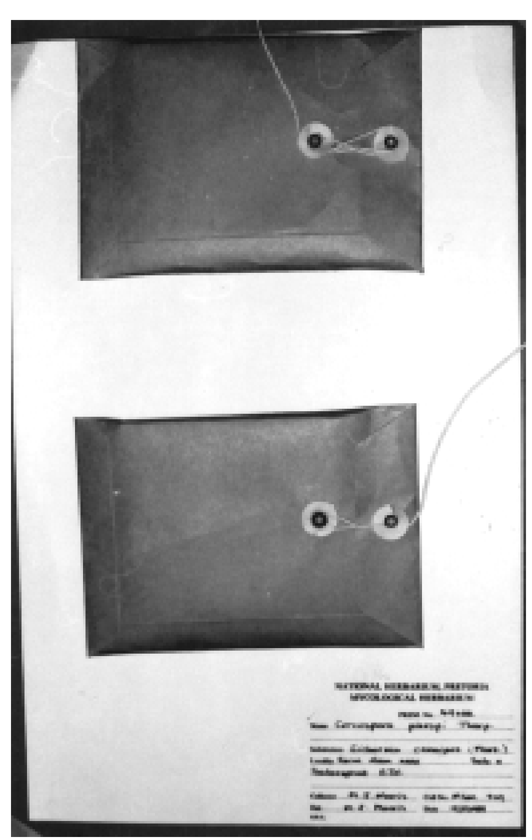
Mounting-board with envelopes containing material





Example of a mounting-board label

G.P.-S. 020-0326 BLW 14/31

NATIONAL COLLECTION OF FUNGI
PRETORIA

PREM No. ....

Name ....

....

Substratum ....

....

Locality ....

....

....

Collector .... Coll. No. ....

Det. .... Date ....

I.D. confirmed by ....

10.4

Materials for the mounting of specimens

Materials for use in the collection should ideally be of archival quality, or the best that can be afforded. Always maintain stocks, and order samples well in advance.

Inks are used on labels, genus covers, species covers and type covers. Inks must be permanent and preferably waterproof or water-resistant. Choose black: other colours should be avoided. Disposable pens with water-resistant and spirit-resistant ink and with various nib sizes are available. Some inks will fade if exposed to light. Black waterproof inks will usually be light-resistant. Ink for stamp pads should also be of permanent quality.

Pencils with medium to soft lead, such as HB, B, 2B or F, should be used. Handwriting in too hard a pencil is difficult to erase, and too soft pencils result in smudging.

Glues are used for mounting specimens, attaching labels, envelopes and photographs etc.

- ☞ Use a permanent adhesive, but one that can be removed if necessary.
- ☞ Wood-working adhesive (PVA — polyvinyl acetate) or gum-arabicum are recommended and are long-lasting, opaque but translucent when dry and easy to apply.
- ☞ Avoid sticky tape because it becomes brittle and stains with age.

String and thread are used to tie parcels, secure bundles for transit and storage, and reinforce the mounting of bulky specimens.

- ☞ Use strong, smooth, medium-weight string that will retain the correct tension, or strong linen thread, such as used by bookbinders.

Mounting-boards or specimen sheets are used to permanently support specimens in the collection:

- ☞ The best white or cream cartridge-type paper available must be used. Paper with all the lignin removed and then bleached for good colour (wood-free) or paper made from cotton, linen or hemp (100 % rag) is recommended. Mechanical wood or ground wood-pulp is not recommended as it becomes acidic, discolours and deteriorates. Sulphur-free paper is recommended if an insecticide containing mercury is to be used, as mercury will react with sulphur and blacken the mounting-sheets.
- ☞ A 'matt' surface, known as 'NOT', allows glues to stick better.
- ☞ The paper must have the correct thickness or weight. As a simple test, it should be possible to hold a sheet of paper horizontally at one end without warping.

Contents

☞ Labels can be printed on the boards beforehand (see Section 10.3).

☞ A size A3 is ideal for mounting-board but it is most important that they should fit easily into the storage cabinets.

Genus covers are used to group specimens of one genus and to protect the specimens. Subgroups of species covers are placed within them.

☞ Use acid-free thin card or thick paper cut with grain running lengthwise. When folded the size of the file should be somewhat larger than the mounting sheet. The better the quality the easier it will fold.

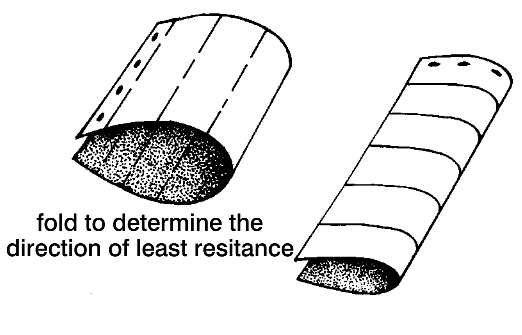
☞ To test the grain of paper:

- Take a few sheets of paper and lightly bend across the length and across the width — **more resistance will be felt when the grain runs at right-angles to the fold**.
- Tear the corner of a piece of paper in both directions. **The tear made in the direction of the grain will be straighter and more easily made**.
- Place a small piece of paper on a damp surface. The paper will **curl at right angles to the direction of the grain**.

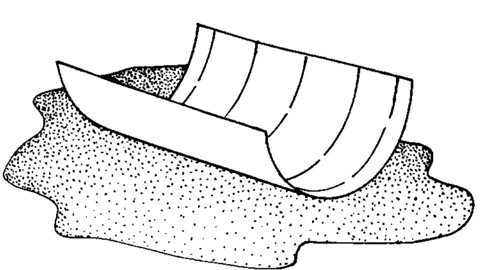
☞ The colour should be buff or some other neutral colour.

☞ Genus covers can be re-used if the information has been entered incorrectly, by turning them back-to-front or upside-down.

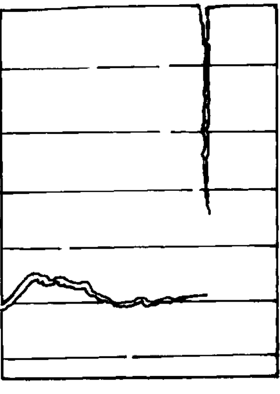
Testing the grain of paper



fold to determine the direction of least resitance



curls at right angle to the grain on damp surface



tears more easily in the direction of the grain

Species covers are used to **group and protect specimens of the same species in the collection**. A species cover containing specimens is placed inside the genus cover.

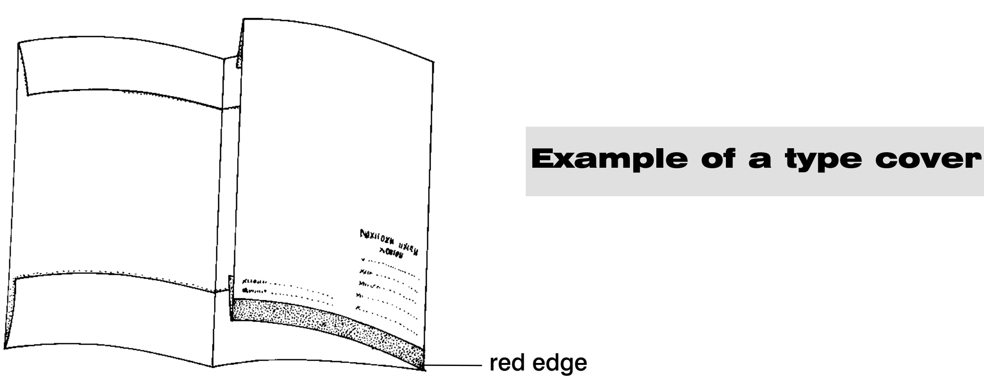
☞ Use light-weight, acid-free paper with the grain running lengthwise (before folding it). When folded in half, they should be slightly larger than the mounting boards.

