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Diagnostic manual for plant diseases in Vietnam

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Australian Centre for International Agricultural Research
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Foreword

Plant diseases continue to cause significant crop losses in Vietnam and other regions of tropical South-East Asia. The recent epidemic of rice grassy stunt virus and rice ragged stunt virus in southern Vietnam highlighted the significant socioeconomic effects of crop diseases at a national level.

Outbreaks of disease of valuable cash crops can also have a major impact on small farmers in localised areas where there are few suitable alternative crops—an example being ginger wilt complex in Quang Nam province.

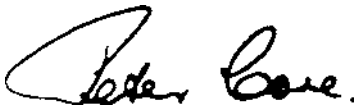
The accurate diagnosis of the cause of a disease is essential to the success of control measures. However, many diseases produce similar symptoms, making diagnosis in the field difficult or impossible. Hence, diagnostic laboratories are an essential component of a plant protection network. Staff assigned to diagnostic work require intensive training at the undergraduate and graduate level in both field and laboratory skills, and in the basic concepts of plant disease and integrated disease management.

Accurate diagnosis of diseases is also essential to the development of a scientifically sound national database on plant diseases. A database on diseases in Vietnam will be a critical part of successful plant quarantine operations. Furthermore, a national database is a critical element of the biosecurity measures that relate to trade in agricultural products, especially for members of the World Trade Organization.

This manual is designed to help plant pathologists develop basic skills in the diagnosis of the cause of diseases, focusing on fungal diseases of the roots and stems. These diseases are insidious, and cause significant socioeconomic losses in Vietnam.

The content of this manual is based on the experience of the authors and many colleagues in Australia and Vietnam in training programs associated with various projects funded by the Australian Centre for International Agricultural Research (ACIAR), AusAID Capacity Building for Agriculture and Rural Development, and Academy of Technological Sciences and Engineering Crawford Fund.

The manual complements other publications produced by ACIAR and various colleagues in Vietnam.



Peter Core
Chief Executive Officer
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Preface

This manual is designed to provide a basic introduction to diagnosing fungal diseases of crops in Vietnam. The content is based primarily on experience gained during two Australian Centre for International Agricultural Research (ACIAR) projects in northern and central Vietnam.[1] It takes into account other manuals published or in press.

Four low-cost diagnostic laboratories were established in the central provinces of Vietnam during the current ACIAR project.[2] These laboratories are located at the Plant Protection Sub-departments (PPSDs) in the provinces of Quang Nam, Thua Thien Hue and Nghe An, and at Hue University of Agriculture and Forestry. They have the equipment needed to isolate and identify common genera of fungal and bacterial pathogens that persist in soil, and common foliar fungal and bacterial pathogens. They also have facilities for pathogenicity testing newly recognised pathogens in Vietnam. The staff in these laboratories have had basic laboratory training through workshops at Hanoi Agricultural University and in the Quang Nam PPSD, where a teaching laboratory has been established. Staff have also been involved in regular field surveys of disease and have diagnosed diseases collected by farmers.

Each laboratory has a small library and a computer for accessing web-based information, which are essential resources for diagnostic plant pathologists.

Small greenhouses have been established in each province, both for pathogenicity testing and for the evaluation of fungicides and soil amendments for disease suppression. The design and operation of greenhouses for experimental work

1 CS2/1994/965 Diagnosis and control of plant diseases in northern Vietnam (1998–2001) and CP/2002/115 Diseases of crops in the central provinces of Vietnam: diagnosis, extension and control (2005–2008).

2 CP/2002/115 Diseases of crops in the central provinces of Vietnam: diagnosis, extension and control (2005–2008).

and the production of pathogen-free planting material have been the subject of training activities in Vietnam and Australia. Dr Ngo Vinh Vien, Director of the Plant Protection Research Institute, has recommended that all staff receive training and professional development in these areas. The team from the current ACIAR project visited nurseries in Dalat as part of the activities.

The integration of English teaching with training in plant pathology has been a critical aspect of staff development in the current project. Many of our colleagues in the current project can now seek advice by email (with the aid of digital images) on new disease problems.

Colleagues from Vietnam and Australia have contributed images and text for this manual—these contributions are acknowledged individually.

Diagnostic work provides a basis for designing field trails on disease control, and developing control measures for extension purposes. The accurate diagnosis of a wide range of diseases and the identification of pathogens to species level depends on broad experience over many years. We hope this manual will assist our early-career Vietnamese colleagues with their first field and laboratory studies on plant disease diagnosis.

Acknowledgments

The authors sincerely thank Dr T.K. Lim for suggesting the concept of a diagnostic manual for plant disease in Vietnam and the Australian Centre for International Agricultural Research (ACIAR) for financial support for the initiative. The senior author also acknowledges the invaluable support and encouragement provided by ACIAR for diagnostic, research and capacity building activities in Vietnam for over 12 years.

The authors also sincerely thank successive rectors, our colleagues in plant pathology and staff of the international office at Hanoi Agricultural University for their support since 1992. Similarly the authors are indebted to staff of the Plant Protection Research Institute for guidance and support, especially the Director, Dr Ngo Vinh Vien.

The assistance of staff at The University of Sydney, Royal Botanic Gardens and Domain Trust, and the New South Wales Department of Primary Industries with teaching and research activities in Vietnam is also gratefully acknowledged.

We are also indebted to the generosity, hospitality and support provided by colleagues in the Plant Protection Sub-departments in Quang Nam, Thua Thien Hue, Nghe An, Quang Tri and Lam Dong, the Hue University of Agriculture and Forestry, Centre for Plant Protection Region 4, and the collaborating farmers in these and other provinces. Our current project has been especially rewarding to all concerned.

The following colleagues in Vietnam and Australia have contributed to this manual through images of plant diseases, associated comments and editorial advice. However, the authors bear final responsibility for the content and illustrations.

Australia—Barry Blaney, Julian Burgess, Eric Cother, Norma Cother, Nerida Donovan, Phillip Davies, Mark Fegan, Col Fuller, David Guest, Ailsa Hocking, Greg Johnson, Edward Liew, Suneetha Medis, Dorothy Noble, Tony Pattison, Brett Summerell and Ameera Yousiph.

Vietnam—Dang Luu Hoa, Dau Thi Vinh, Ho Dac Tho, Hoa Pham Thi, Hoang Thi Minh Huong, Huynh Thi Minh Loan, Luong Minh Tam, Ngo Vinh Vien, Nguyen Kim Van, Nguyen Thi Nguyet, Nguyen Tran Ha, Nguyen Vinh Truong, Pham Thanh Long, Tran Kim Loang, Tran Thi Nga and Tran Ut.

1 Introduction

Plant diseases cause serious income losses for many farmers in Vietnam, by reducing crop yields and the quality of plant products. The costs of control measures such as fungicide can further reduce a farmer's income.

Some diseases are caused by fungi that produce mycotoxins, such as aflatoxin, which can contaminate food products (e.g. maize and peanuts). Contamination by mycotoxins can have adverse effects on human and animal health.

Occasionally diseases spread in devastating epidemics through major crops. Such epidemics can have serious economic and social impacts on an entire region or country. In 2006, for example, rice grassy stunt virus and rice ragged stunt virus caused major losses to rice crops in the Mekong delta, affecting one million hectares across 22 provinces. This epidemic directly affected millions of farming families.

The Vietnamese Ministry of Agriculture and Rural Development has long recognised the importance of plant disease in agriculture. It has an extensive network of research centres and a network of plant protection staff at provincial and district levels across Vietnam. These resources provide diagnostic support and information on control measures for disease. This service is a major challenge, given the diversity of crops and diseases, and the range of climatic regions in Vietnam.

Successful control of disease depends on accurate identification of the pathogen and the disease. Some common diseases can be diagnosed accurately in the field by visual symptoms. For example, boil smut of maize, Sclerotinia stem rot, root knot nematode, club root and peanut rust all have symptoms that are distinct and obvious to the unaided eye. However, there are many diseases that have similar non-specific symptoms (e.g. wilting, stunting, leaf yellowing). Some of these can be identified accurately in the laboratory by examining samples using a microscope. Many fungal pathogens and parasitic nematodes can be identified in this way.

However, some fungal and bacterial pathogens can only be identified by isolation into pure culture. Once isolated, pure cultures can be identified using a microscope and, if necessary, identification can be confirmed using molecular and other more costly techniques. Most of the fungal pathogens that cause root and stem rots can only be identified by isolation of the pathogen into pure culture. Most plant virus diseases can only be identified accurately in a virology laboratory. Diagnostic kits are available that enable fast and accurate diagnosis of some viral and bacterial diseases in the field; however, these kits are relatively expensive.

This manual was designed to assist in the establishment and operation of small laboratories for diagnosing common fungal diseases at a provincial level in Vietnam. It is particularly concerned with the fungal root and stem rot diseases that cause significant losses to many Vietnamese farmers every year. Many of these diseases are yet to be properly identified.

In this manual the terms fungi and fungal are generally used in the traditional sense as is common practice in Vietnam at present. Thus these terms are used to refer to the true fungi as well as fungal-like filamentous species in the Oomycetes, and the endoparasitic slime moulds. However the importance of understanding the modern approach to the taxonomic treatment of these organisms is emphasised in the text. An outline of one of the modern taxonomic systems of classification of these various organisms is included in the manual.

Fungal diseases are useful for diagnostic training. The Australian Centre for International Agricultural Research (ACIAR) has supported the establishment of four diagnostic laboratories at the provincial level, including considerable training in the field and laboratory for staff. There has been encouraging progress, although it takes many years of experience and practice to become familiar with diagnosing diseases caused by all plant pathogens—fungi, bacteria, viruses, mollicutes and nematodes.

The staff in a diagnostic laboratory must keep accurate records of diagnoses in an accession book and every sample should be recorded. Information on the occurrence of diseases can then be entered into a national database on diseases, which is a key element of biosecurity processes supporting the export of agricultural produce. The national database will be very important now that Vietnam has joined the World Trade Organization. A national database of plant diseases and a network of diagnostic laboratories will help Vietnam to meet the challenges of establishing and maintaining biosecurity. Ideally, laboratories should maintain a reference culture collection and a herbarium of disease specimens (see Shivas and Beasley 2005).

Disease is only one factor affecting plant health and, consequently, crop yields. It is important for the diagnostic plant pathologist to be aware of all the factors that affect plant health and interact with disease—pests, weeds, pesticide use, soil characteristics, local climate and other environmental factors.

The successful diagnosis and control of disease is facilitated by close collaboration between plant protection staff and farmers. Farmers can be very observant and can provide important information to assist in diagnosis from their own observations and experience.

This manual is organised into the following sections:

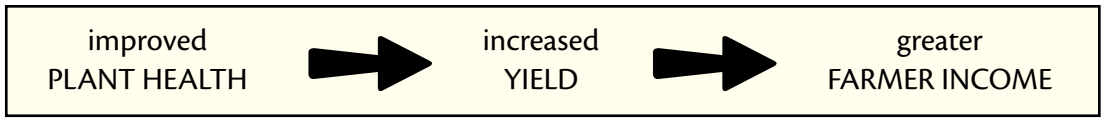
- general plant health and factors that can affect it
- field and laboratory procedures for diagnosing the causes of a disease
- symptoms of plant disease
- procedures and equipment for working in the field
- procedures and equipment for working in the laboratory
- a brief introduction to fungal taxonomy
- methods for pathogenicity testing
- integrated disease management
- diseases caused by fungal pathogens that live in soil
- common diseases of some economically important crops
- health implications of fungal pathogens
- design, development and operation of diagnostic laboratories and greenhouses
- appendixes on making a flat transfer needle, maintaining health and safety procedures, as well as recipes for media, sterilisation methods, and methods for preservation of fungal cultures
- a suggested reference library for diagnostic laboratories.

1.1 References

Shivas R. and Beasley D. 2005. Management of plant pathogen collections. Australian Government Department of Agriculture, Fisheries and Forestry. At: <http://www.daff.gov.au/planthealth>.

2 General plant health

Plant health is a determining factor in crop yield and consequently in the income of the farmer. Therefore, it is very important to manage the health of the crop so that profits are maximised.



Disease is only one of the factors that can affect the health of crop plants. Other factors include pests, weeds, nutrition, pesticides, soil conditions and the environment (Figure 2.1). All of these factors must be considered during the diagnostic process as each can affect the plant and cause symptoms similar to those caused by disease. Each factor can also potentially affect the development of disease in the plant.

Diagnostic plant pathologists should have an understanding of all of the factors that affect plant health and disease. In the field, the pathologist should record information on all of the relevant factors (see field sheet in Section 5), and discuss the history of the field and crop management with the farmer.

Vietnam has a wide range of agroclimatic regions. For example, the central and northern provinces experience a cool to cold winter that favours temperate pathogens. The low temperatures inhibit growth of some crops making them more susceptible to seedling and other diseases. Furthermore, the yearly weather cycle includes very wet as well as dry periods. Such weather can also lead to crop stress and favour some diseases, especially of the roots and stems caused by pathogens that survive in soil. Indeed waterlogging and poor drainage are major factors favouring these diseases in Vietnam. Therefore high raised beds and good drainage are critical practices in integrated disease management. A diagnostic pathologist must understand these effects.