Species file inserted into a genus file

Type covers are used to protect type specimens, retain any fragment that may fall off them, and draw attention to the specimen.

☞ Use acid-free, strong paper slightly larger than a mounting-board. Type covers should, when folded, overlap at all four edges. The top and bottom edges need only be about 3 cm wide; the left-hand overlap should entirely cover the specimen, while the right-hand cover (outside) overlap could be 10 cm or more wide. This top half flap should contain the relevant information about the specimen and have the word 'TYPE' and the name of the collections printed on it.

☞ The colour of these covers should be in contrast with that of the genus and species covers, or have top and bottom edges printed with bands of a bright colour such as red.



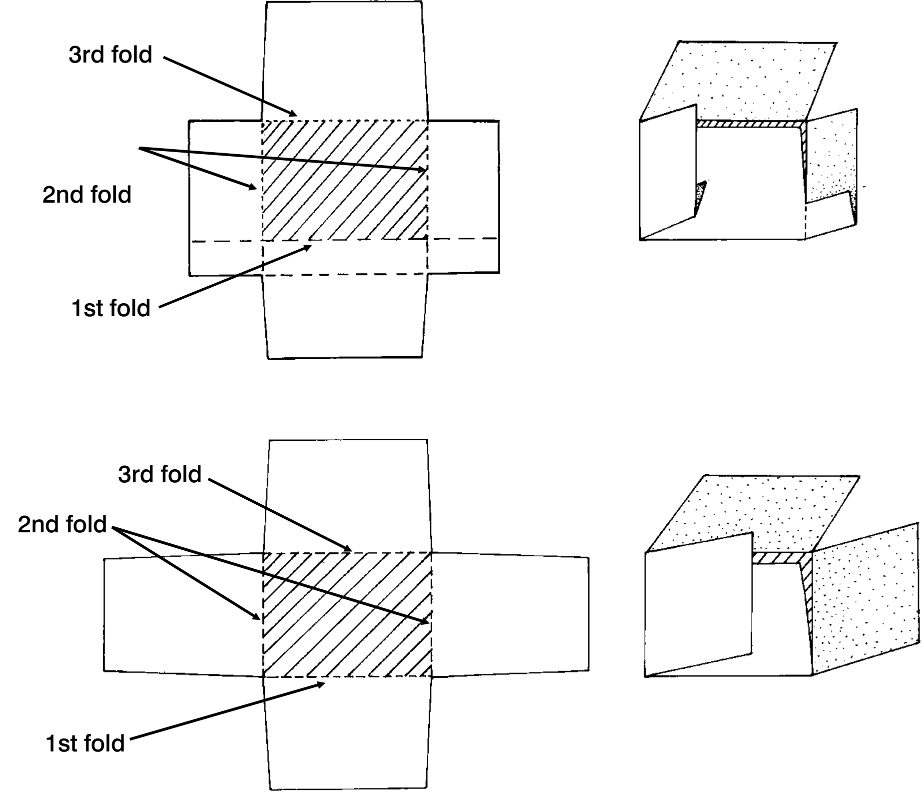
Example of a type cover

Contents

Envelopes or paper capsules are glued to mounting-boards to contain and protect small portions of the specimen, or very small specimens.

- ☞ Use acid-free white or buff-coloured paper.
- ☞ Paper envelopes of various styles and sizes can be used but when folded and glued to the mounting-board they should open flat, and stay closed without the aid of a paper-clip, and not allow small portions of material to escape from the bottom corners. The recommended size is 15 × 10.5 cm. They can be bought, or folded from rectangular pieces of paper such as a sheet from a acid-free writing pad. Do not fold the sides in underneath them because opening these could damage delicate structures. Paper-clips can be used to secure them. Plastic continual-loop or copper clips are best. Do not use ordinary office clips because they rust and stain the paper. Conventional envelopes are not recommended because they do not

Different methods of folding envelopes



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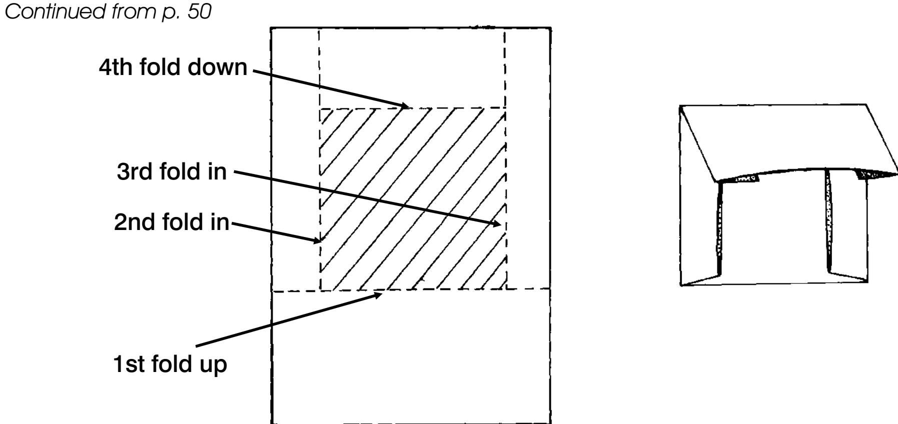


open flat. Material removed from them can be damaged. Strong envelopes of good quality paper can, however, be glued onto mounting-boards and used in flat boxes containing dried cultures.

Specimen boxes are used for large specimens, fruit-bodies, fragile specimens and dried cultures. These boxes should be of strong, thin (1.2 mm thick) cardboard and should not collapse or have the bottom drop open when handled. Ideally they should be made from a single piece of cardboard, folded and stitched together. Boxes of various sizes should be obtained as needed, and must fit into the cabinets used for the collection. Large boxes of 430 × 275 × 125 mm are convenient and can hold several smaller boxes, or the specimens directly. Flat cardboard boxes are used for microscope slides 91 × 41 × 8 mm and boxes of 115 × 112 × 5 mm for dried cultures.

Translucent packets, also called glassine packets, are used to store small specimens such as seeds, or when small specimens are forwarded on loan. They should have a sealable flap. Avoid cellophane packets since they are not long-lasting, as well as polythene bags, which cannot be written on and also generate static electricity that makes specimens cling to them.

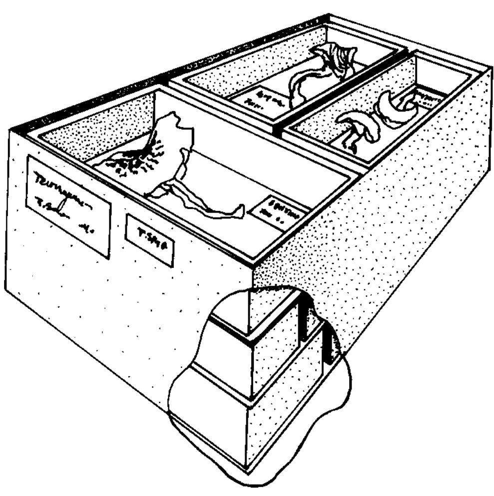
Labels are important because without the information on them, specimens are of little use. The information on labels is dependent upon the amount of detail provided by the collector.

☞ **Design and preparation:**

- Labels can be pre-designed and printed on mounting-boards or on separate pieces of paper.
- Use paper of good quality, unglazed and acid-free if separate labels are printed.

Contents

Boxes with specimen boxes packed into them



- Use a typewriter or computer to print information on the labels if possible. Printed labels are easier to read than handwritten ones. If handwritten, use permanent black ink.
- Duplicate labels can be photocopied, carbon copies are often of poor quality.