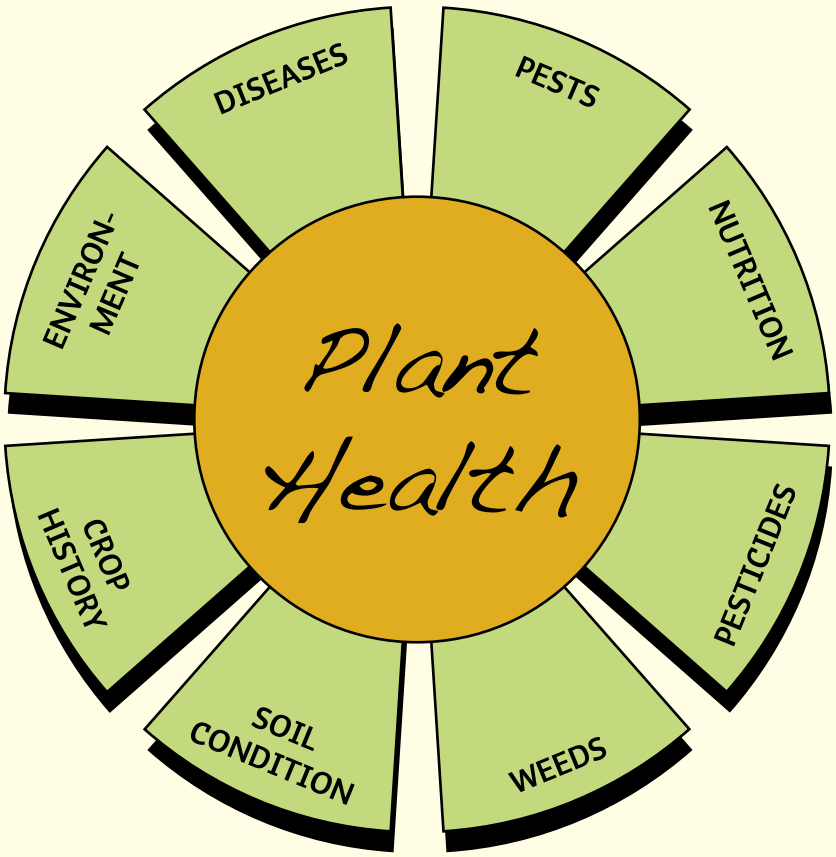


Figure 2.1 Key factors in maintaining plant health

2.1 Weeds

Many pests and pathogens persist on weed hosts when the susceptible crop host is absent. Therefore, effective weed control is an important control measure and a key part of integrated disease management (IDM). In addition, weeds growing with a crop will compete for water, nutrients and light, which will stress the crop and increase disease severity.

2.2 Pests

Feeding by invertebrate pests can cause damage to the plant similar to disease symptoms (Figure 2.2). For example, aphids, leaf hoppers, thrips, mites and whiteflies can cause damage to the leaf similar to the symptoms of some foliar diseases. These pests also can act as vectors of viruses and bacteria. Stem borers and root grubs affect water uptake and can cause wilting that is similar to wilting caused by vascular wilt and root rot diseases.

2.3 Pesticides

The application of pesticides can cause leaf damage, such as leaf burn and leaf spots. These symptoms can be confused with symptoms of leaf blight and leaf spots caused by many fungal and bacterial pathogens. Herbicides may stress plants, affecting their susceptibility to a pathogen.

2.4 Nutrition

Poor nutrition commonly causes stunting and poor root growth (Figure 2.3). These symptoms are also caused by root rot pathogens. Other signs of mineral deficiencies and toxicities can also be similar to the symptoms of some diseases. For example, nitrogen deficiency causes leaf yellowing, particularly of the lower leaves. Leaf yellowing is also a symptom of root disease, which can also disrupt the uptake of nitrogen. Mineral deficiencies or toxicities can affect the susceptibility of plants to some pathogens.

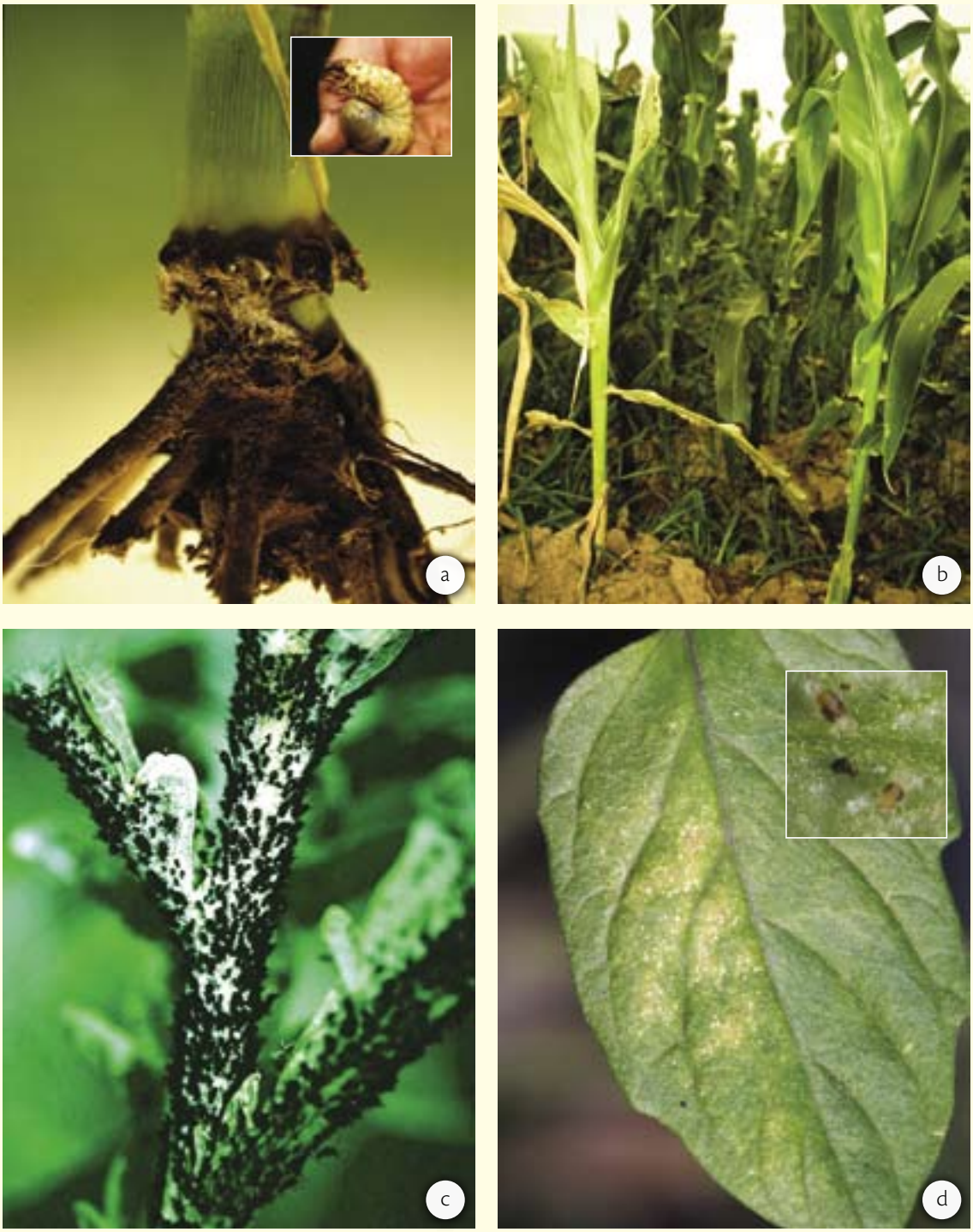


Figure 2.2 Invertebrate pest damage: (a) white grub (inset) damage to maize roots, (b) wilting maize plant affected by white grub, (c) aphid infestation, (d) typical bronzing of leaf caused by mites feeding on the underside of the leaf (inset)

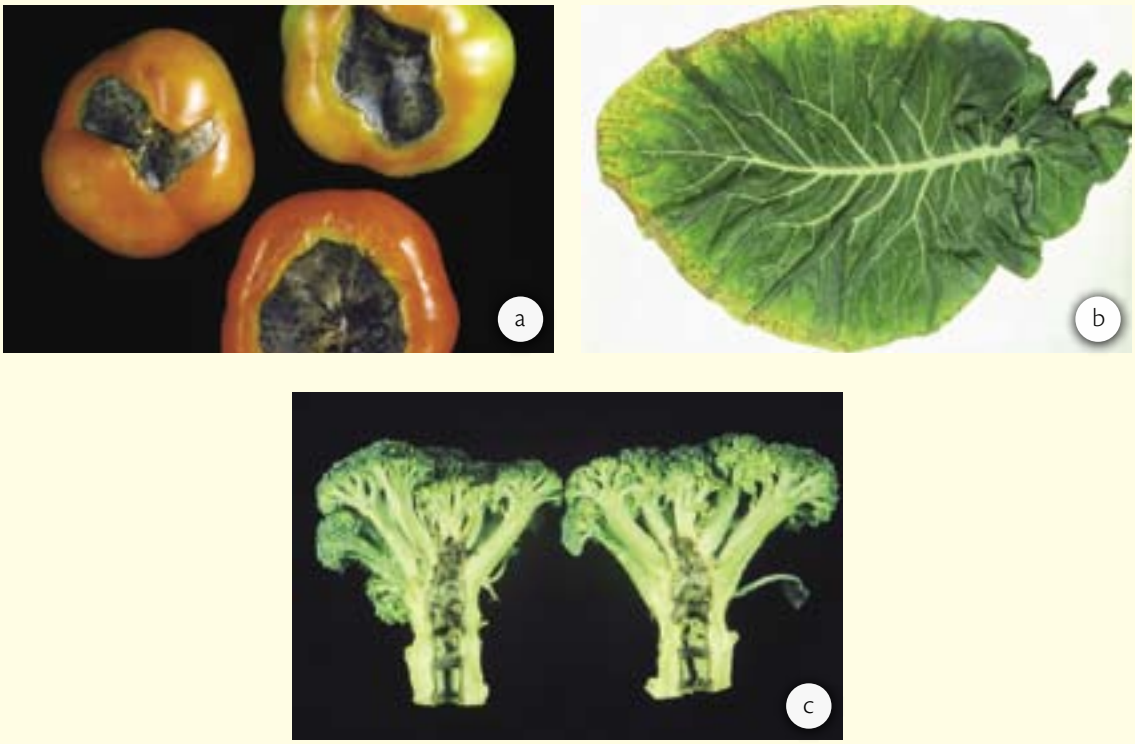


Figure 2.3 Nutrient deficiencies causing disease-like symptoms: (a) blossom end rot due to calcium deficiency of tomato, (b) potassium deficiency of crucifer, (c) boron deficiency of broccoli

2.5 Soil conditions

Waterlogging (poor drainage), poor soil structure, hard clay soils and 'plough pans' (hard layers in the soil profile) can interfere with root growth. Stunting of the roots decreases the uptake of water and nutrients, causing stress on the whole plant. Stunting of the roots can also cause wilting and yellowing of the leaves, changes which are similar to the symptoms of many plant diseases. A plough pan can cause roots to grow laterally (turn sideways) (Figure 2.4), reducing root function and growth; this stresses the plant, leading to favourable conditions for some pathogens.

Figure 2.4 Lateral root growth caused by a hard layer in the soil profile (plough pan)

2.6 Environment

A variety of weather conditions can cause damage and stress to plants, and thus be detrimental to plant health. These conditions, including extremes of temperature, humidity and rain, as well as hail, flooding, drought and typhoons, lead to increased disease incidence and severity. High temperatures, low humidity and drought can cause severe wilting and plant death. Wet windy conditions facilitate infection and the spread of many fungal and bacterial leaf pathogens. Wet soil conditions favour Phytophthora and Pythium root rot diseases. Drought stress facilitates some root diseases, and stem and stalk rot problems. The combination of root rot disease and dry soil can kill plants.

There is evidence that typhoons or gale-force winds that severely shake trees cause damage to the tree root systems. Such damage can facilitate higher levels of infection by root rot pathogens and cause decline and death of the trees. For example, typhoons or high winds are the suspected cause of tree decline in some coffee and lychee trees in Vietnam.

2.7 Crop history

An understanding of the history of the crop can help with the diagnosis of a disease. For example, the origin of the seed and whether it was treated with fungicide can provide an indication of whether a seed-borne pathogen may be affecting the crop. As discussed above it is important to understand the history of weather conditions prior to a disease outbreak. Cool wet conditions favour many root rot pathogens but the plant may tolerate some damage to the roots under these conditions as transpiration rates are low. However, if the weather turns hot and transpiration rates are high, the diseased plant can quickly wilt and die.

An earlier infestation of a virus vector in a crop could indicate that a virus carried by the vector has infected the crop and is responsible for the symptoms observed.

Knowledge of the previous crops and their diseases can also provide a guide to potential diseases in the current crop. For example, some rotations will increase the severity of particular diseases caused by soil-borne pathogens. For example, successive crops in the family Solanaceae are likely to increase bacterial wilt caused by *Ralstonia solanacearum*.

CASE STUDY

Weeds as alternative hosts for *Ageratum conyzoides*

Weeds can act as alternative hosts of many important crop pathogens.