Contents

☞ **Types of labels:**

Data labels are attached to each specimen and must contain at least the following:

- the name of the collection;
- accession number;
- scientific name of the organism and author citation;
- collection number or collector's number;
- name of host or substratum and/or the culture medium (if applicable);
- locality where collected in as much detail as possible, e.g.: country, province, farm, district, town, grid reference, latitude and longitude and altitude (if available);
- habitat / ecological notes;
- date of collection;
- collector's name (not necessarily the same as the donor's).

- **Determinavit and confirmativit labels** indicate that a specimen has been referred to in a special research project.
- **Pieces** removed for anatomical or chemical studies can also be indicated on labels.
- **Type labels** indicate that the specimen is a type of the name shown on the label e.g. Holotype, Isotype.
- **Other: Genus, family, geographical regions, reference to literature**, can also be indicated by labels and can be colour-coded.

All labels can be pre-gummed. It is preferable, however, to have pre-designed labels printed on the mounting-boards. If the sample is large it is often possible to divide it into a number of duplicate packets. The label on each duplicate packet bears the same information and number as the packet from which it was taken, with reference to the existence of the other packets. These can be exchanged for named specimens in other collections.

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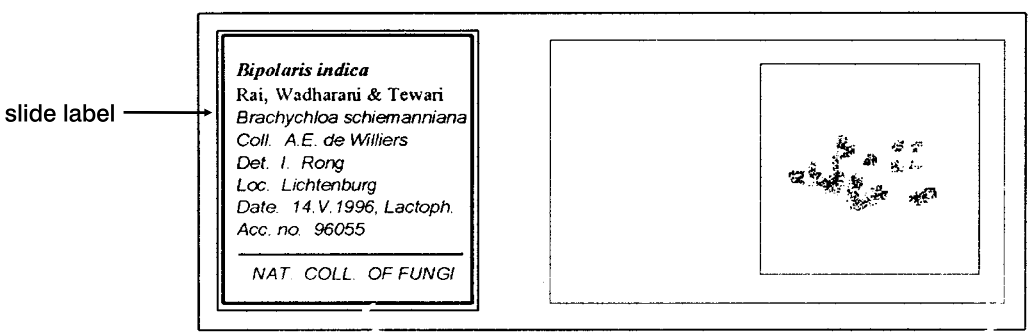
Different types of labels



labels that may be affixed to envelopes

DET .... 19 ....

Mycobiota of Gauteng

Det. Date

HERBARIUM PREM

Drawing prepared for "Bioscience"
Vol. , No. ( ).

Verified
E.M. Doidge 1941

Seen B.C. Sutton
for "The Coelomycetes:

11. Reference collections: Data storage and retrieval

The way in which data is dealt with in the South African National Collection of Fungi is outlined here. Although systems vary from one collection to another, all share important principles. Specimens and their data, for instance, must be easily retrievable. For this reason, each specimen should be given its own reference number (e.g. **PREM 5001**), the accession number.

Conventionally, information is written in an accession register, in fungi and host indexes, and on labels on the specimens themselves. Computers are also increasingly being used for data storage and retrieval.

11.1 Accession registers

The register itself can be a strong ledger or bound book which is stored safely in the herbarium.

When newly entered, the name of the fungus is recorded in the **specimen register** against a unique number. This number, as well as the collection's international symbol (or acronym), must be noted in publications, e.g. PREM 5001. PREM is the acronym for dried specimens in the National Collection of Fungi, South Africa (**Pre**toria, **M**ycology). Other well-known international collections are the IMI (**I**nternational **M**ycological **I**nstitute, United Kingdom), the culture collection of the CBS (**C**entraal **B**ureau voor **S**chimmelcultures, in The Netherlands) and ATCC (**A**merican **T**ype **C**ulture **C**ollection) in the United States of America.

Entries must be in numerical order (e.g. PREM 50 001, 50 002 etc.), in sequence of acquisition. The number against which a fungus is recorded in the register is the permanent specimen number. The international code for the collection e.g. 'PREM' is written before the number: PREM 50 001 when used in letters, reports or publications.

Allocate different numbers for specimens:

- ☞ from different / distinct geographical localities;
- ☞ collected at different dates but from the same locality and substrate;
- ☞ collected by the same person;

☞ with more than one fungus on the same material, but give consecutive reference numbers to the different fungi present.

Allocate the same number to parts of specimens comprising more than one object, for example the original leaves from which a fungus was isolated, the dried culture and a microscope slide.

The data for each specimen are noted in a register next to its unique reference number. The reference number usually appears in the extreme leftmost column of the page, with the rest of the information following it. These data can be arranged in various formats but should include the collector, the locality, the hosts or substrate, the person who identified it and an accession date.

11.2

Card index of fungi

In the card index of fungi the fungus genera are alphabetically arranged, and the species are in alphabetical order in each genus.

☞ If the fungus is of a **species already represented** in the collection, enter the number and details of the new specimen on the existing species card in the index of fungus names.

☞ **If the existing species card is full**, start a new one, writing the full name of the fungus and its author/s on the top line. Number the new card '2', or whatever the number should be in the sequence, in the top right-hand corner.

☞ Maintain continuity when numbering the list of records: i.e. do not start with '1' on the new card, but continue numbering from where the list of records ended on the previous card. Card 2 is placed in front of card 1 in the card index, and so on.

☞ If the fungus **species has not yet been recorded in the collection,** prepare a new card with the full name of the fungus and its author/s on the top line, record the necessary details on it and place it in alphabetical order behind the correct genus card in the card index.

☞ In the case of a Type specimen, write a small 'T' or 'Type' above or to the left of the reference number.

☞ If the **genus has not yet been recorded in the collection:**

- Prepare a new shoulder card (used to separate different genera) for the genus and a new index card for the species. Place the genus card in the correct position (alphabetically) in the card index and the species card behind it.
- If a taxonomic system is used, the **genus must be given a number** according to its family. If the genus is not listed in

Contents

Cabinets containing card indexes of fungi and hosts

the collection list of numbered families and genera, consult 'The Dictionary of the Fungi' or other relevant literature, or the curator or specialist mycologist concerned to find the most appropriate placing. **NB:** Add the genus name and family number to the collection list.

- **Multiple specimens:** Enter data on the cards in the same way as for the specimen sheets.

11.3 Card index of hosts

The card index of hosts / substrates is an index in which hosts or substrates are alphabetically arranged in the same way as in the index of genera and species of fungi:

☞ If the fungus occurs on a host plant or part of a host plant (e.g. seeds), enter information regarding the fungus on the appropriate host card.

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Use the scientific name; cross-reference common names.

☞ Substrates other than plants (e.g. soil, manure, insects) are also recorded in this index.

☞ If the existing host / substrate card is full, start a new one as described for a species card.

☞ When the host or substrate has not yet been recorded in the collection, complete a new card (as described for species and genera cards), and place in alphabetical order.

Note: Specimens mounted on sheets are filed in cabinets after their collection details have been entered in the register and card indexes.

11.4

Computer database

A computer database works like any other data indexes such as described above. Another example is a telephone directory. In a telephone directory, the name and address of a person can be traced if the telephone number is known. This information, however, is available only after one has paged through many records listed in alphabetical order. Computers can do this type of searching much more efficiently. In addition, data can be retrieved much more readily than with card indexes if the computer database is configured correctly.

☞ **Computers can be used to:**

- Record incoming material.
- Prepare labels.
- Retrieve data such as a list of the species in a specific genus or family or species from a specific location or host etc.
- Manage loans.

☞ **Set up a computer system by:**

- Planning the database before buying any equipment. One must know what information will be extracted from the database.
- Use a personal computer instead of mainframe computer or mini-computer. They are by far the best option for small or medium-sized herbaria or for those with a limited budget.

- Carefully select the computer program or software for the database, considering aspects such as data security, ease of use and ability of users.