Ageratum conyzoides is a common weed in Vietnam (Figure 2.5), growing within crops, in fallow areas between crops and alongside footpaths. It is an alternative host of several important pathogens and provides a source (reservoir) of inoculum of these pathogens to infect new crops. If this weed is present, the farmer can lose the benefit of crop rotation for controlling pathogens in the soil.

Ageratum conyzoides is a host of *Ralstonia solanacearum* (which causes bacterial wilt), root knot nematode and possibly aster yellows, which is a disease caused by a phytoplasma transmitted by leaf hopper vectors to susceptible crops such as asters, potatoes, carrots and strawberries.

Controlling weeds acting as alternative hosts is extremely important.

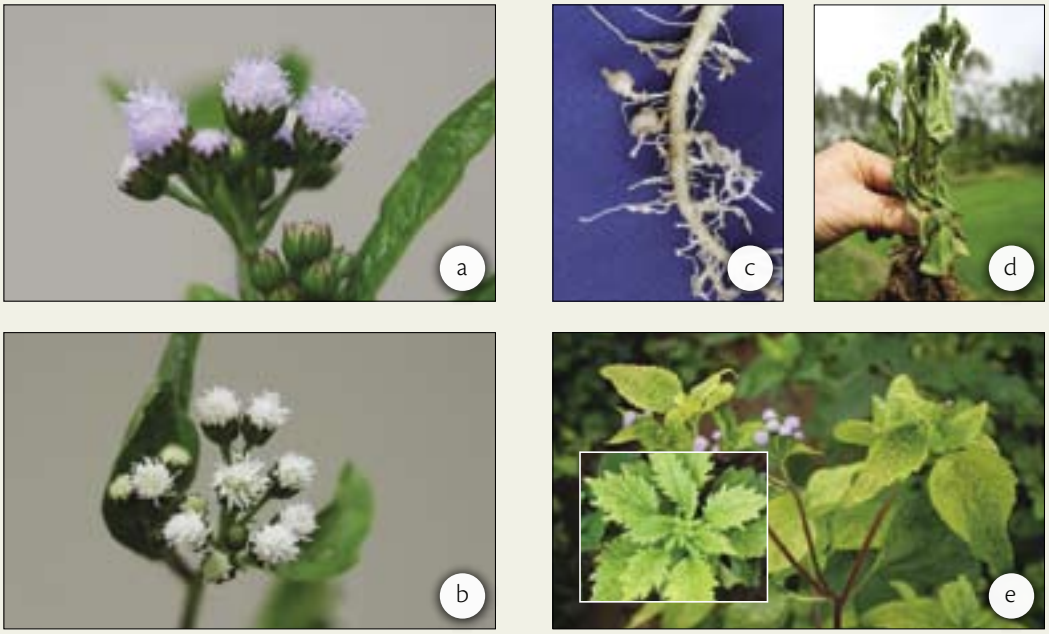


Figure 2.5 *Ageratum conyzoides*: (a) blue flowered variety, (b) white flowered variety, (c) *Ageratum conyzoides* root affected by *Meloidogyne* spp. (nematodes) causing root knot symptoms, (d) wilting *Ageratum conyzoides* caused by *Ralstonia solanacearum* (a bacterium), (e) aster yellows-like symptoms on *Ageratum conyzoides* (inset: the aster *Callistephus chinensis* showing aster yellows symptoms)

3 The diagnostic process

The main activities involved in the diagnostic process are:

- collecting diseased plants in the field
- examining collected plants in the laboratory
- pathogenicity testing
- disease diagnosis.

These activities are shown in Figure 3.1.

3.1 Case studies

In this section, two case studies are presented to provide an illustrated overview of the diagnostic process:

- diagnosing the cause of pineapple heart rot—*Phytophthora nicotianae*
- surveying a complex disease—ginger wilt caused by bacterial and Fusarium wilts.