☞ **Disadvantages are:**

- Initial as well as subsequent maintenance costs are high. The database can only be used once all specimens in the collection have been entered.
- Errors occur with data entry and the information entered must be checked by a specialist.
- The software must often be written by the staff themselves.
- A steady supply of electricity is needed. This can be overcome by the addition of an unit that will give an uninterrupted power supply.
- A strict back-up programme must be followed to allow for any loss of information due to software and human errors, hardware failure etc. Regular printouts must be made.
- Computer databases are just as good as the data that are entered into them. Variation must be avoided. For example dates can be written in many ways: 12/06/1996; 12th June 1996; 06/25/96; 25/vi/1996. Information must be entered into the database in a standard way.

12. Arrangement of specimens in a collection

The arrangement of a collection is a matter of choice. Arrangement can be alphabetical or according to taxonomic groups. A combination of both can also be used. Many fungal collections are still arranged according the taxonomic groupings of Saccardo. Within this system genera and species are arranged alphabetically. Some larger collections also have geographical groupings in addition to the taxonomic arrangement.

12.1 Alphabetical arrangement

It has the advantage that it is easy for non-specialists to maintain. The disadvantages are that related and similar groups are placed far apart so that identification by matching becomes difficult. Filing errors are more easily made by unskilled personnel, or also occur when specimens are filed under old names.

12.2 Taxonomic arrangement

It has the advantage of similar groups being placed together but the disadvantage is that specialist knowledge is required to maintain it. It is also labour-intensive to implement major systematic changes. Classes and families are arranged in an evolutionary order ('phylogenetically'), from the 'lowest' to the 'highest'. The families are numbered, from No. 1 onwards, and arranged numerically in the cabinets.

12.3 Geographical arrangement

This depends largely on the size of the collection, the area it serves and the diversity of the area. Such an arrangement helps with the naming of specimens within a certain area. It is not a practicable arrangement for smaller collections.

Foreign specimens are often separated from local ones within a genus, particularly in large genera. They are placed after the local material in separate genus files. Mark these with an 'F' before the genus name.

12.4
Special collections

Special collections must have their own individual index or register.

These collections can be:

- ☞ **Historical** and are kept separate because specimens are fragile or of special interest to a specialist. Historical collections should be arranged in their original sequence.
- ☞ **Type collections** are irreplaceable and need special curation. When kept separately, they can easily be removed in a crisis and will be handled less often. Place type specimens in type covers with their labels. For each type specimen place a sheet in the species cover referring to it in the general collection. In smaller collections filing types separately is impractical — they are then filed first in the relevant species cover.
- ☞ **Spirit collections** should be kept in separate cabinets but near the main collection. The best arrangement is by consecutive numbering, with a series of reference numbers reserved for each size of jar. Jars of similar size are stored together. Sheets in species covers should contain the reference numbers to these jars.
- ☞ **Photographs, illustrations and microscope slides** can be mounted on similar mounting-boards as part of the main collection. The arrangement of these should follow the arrangement of the main collection. Large specimens or microscope slides, if kept separately, must be cross-referenced to the specimen sheet, as done for types or large specimens in boxes.

12.5
Horizontal versus vertical storage

Horizontal arrangement of mounting-boards keeps the specimens secure to the mounting-board. When cabinets are too full, the weight of other, heavier specimens on top may damage specimens at the bottom. Upright storage can be implemented by placing envelopes in boxes that slide into cabinets. This method uses less material for mounting, but specimens tend to slide to the bottom of paper envelopes, and this can result in damage to specimens.

13. Filing of specimens and loans

Specimens that have been entered into the register, mounted and put into boxes and whose details have been entered on the appropriate fungus and substrate index cards (as set out above), may be filed.

13.1 Numerical order

Specimen sheets are arranged in numerical order, with the highest reference number, or the most recent specimen, on top. When the specimen is of a species already represented in the collection, place it with the other specimens of that species in the correct folder.

- ☞ Place larger specimens, in their boxes, in the cardboard container in the correct position (i.e. close to where the specimens sheets are placed) in the cabinet. Add their reference numbers to the list in the cardboard container. Note on the specimen sheet ‘Specimen is in box’ and file the accompanying specimen sheet in the usual way.
- ☞ If the specimen is of a **species not yet represented in the collection,** clearly print the name in ink on the outside, lower right-hand corner of a species folder (**opening to the right**), e.g. *‘Alternaria alternata’.* Place the specimen sheet in the species cover and then file it in the correct alphabetical position in the appropriate genus folder (**opening to the left**).
- ☞ If the specimen is of a **genus not yet represented in the collection,** clearly print the generic name in ink in capital letters in the lower right-hand corner of a genus folder, and the family number in the lower left-hand corner (if a taxonomic arrangement is followed). Place the specimen, in its species folder, inside the genus folder and file it in alphabetical order with the other genera in its family number.
- ☞ **Type specimens** (if kept in a separate collection): Place the specimen sheet in a Type species folder (with a red border) and then file it in the Type collection. Genera are alphabetically arranged and species are in alphabetical order within each genus. Also file a specimen sheet in the species cover with a note: ‘Specimen in Type collection’.

Specimen cabinet

13.2
Multiple specimens

When **more than one fungus** has been identified in a sample, insert the separately numbered specimen sheets for each fungus in each of the respective species folders.

Additional information, material or literature relevant to a specimen and which may be of use or interest to future research, may be filed with the specimen in question. This could include field notes, notes on pathogenicity or toxicity, drawings or photographs, reprints, or references to publications in which the specimen has been cited.

13.3

Loans to and from other institutions

International conventions

There are two kinds of people, a famous writer once claimed: those who borrow and those who lend. Staff of biosystematic reference collections need efficient systems to deal with both. Why?

A reference collection is associated with professional taxonomists. This means that it will include specimens that have been mentioned in research publications. Researchers preserve examples of the organisms involved in their work because one of the basic principles of scientific research is that it must be repeatable. To ensure repeatability, such specimens — known as reference or voucher specimens — must be:

☞ publicly and internationally available for study to all researchers, both local and foreign, and permanently safeguarded in a collection in a recognised institution.

Scientists from various institutions may wish to examine these reference specimens. It may not be possible for them to visit the collection themselves, so they will ask for the material to be sent to them on loan. For similar reasons, taxonomists in your organisation may wish to borrow study material from other collections.

Taxonomists worldwide accept that they:

☞ will be allowed to borrow reference specimens, free of charge;

☞ must treat borrowed material with care; and that they

☞ are expected to comply with the instructions they receive.

Institutions need to protect and keep track of specimens sent out on loan, so it is important for them to keep careful records. There are many aspects to consider: some of these are mentioned in the following sections.

Regulations regarding loan policy

You may believe the saying that the only golden rule is that there are no golden rules. In a reference collection, however, there is certainly one: no reference specimen can be replaced. Because each specimen is unique, all major institutions have instructions or guidelines to protect material that is sent out on loan. The most important points concern time limits, safety and conservation.

☞ **Duration of loan:** This can range from three to six or twelve months. Borrowers should be informed of the following:

- the loan period;
- that, if they need the material for a longer time, they must ask for an extension in writing (letter, fax, e-mail) before the loan period expires, and that they will receive a written reply. Extensions are usually granted unless the material is urgently needed by another researcher.

☞ **Use: restrictions and conditions:** Each specimen is limited by its size and quality, so it should be used non-destructively or, at least, conservatively.

When pieces must be taken for slides, analysis or electron microscopy, the:

☞ minimum material should be used, and

☞ any slides or photographs that result must be preserved with the rest of the specimen for future use. This will obviate the need for repeated removal of parts of the material. Some specimens may already be too depleted to allow certain methods of study, or even to allow it to be sent on loan.

Institutions usually also ask for a reprint of any publications that result from a loan, in exchange for the use of the material.

If a change of scientific name is recommended, the borrowing taxonomist should indicate this on a small piece of paper on which his or her name and signature are written, and the date. (This is a 'det.-slip': short for 'determinator's slip of paper').

☞ **Return:** Loan instructions usually ask that material be returned by airmail, and that the original packaging material be used. Fast delivery services limit the time that specimens spend outside the protection of a research laboratory, and this limits possible damage.

Many institutions also request that parcels should be registered, to reduce the possibility of loss.

13.4

Selecting material

Ideally the whole specimen should be sent. Because of the real danger of possible loss in transit, however, or non-return for other reasons, most institutions send only a part. This part must be representative of the whole specimen, and a photocopy of the original label and any notes should be included. There is one problem though: if the material actually represents a mixture of taxa and only one of these is included in the loan, confusion can result.

13.5

Documentation

Details of each specimen sent out on loan must be carefully recorded. Institutions usually have at least:

☞ a loan register or computerised record, and

☞ loan forms.

The responsible parties

A loan is an official undertaking, not between individual researchers but between institutions:

☞ The Curator must approve all loans.

☞ All correspondence should therefore be sent from the Curator of the lending institution, and addressed to the Curator of the borrowing collection for the researcher who needs the material.

The institution that borrows material must undertake to ensure its safety; in other words, to see that it is:

☞ carefully safeguarded, for example from insect attack,

☞ handled with care, and

☞ promptly returned.

Loan register

A separate book or computerised record system is used to list:

☞ each specimen sent on loan, with its name and reference number;

☞ the name and address of the person who borrowed it;

☞ the date when it was sent;

Contents

- ☞ the date on which the person who borrowed the material received it;
- ☞ the date on which it was returned, and
- ☞ whether all the material was returned in good order.

Some collections keep a central record of incoming loans as well as of outgoing ones. This is useful as it encourages the rapid return of material borrowed from other institutions.

Loan forms

At least two copies are made of a loan form with:

- ☞ Details of the contents of the loan: list each specimen,
- ☞ the full name and address of the borrower,
- ☞ the date sent,
- ☞ the duration of the loan period, as well as
- ☞ space where the borrower can enter:
    - The date on which the material arrived, and whether it arrived in good condition. The borrower should be asked to supply this information on one copy of the form as soon as the loan has been received;
    - the date on which it was returned. The borrower should be asked to return this copy of the form separately from the specimens.

Loan notices

In the collection itself, in the place of a borrowed specimen, a note should be placed to show that the specimen, or part of it, has been sent out on loan. This note should be left there until the material has been returned.

13.6

Packaging

All material must be securely packed so that it cannot be easily damaged by the rough handling that it could receive in transit.

Specimens should be protected from the effects of:

- ☞ pressure: to prevent crushing, pack them in a rigid but lightweight box preferably made of corrugated cardboard;
- ☞ friction: to prevent specimens from rubbing against one another, pack them securely, supporting them with soft wadding or foam sheets in the spaces between and around them;

- ☞ damp: wrap the contents of a package in waterproof polythene material before wrapping the parcel.

Finally, every parcel must be strongly wrapped, securely tied with string and clearly labelled, in accordance with standard postal instructions, including:

- ☞ the name and address of the sender, and
- ☞ the name and address of the recipient, as well as
- ☞ a description of the contents: for example, ‘Dried reference specimens. Of no commercial value: for scientific study only’.

14. Pest control in collections

14.1 Strategy

Any organism capable of damaging reference material can be judged to be a pest. Rats, mice, mites and fungi are common culprits, but insects usually cause the most problems.

In the past, collection managers attempted to eliminate pests with toxic chemicals. Their approach was one of total pest eradication. There is a greater awareness now, however, of the damaging effects that pesticides have on the environment and on human health. Researchers have explored alternative ways of control and found that it is best to combine various methods into a pest management strategy.

A four-pronged pest control strategy is the best.

- ☞ House the collections in a building especially designed for the purpose.
- ☞ Be aware of known pests and regularly monitor the building for them: remove any infestation as soon as it is detected.
- ☞ Allow only uncontaminated material into the collection room.
- ☞ Fumigate at least once a year.

Each institution should **appoint a pest control coordinator** to regularly monitor the collection. The **duties** of such a person should include:

- ☞ collecting and identifying pests;
- ☞ detecting and recording infestations and counter-measures;
- ☞ ensuring safe, effective use of insecticides and repellents;
- ☞ keeping records of the poisons in use and having information available on first-aid treatment.

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14.2 Common pest insects in temperate regions (Hall, 1987)

Cigarette beetles (tobacco beetles)

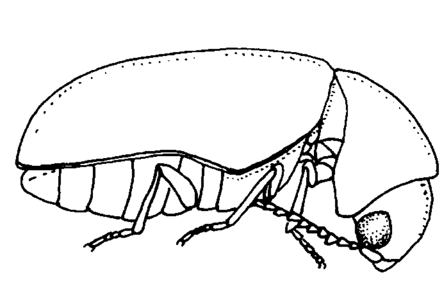
Adults brown, 2–3 mm long, head and front parts strongly curved downwards; larvae curved into a C-shape, up to 4 mm long, hairy but without long tail hairs, head pale brown.

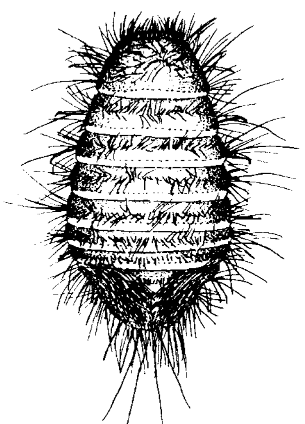
☞ **Notes:**

This is the major pest in herbaria, especially the larval stage. Larvae feed on a wide range of materials and severely damage herbarium specimens and books. They thrive in dried vegetable matter and are able to absorb moisture from air with a relative humidity as low as 43 % . Adults are attracted to light. They are strong fliers and are usually active in the late afternoon and on cloudy days, leaving and entering buildings through open windows.

☞ **Control:**

Fumigation; silica aerogel insecticidal dust; sticky traps; spray incoming fresh material with activated pyrethrin aerosol; place fine mesh screens over windows, doors and ventilation ducts to exclude adults; good housekeeping.





Cigarette beetle (tobacco beetle) with larva

Dermestids (carpet beetles, especially the varied carpet beetle)

Adults mostly black with various markings, 2–3 mm long, head and front parts not strongly curved downwards; **larvae** not curved or C-shaped; up to 6 mm long, densely hairy and generally with long tail bristles.

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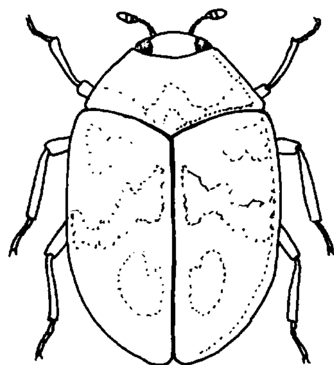

Dermestid (carpet beetle)

☞ **Notes:**

They can severely damage herbarium specimens and book bindings. The larval stage of the life cycle is the most destructive because it is relatively long: 200–300 (up to 600) days. In herbaria the chief problem species is *Anthrenus verbasci,* which has a preference for daisies (Asteraceae) and blue or white flowers; it also occurs in birds' nests where it feeds on feathers, food scraps and excrement.

☞ **Control:**

Fumigation; spray incoming fresh specimens with activated pyrethrin aerosol; freeze or microwave incoming material; remove potential sources or attractants from the vicinity of the building; good housekeeping.

Fishmoth (silverfish) and their allies

Greyish, wingless, fast-moving creatures, 11–18 mm long, tapering to the rear where there are 3 long tail bristles.

☞ **Notes:**

Fishmoth and firebrats hide in cracks or between undisturbed papers during the day and are active at night, feeding on paper and starch, eating through specimen labels to reach the glue, and damaging index cards, book bindings and photographs. They like damp places because they can meet their water requirements by absorbing moisture from the air (firebrats do this down to 15 % relative humidity). They also occur in birds' and insect nests, and under bark on trees.