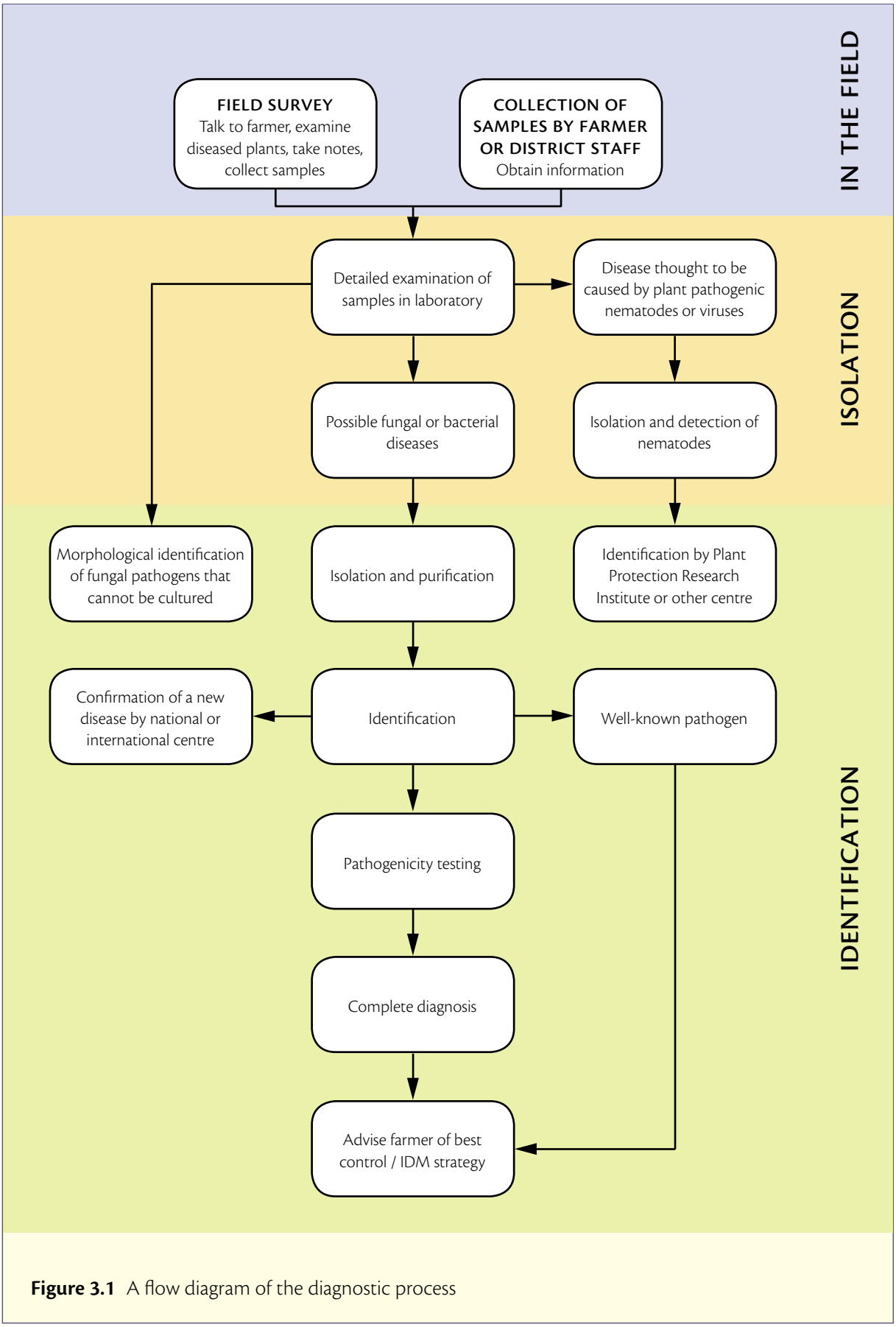


Figure 3.1 A flow diagram of the diagnostic process

DIAGNOSTIC CASE STUDY 1

Diagnosing the cause of pineapple heart rot—*Phytophthora nicotianae*

Figure 3.2 provides an example of the steps to follow during the diagnostic process.

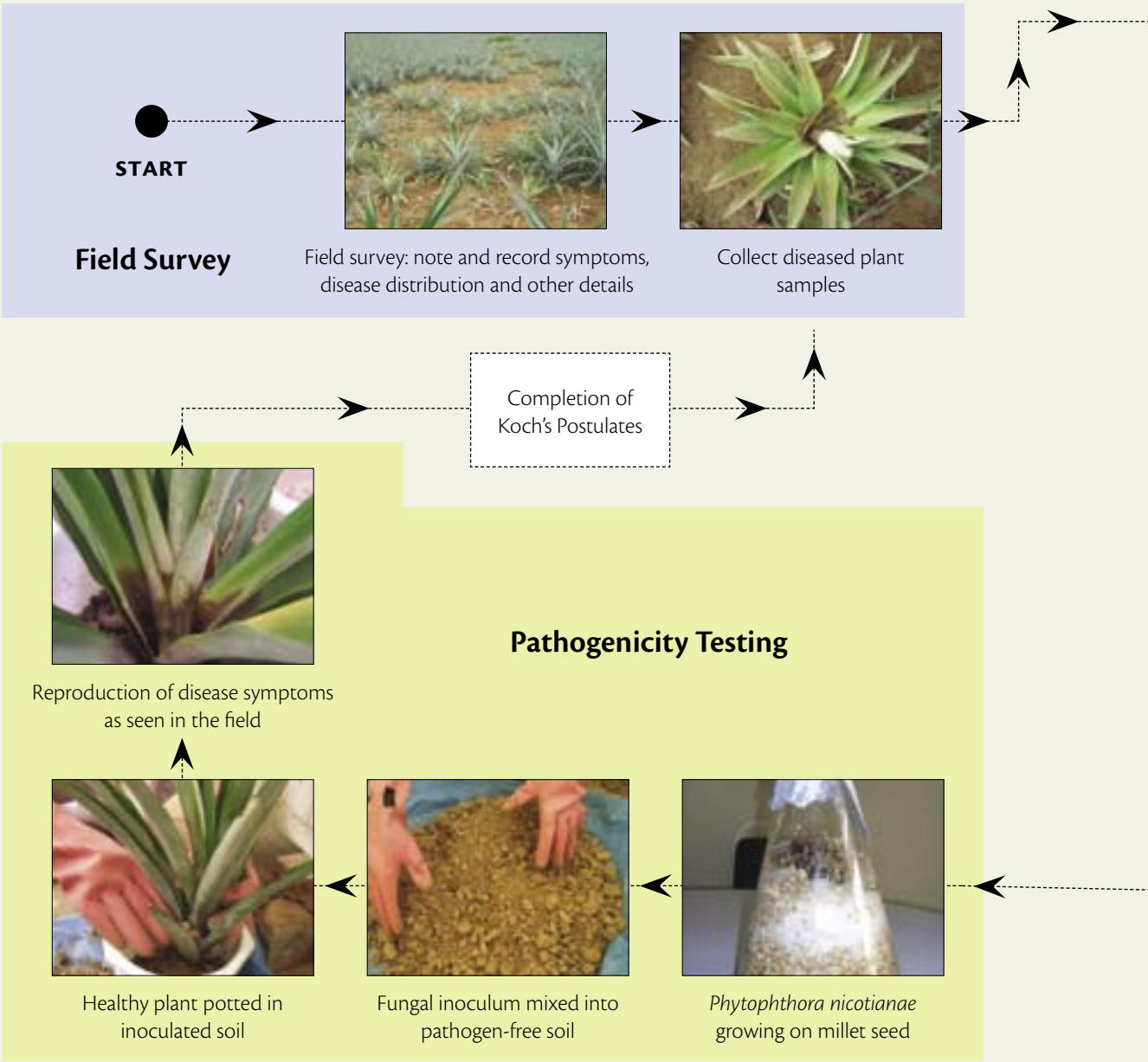
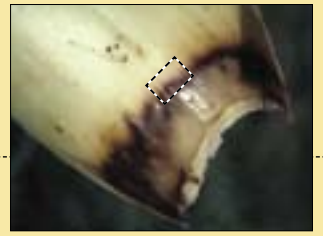


Figure 3.2 Steps involved in the isolation, purification, identification and pathogenicity testing of the pineapple heart rot pathogen, *Phytophthora nicotianae* (Images provided by Dang Luu Hoa)



Selection of material on margin of necrotic tissue

Wash and surface sterilise samples

Small segments cut and transferred aseptically

Plate segments on selective medium

Laboratory

Isolation and Purification

Incubation of plates

Colonies growing from segments

Subculture of fungal colony and hyphal tipping on water agar

Identification of pure culture from hyphal tip (*P. nicotianae*)

DIAGNOSTIC CASE STUDY 2

Surveying a complex disease—ginger wilt caused by bacterial and Fusarium wilts

Introduction

Ginger wilt was first recorded officially in Quang Nam in 2000. The disease has caused severe losses, with many farmers losing 100% of their crop. A preliminary study in 2006 indicated that bacterial wilt and Fusarium wilt were involved. A systematic survey of the disease complex was made in January 2007, as a part of the Australian Centre for International Agricultural Research project CP/2002/115, Diseases of crops in the central provinces of Vietnam: diagnosis, extension and control (2005–2008).