☞ **Control:**

Barium fluorosilicate baited with starch and sugar, and fixed with glue, painted onto wooden shelving where it is has a prolonged action;

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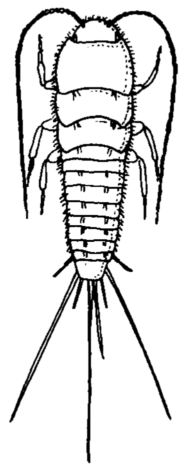
Fishmoth (silverfish)



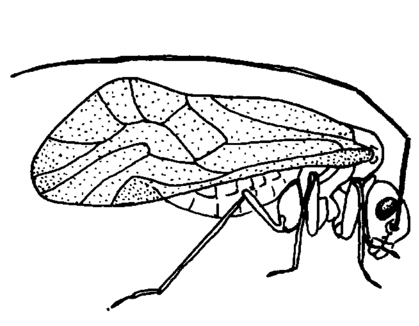
dust storage areas with silica aerogel; reduce relative humidity to less than 40 %; remove potential sources or attractants from the vicinity of the building; good housekeeping.

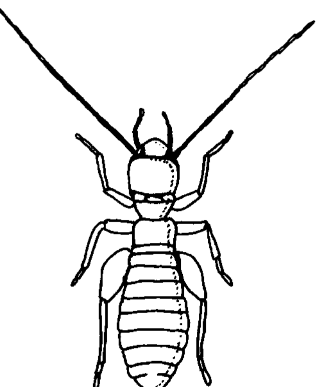
Psocids (booklice)

Pale, dull brown or whitish, soft-bodied, wingless and tiny but fast-moving: 1–2 mm long.

☞ **Notes:**

They are said to feed on fungi, but they damage glues, for instance in book-bindings, and damp or mouldy paper, as well as dried herbarium specimens, particularly Asteraceae, Brassicaceae and petaloid monocotyledons. They avoid well-lit areas and thrive in warm, damp conditions. They absorb water vapour through their





Psocids (booklice)

outer covering and move away when the relative humidity is too low. In nature they thrive in moist piles of plant debris.

☞ **Control — Difficult!**

Restrict humidity and dampness, e.g. reduce relative humidity to below 60 %; fumigation followed by prevention of entry; freeze or microwave all incoming material; spray with pyrethrin aerosol; use naphthalene (mothballs) as a cabinet fumigant; eliminate sources near the building.

14.3 Clean-entry policy: decontamination methods

The best way to deal with pests is to prevent them from getting in. Any building housing a collection should be designed to stop pests from easily gaining entry or finding breeding places (see section on housing of collections). Further, only clean, uncontaminated material should be allowed in. Newly collected specimens as well as in-coming parcels must be disinfested.

There are various ways to disinfest material.

Deep freezing

Dried material is frozen at about –18 °C, but preferably down to –27 °C, in a standard, large, domestic chest refrigerator.

Method:

- ☞ place thoroughly dried material in bundles of not more than 15 cm thick;
- ☞ preferably enclose each bundle in a sealed polythene bag;
- ☞ freeze as rapidly as possible: some insects can increase their cold resistance fast enough to survive if the temperature drops slowly;
- ☞ freeze for 48 hours;
- ☞ on re-warming, circulate dry air around the parcels to prevent condensation, for instance in a drying cabinet, especially if they are unwrapped;
- ☞ label the material with a note of the time for which it was frozen;
- ☞ for resistant pests such as dermestid beetles, follow the above steps and then freeze the material again.

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- ☞ **Good points:** Non-toxic.
- ☞ **Bad points:** Relatively time-consuming; dampness may occur as a result of condensation upon thawing if suitable precautions are not taken, and this encourages growth of fungi.

Fumigants

Fumigation has been replaced by freezing in most institutions because it is a cheaper and safer method of disinfesting incoming specimens.

- ☞ **Good points:** Fumigation with a deeply penetrating poison, such as methyl bromide or ethylene oxide, is an effective annual defence against insect infestations in humid areas.
- ☞ **Bad points:** Highly toxic; may damage sensitive materials, such as fastenings and leather bindings, and cause acid-catalysed decay of certain kinds of paper.

Note: Do not use

- ☞ Gamma-BHC or Lindane in contact with paper or specimens: it produces hydrochloric acid as a breakdown product and this damages paper and specimens.
- ☞ Phosphine or chlorpyrifos corrode copper and can render electrical fittings unsafe.

Insecticides

Domestic insecticides with rapid killing properties, such as activated pyrethrin, may be used on fresh specimens.

Method:

- ☞ place material in an enclosed area, such as a fume cupboard with a duct leading to the exterior of the building, or in a large cardboard box in a well-ventilated place;
- ☞ spray.

- ☞ **Good points:** Effective for rapid knock-down; stops dermestid beetles from infesting dense material such as inflorescences of Asteraceae.

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☞ **Bad points:** Toxic; some people become allergic to pyrethrins.

Note: There should be an extractor fan to extract the fumes from the building.

Microwaves

Domestic units kill pests and their eggs by heating the water and oils in their tissues to lethal levels.

Method:

☞ microwave the material on the highest setting and

☞ according to thickness, for instance for packs of mounting sheets:

- up to 50 mm: 75 seconds;
- up to 90 mm: 95 seconds;
- up to 150 mm: 120 seconds.

☞ **Good points:** Very quick, so it can be used when a visitor arrives with a specimen for immediate identification.

☞ **Bad points:** In fungi, important cell properties may be damaged; in plants, seeds are killed; steam from residual water can cause structures in the specimen to split; metal pins or clips may become overheated and cause damage.

14.4 Fumigants and insecticides used in specimen cabinets

Fumigants

Of all fumigants, methyl bromide is preferred. Fumigation should be carried out at least once a year. Certain materials can be damaged by this and should not be kept in areas likely to be fumigated.

Insecticides and insect repellents

Any poison for permanent use in specimen cabinets must be carefully chosen,

with due attention to:

- ☞ its effect on people working in the collection;
- ☞ safe handling procedures and precautions;
- ☞ the most recent information on it.

Naphthalene (mothballs): This has a slightly insecticidal but predominantly repellent vapour. It sublimates slowly, requiring replacement only about once a year. Although direct contact with high concentrations of naphthalene is known to be hazardous, there is less clarity about the long-term effects of inhaling the fumes. Nevertheless, some people are sensitive to this substance and it is no longer commonly used. **Where it is used, there must be good ventilation**.

Long-acting silica aerogels with pyrethrins appear to have the most advantages. They are sprayed onto shelving and into cabinets. Skin contact should be avoided.

15. Housing of collections

Collections should be accommodated in a building that provides:

- ☞ permanent security for its specimens, and
- ☞ a safe working environment for its users.

15.1 Construction

Ideally, collections should be safeguarded in housing that has been purpose-designed. Where this is not possible, such housing must at least be:

- ☞ structurally stable and sturdy;
- ☞ waterproof;
- ☞ fireproof, with sufficient and adequate fire-doors;
- ☞ sufficiently spacious to allow:
  - a sensible arrangement of specimens, and
  - future growth of the collection.

15.2 Site and design as aids to pest control

Modern buildings that are specially planned for collections, provide for the physical exclusion of pests. Older buildings rely on pest control by means of toxic chemicals or by the expensive, artificial regulation of the interior environment.

Buildings or rooms that house collections should be separate from others. This simplifies:

- ☞ **fumigation:** otherwise adjoining rooms have to be vacated;
- ☞ **pest control:** it reduces sources of contamination.

Avoid or remove sources of infestation near the building by:

- ☞ preventing birds from nesting in, on or near the building;

- ☞ removing old trees close by;
- ☞ not planting asteraceous plants or plants with white or blue flowers that attract cigarette beetles in the vicinity, especially near herbarium windows; and
- ☞ avoiding the accumulation nearby of piles of decomposing plant litter.