The objective was to isolate and identify potential pathogens associated with diseased ginger plants, and determine their relative importance. Ten diseased plants were collected from each of 10 crops from two districts, Phu Ninh and Tien Phuoc, which are the main areas of ginger production in Quang Nam. Crops were selected on an ad hoc basis for sampling before inspection.

In the field

Information was collected from the farmers at each crop site (Figure 3.3). The farmers maintained that there were two types of wilt: quick wilt and slow wilt. The leaves of plants with quick wilt appeared to have been 'boiled in water' and were translucent. In contrast, the leaves of plants with slow wilt appeared yellow (Figure 3.4). These comments suggested that two diseases were involved and the symptoms described were assumed to correspond to bacterial wilt (quick wilt) and Fusarium wilt (slow wilt).

Figure 3.3 Discussions with farmers on ginger wilt

Figure 3.4 A ginger wilt survey in Quang Nam in January 2007: (a) ginger with symptoms of quick wilt, (b) ginger plants with yellowing, a symptom of slow wilt, (c) adjacent crops, one crop with quick wilt, the other symptomless, (d) and (e) plants being removed carefully using a machete, keeping the root systems intact, (f) sample bag labelled with site number, farmer's name and date

DIAGNOSTIC CASE STUDY 2 (CONTINUED)

In the laboratory

Specimen preparation

Plant roots were washed carefully to remove soil. The plant was then examined and small samples from diseased areas on the plant were taken into the laboratory for microscopic examination and isolation of the pathogens (Figure 3.5).

Gently washing the soil from the roots

Examining the plant (leaves, shoot, rhizome, roots) and recording symptoms

Root knot nematode (*Meloidogyne* sp.) present in some root systems

Cut rhizome from root and stem

Figure 3.5 Preparation and examination of plants with ginger wilt for the laboratory

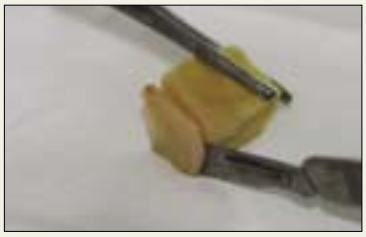
Isolation of potentially pathogenic organisms from diseased tissue

Ginger rhizomes were surface sterilised, peeled and surface sterilised again. A disc was removed from each rhizome, from which segments were cut and plated on peptone PCNB (pentachloronitrobenzene) agar and *Phytophthora* selective medium. Another segment was macerated and streaked on a plate of King's B medium to isolate bacteria (Figure 3.6).

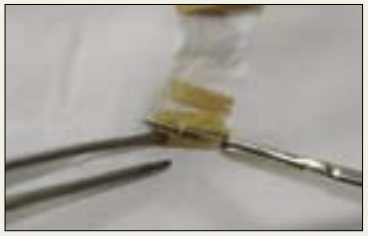
Rhizome was surface sterilised in 70% ethyl alcohol for 5 seconds and flamed

Rhizome was peeled (outer layer removed) and then surface sterilised again in 70% ethyl alcohol

Peeled rhizome was then flamed and a disc from the top of the rhizome was then aseptically removed



Five small segments were cut from the disc and two were plated on PPA and two on PSM



The remaining segment was macerated in sterile water on a glass slide and streaked onto a plate of King's B medium for isolation of bacteria

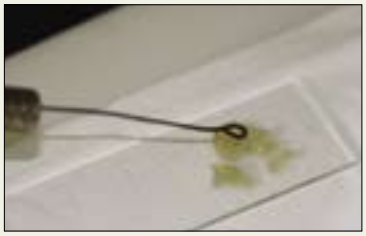


Figure 3.6 Isolation procedure for potential plant pathogenic organisms from ginger rhizome

DIAGNOSTIC CASE STUDY 2 (CONTINUED)

Recovery of potential pathogens

Fusarium spp. were isolated from rhizomes (Figure 3.7) from plants identified as having slow wilt—yellowing and root knot nematode symptoms—from some sites. The isolates were purified by single spore isolation and identified as *Fusarium oxysporum*.

The colonies of *F. oxysporum* were identical in culture on carnation leaf agar and potato dextrose agar, indicating that they could be pathogenic. (Cultures of saprophytic strains of *F. oxysporum* are usually quite variable in culture.)

Figure 3.7 Isolation of *Fusarium oxysporum* from some segments of ginger rhizomes on selective isolation medium (peptone pentachloronitrobenzene agar) for *Fusarium*

Phytophthora species were not isolated from the ginger rhizome.

A Pocket Diagnostic® Kit test for *Ralstonia solanacearum* was positive for rhizomes from three plants identified as having quick wilt, which indicates that this bacterium was present in the rhizome tissue. The kit test was negative for a rhizome from a plant identified as having slow wilt symptoms (yellowing)—*F. oxysporum* was isolated from this rhizome.

A variety of bacterial colonies were isolated on King's B medium and it was not possible to identify putative colonies of *R. solanacearum* with confidence. Because the plants that were sampled had obvious to severe symptoms and had been subjected to very wet soil prior to sampling, conditions would have favoured colonisation of diseased tissue by non-pathogenic bacteria.

Consequently, a bioassay procedure was used in an attempt to recover cultures of *R. solanacearum* (Figure 3.8). The inner tissue of additional rhizomes from plants previously sampled at six sites was cut into segments and shaken with 30 mL of sterile water. The water was then poured into small containers with freshly cut tomato and chilli stem cuttings—bait

seedlings—which were kept in a greenhouse at 25–30 °C. Within 4–8 days, some of the cuttings in the water extracts wilted and some of these showed signs of bacterial ooze. Control seedlings in sterile water remained healthy.