Prevent pests from entering by:

- ☞ effectively sealing the building — not just the rooms housing the collection — at all points of entry. For instance, fine-mesh screens over windows, doors and ventilator intakes prevent entry of flying insects such as the notorious cigarette beetle;
- ☞ sealing communication routes along plumbing and electrical ducts.

Prevent dampness from leaks or condensation

15.3
Special facilities

Provision should be made for receiving, decontaminating and drying incoming specimens. The ideal is to have separate rooms for these activities, away from the main collection rooms. Each of these rooms should have its own ventilation to the outside air via extractor fans.

15.4
Temperature and humidity

Air-conditioning that maintains a low relative humidity and provides filtered, clean and insect-free air is expensive, but it will reduce pest infestations in collections. The air-conditioning unit should be separated from laboratories and offices.

Reduction of relative humidity to a level lower than:

- ☞ 43 % inhibits the growth of larvae of the destructive cigarette beetle;
- ☞ 45 % inhibits the growth of fishmoth and firebrats;
- ☞ 50 % inhibits fungal growth, thereby eliminating the main food source of psocids.

In some areas, simply heating the air in winter may be enough to reduce relative humidity. Recommended levels thus lie between 40 and 55 %, with not more than 5 % fluctuation.

A temperature between 20 and 23 °C is ideal for the collection and for staff efficiency.

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15.5 Ventilation

Good ventilation is important, both in terms of human health and pest control. This should not, however, increase the loss of pest-preventative chemicals or the entry of humid air, insects or dust.

Forced-draught ventilation:

- ☞ prevents the development of damp corners (this is a problem in humid regions);
- ☞ reduces the exposure of users to pesticides or to repellents used in the cabinets.

15.6 Internal layout and design

All interior finishes should be of a kind that limits cracks and crevices.

Floor:

- ☞ Should be of hospital design standards, made of smooth but non-slip vinyl, with rounded edges and corners — not skirtings — for easy cleaning. Exclude carpets as they serve as habitats for insects and fungi.
- ☞ Metal shelving and cabinets also help to keep humidity low.

Cabinets:

- ☞ The doors of steel cabinets should be sealed by neoprene rubber that contains magnetised strips (natural rubber is attacked by some fumigants).
- ☞ Steel panels should be closely welded to prevent entry by insects.
- ☞ Although wooden cabinets are difficult to seal they are better than steel ones at insulating their contents against fire damage.

Work surfaces should be:

- ☞ large enough to allow folders to be spread out on them easily;
- ☞ as close to the cabinets as possible;
- ☞ high enough so that one can comfortably stand and work at them.

There must be no damp, dark, airless corners, no unsealed panelling, loose wallpaper or cracks in floors or walls. These serve as breeding places for pests.

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15.7
Lighting

Adequate lighting is essential. Preferably this should be natural, supplemented by fluorescent tubes carefully positioned to reduce shadow areas between cabinets or over work areas. Ultra-violet filtration is preferable.

15.8
Fire precautions

Dried specimens and collection materials are flammable so strict precautions must be enforced.

- ☞ Electrical wiring and equipment must comply with the highest safety standards and be well-maintained.
- ☞ No smoking or naked flames should be allowed in collection rooms.
- ☞ Fire extinguishers and a fire-alarm system must be installed in accordance with advice from experts.

15.9
Good housekeeping

- ☞ All areas should be kept clean and free of lint, dust and particles of plant material that serve as food for pests.
- ☞ The structure of the building and the arrangement of the furniture and fittings must allow regular cleaning, particularly along window ledges and below cabinets, and circulation of dry air.
- ☞ There should be regular vacuum-cleaning to remove lint and food sources.
- ☞ Food should be excluded.

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16. Collection management policy

16.1 Purpose

There is an African philosophy known in the south as Ubuntu. It unites the concept of 'goodness' with 'sharing' and 'interdependence'. These are ideals that all custodians of biosystematic reference collections also uphold. The precious material in their care, however, demands special protection. Reference collections are expensive both to build-up and to maintain. But without them, few areas of biological research have a solid, continuous, scientific base. Consequently, carefully maintained collections are essential in terms of international agreements to protect, conserve and sustainably use the diversity of life on earth. That is why they are considered part of each nation's scientific inheritance.

Each reference collection should have a carefully considered management plan. In fact, it should have a written statement that clearly outlines its purpose, goals and principles. This is known as a **policy document** and it has three **main aims**:

- ☞ To protect a collection and to guarantee its future. The organisation in overall charge of the collection (or its controlling body) must therefore support this policy.
- ☞ To publicly state the nature and purpose of the collection.
- ☞ To provide guidelines on maintaining the highest professional standards possible. A part of the job description of all employees and future employees should be that they are to manage the collection according to the policy document.

16.2 Contents

A policy document should include concise coverage of all aspects of collections management. It may be supported by detailed protocols dealing

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with specific aspects, for instance loans or accessioning procedures. A framework is provided here.

Statement of intent

☞ Status:

- Is the collection international, national or regional, local or special (such as a university teaching collection, or an historical collection)?
- Does the collection function within a government body, or within a university or private organisation?
- What are its main components, for instance the groups of organisms involved?

☞ Origins:

- When and how did the collection come into being?
- Mention any significant steps in its development.

☞ Purpose and goals:

- For what reasons did the collection come into being?
- What is planned for the future?
- Have its purpose or goals ever changed, and if so, why?

☞ Responsible persons and their delegates:

- Does the final responsibility lie with the relevant Board of Control, Head of Department, Director of Institute?
- Is this responsibility delegated, for instance to a collections manager, curator or head curator?

Care, maintenance and protection measures

☞ Housing:

- How is the collection accommodated?
- How should this accommodation be maintained?

☞ Security:

- What security measures are in place: are there alarm systems, burglar-proofing, controlled access?

☞ Protection against fire:

- Biennial inspections should be carried out, and there must be an awareness of fire hazards in the collection.
- There should be a staff training programme in the use of fire-fighting equipment.

- Fire-drill procedures should be planned, and they should be regularly practised by all staff.

☞ Pest management:

- There should be a set of procedures that ensures effective pest management.
- Inspections should be carried out at specified intervals.

☞ Storage facilities:

- What methods and procedures are followed? Guidelines must be set out for the handling of specimens.
- Type specimens should be kept separate from the rest of the collection.

☞ Emergency procedures:

- There should be a plan stipulating procedures to be followed in the case of fire or any other emergency.
- All staff should be aware of this plan and it should be on display.

Deposited specimens

☞ Compliance with goals of the collection:

- Items obtained through field work, gifts or research deposits, exchanges or other means, and added to the collections, must fit in with the aims of the collection as set out in the policy document.

☞ Quality requirements:

- Specimens must meet specified standards regarding quality, quantity and nature.

Collection data requirements:

☞ The information that is expected to accompany specimens must be clearly indicated.

☞ Conditions imposed by donors:

- Items should be accepted only if there are no restrictions.
- It is not advisable to accept material on long-term loan.
- If the donor imposes conditions, these must be acceptable to the recipient.

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- The collection policy should specify who has the authority to approve conditions set by donors, or deviations from its rules.

Removal or transfer of material

Conditions under which material is transferred to another collection, or permanently removed:

☞ The policy document should specify who has the authority to approve the removal of material, and the **reasons for which material may be removed**, for instance:

- specimens destroyed by insects;
- severely denuded specimens;
- if donated material is to be removed, the donor must be informed;
- appropriate entries must be made in the accessions registers and other record systems regarding the reasons for and methods of removal;
- if an entire collection can no longer be adequately maintained, it must be offered to other appropriate bodies.

Records

☞ System:

- Each collection must have a detailed record system that is precisely maintained according to set rules.
- All materials used should be of proven quality and permanence, and the system must include the following components.

☞ Accession registers:

- These must be bound volumes with sequentially numbered pages.
- Entries must be waterproof.
- Registers should be stored in a fire-proof safe.
- Computer discs or tapes are acceptable only as duplicates and must be stored separately from the primary, printed records.

☞ Card catalogues or computerised species records.

☞ A catalogue of Type specimens.

☞ A loans register.

Access

☞ Who is allowed access to the collections?

- This depends on the nature of the collection.

- In the case of scientific reference collections, it is usually the people who would also be granted loans.
- Users should be supervised by the curator or someone given this responsibility, according to the needs of the situation.

Loans

☞ Who is permitted to borrow specimens:

- To ensure its safety, material is usually loaned only to
  - » researchers at appropriate institutions, or
  - » their students involved in supervised post-graduate studies.
- A loan is always a formal agreement between the curators of two collections on behalf of a researcher.

☞ Documentation:

Conditions under which loans are approved:

☞ These must be set out according to the requirements of the collection, and include aspects such as:

- loan period;
- how the material may or may not be used;
- packaging and transport.

- Loan records must be made according to a set protocol, and loan registers must be regularly checked for overdue loans.

Services associated with the collections

☞ All the services on offer should be outlined.

☞ Recommendations and guidelines for users of these services should be clearly set out.

Fees

☞ Indicate which services are free and which are not.

☞ Formulate a cost sheet (issued separately for easier updating).

References and further reading

Dictionary

Ainsworth & Bisby's Dictionary of the Fungi (HAWKSWORTH, D.L., KIRK, P.M., SUTTON, B.C. & PEGLER, D.N.). CAB International, London, 1995 (8th Edition).

This is an invaluable source of information: not only the definitions of terms but literature references and information regarding various subjects, e.g. classification, collection and storage of specimens, literature, methods, seedborne fungi and plant pathogens.

Handbooks / Reference books

General

ALEXOPOULOS, C.J. & MIMS, C.W. (1979). *Introductory Mycology.* 3rd Edition. John Wiley & Sons, New York.

DOIDGE, E.M. (1950). South African fungi & lichens to the end of 1945. *Bothalia* 5: 1–1094.

GORTER, G.J.M.A. (1977, 1981 & 1982). Index of plant pathogens. *Department of Agriculture Scientific Pamphlet* No. 392; appendix to 392 & 398.

GREUTER, W., BARRIE, F.R., BURDET, H.M., CHALONER, W.G., DEMOULIN, V., HAWKSWORTH, D.L., JØRGENSON, P.M., NICOLSON, D.H., SILVA,P.C., TREHANE, P. & McNEIL, J. (1994). International Code of Botanical Nomenclature (Tokyo Code) adopted by the fifteenth International Botanical Congress, Yokohama, August – September 1993. *Regnum Vegetabile* 131: 1–389.

HAWKSWORTH, D.L. (1974). *Mycologist's Handbook.* CMI, International Mycological Institute, London.

JOHNSTON, A. & BOOTH, C. eds. (1983). *Plant Pathologist's Pocketbook.* CAB: International Mycological Institute, London.

Methods

BOOTH, C. ed. (1971). *Methods in Microbiology.* Vol. 4. Academic Press, London.

BRADBURY, S. (1973). *Peacock's Elementary Microtechnique.* Edward Arnold, London.

FORMAN, L. & BRIDSON, D. (1991). *The Herbarium Handbook.* Royal Botanic Gardens, Kew.

HALL, A.V. (1987). Pest control in herbaria. *Foundation for Research Development, CSIR, Pretoria. Occasional Report* No. 27.

SMITH, D. & ONIONS, AGNES H.S. (1994). *The Preservation and Maintenance of Living Fungi*. CAB International Mycological Institute, London.

UNIVERSITY OF THE WITWATERSRAND MUSEUM USERS' COMMITTEE. (Undated) *Collections management policy* — Eleventh draft.

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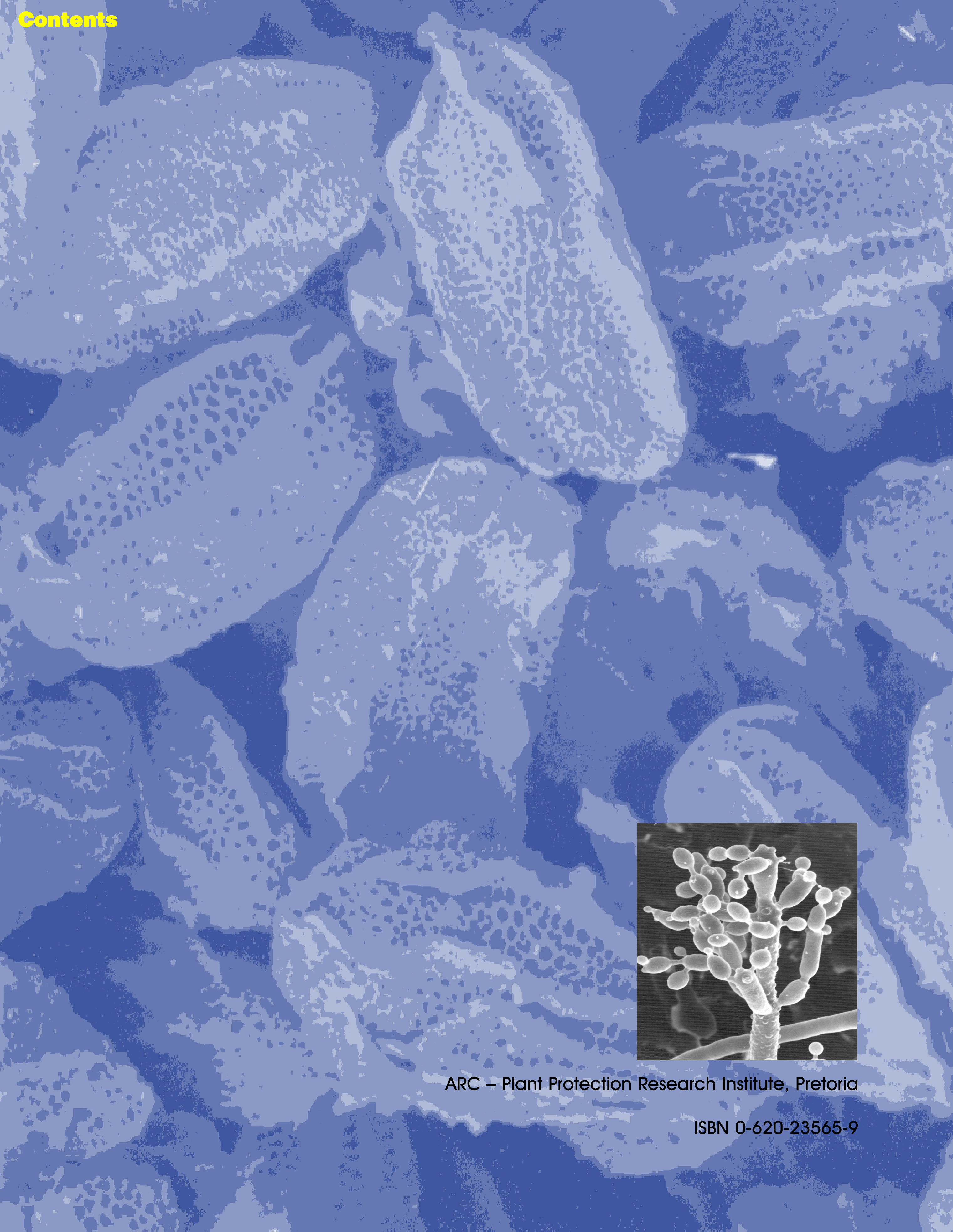

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