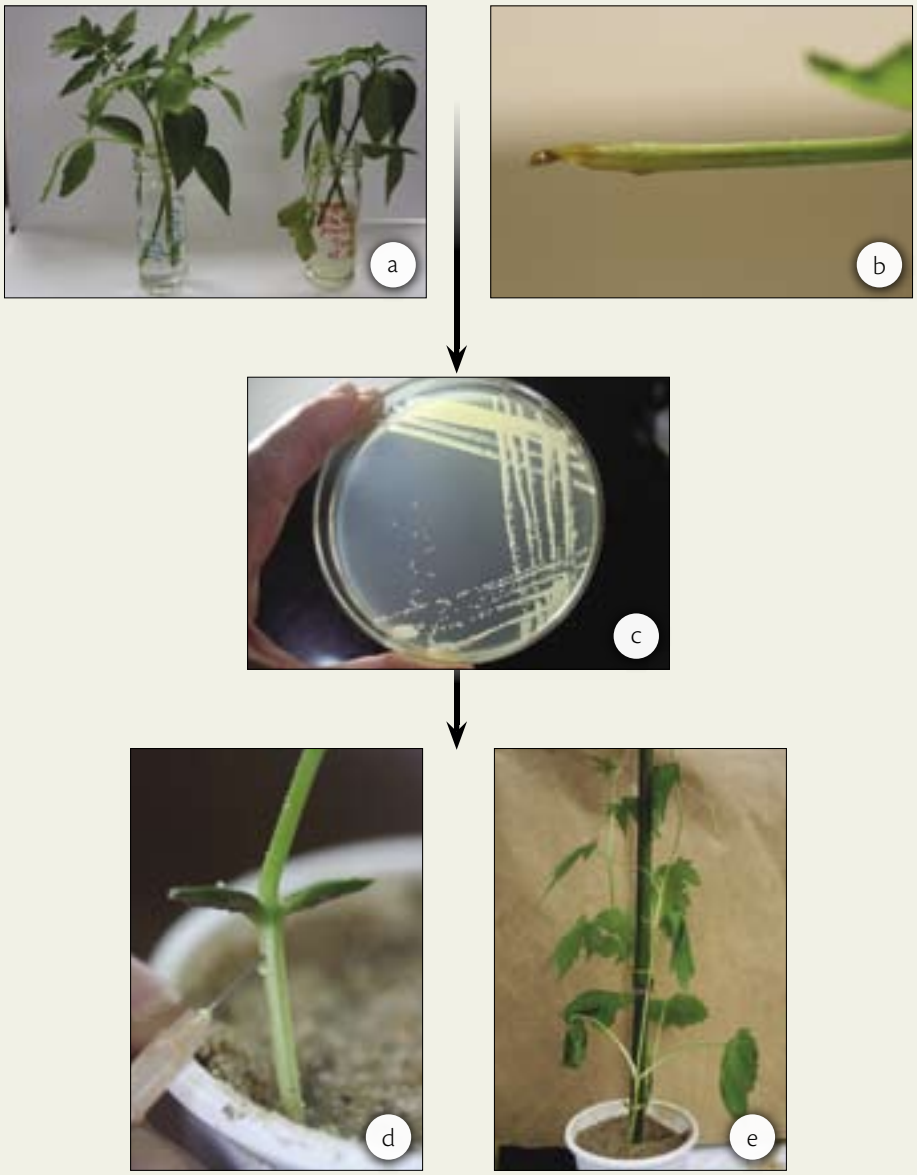


Figure 3.8 Bioassay procedure for isolating *Ralstonia solanacearum* from diseased ginger rhizome: (a) chilli and tomato cuttings in control (left) and wilted cuttings in water extract from rhizome segments (right), (b) wilted chilli cutting showing vascular browning, (c) isolation of *R. solanacearum* from chilli cutting, (d) and (e) pathogenicity test in bitter melon of bacterium isolated in the bioassay

DIAGNOSTIC CASE STUDY 2 (CONTINUED)

A stem section from each wilted bait seedling was macerated in sterile water and streaked on King's B medium. A single colony from this medium was then subcultured for purification and also inoculated into the stem of a 6-week-old bitter melon plant, to assess virulence. Some of the bitter melon plants showed severe wilting and the isolation process was repeated with these stems to obtain pure cultures of *R. solanacearum* for identification at an international reference centre.

Confirming identification with pathogenicity tests

Fusarium oxysporum f. sp. *zingiberi* has been reported widely in many countries as a cause of Fusarium wilt of ginger. However, it was essential that the isolates of *F. oxysporum* from ginger from Quang Nam were tested for pathogenicity in local ginger cultivars, to prove that they were pathogenic isolates and not saprophytic strains. Therefore, representative isolates were grown on millet seed/rice hull medium for use in pathogenicity tests.

Because *R. solanacearum* is also known to cause bacterial wilt of ginger, pure cultures of *R. solanacearum* were also tested for pathogenicity to local cultivars of ginger to complete Koch's postulates (criteria used to establish a causal relationship between pathogen and a disease).

For precise identification, pure cultures of *R. solanacearum* were also sent to an international reference laboratory. This species is variable, including a number of races, which differ in host range and require different crop rotations for control.

Samples of *Meloidogyne* galls were also forwarded to a reference laboratory for precise identification of species.

After proof of pathogenicity is obtained

Once it was established that wilt pathogens are responsible for the wilt disease on the ginger plants, staff developed a supply of pathogen-free ginger rhizomes for planting in small demonstration plots using the pathogen-free rhizomes and soil. The soil was deemed to be free of the pathogen where crops resistant to bacterial wilt (and root knot nematode) had been grown previously. Note that *R. solanacearum* has a wide host range.

Although *F. oxysporum* f. sp. *zingiberi* only causes disease on ginger, it may persist on roots of symptomless non-host plants. Good crop hygiene is important so that soil from diseased fields is not introduced into disease-free fields on shoes and digging implements.

4 Symptoms of disease

Diagnosis begins with careful observation of all parts of the diseased plant—foliage, flowers, fruit, stems and roots. It can be difficult to identify the pathogen responsible because many plant pathogens cannot be identified with the unaided eye. The visible effects of the pathogen on the plant—symptoms—can help in determining the type or types of pathogens present. Symptoms of disease may be caused by:

- damage to the plant tissues
- disruption of the normal physiological functions of the plant:
  - water and nutrient uptake
  - photosynthesis
  - growth.

Symptoms of disease should be recorded carefully in a field notebook and in photographs using a digital camera (if possible).

4.1 Common symptoms

Common non-specific symptoms may be caused by many different types of pathogens. Wilting, yellowing and stunting are common non-specific symptoms (see wilt pathogen sections). Wilting is commonly caused by vascular wilt pathogens, root rots and root galls, collar rots, stem rots and by dry soil. These and many other diseases also cause stunting and yellowing. Therefore, it is important to examine all parts of the plant—most importantly the roots.

Pathogens that damage roots or stem tissues—typically fungi and nematodes—disrupt the absorption of water and nutrients. This can cause wilting, yellowing and stunting, beginning with stunting of the plant and, as the disease progresses, wilting, yellowing and plant death. Bacterial wilts cause wilting and plant death and some virus diseases also cause wilting, stunting and plant death.

Leaf disease symptoms may be caused by fungal pathogens (leaf spot and blight), bacterial pathogens (leaf spot and blight) and plant pathogenic viruses (mosaics or mottling, leaf dwarfing and leaf rolling). Viruses may also cause non-specific symptoms (yellowing, wilting and stunting).

Plant parasitic nematodes mainly affect plant root systems, causing root lesions, root galls, and root proliferation in association with cysts. This leads to stunting, yellowing, wilting and sometimes plant death.

Some symptoms can help in determining the cause of a disease as they are specific to certain pathogens. For example, the distinctive root galls caused by *Meloidogyne* spp. (root knot nematodes) or club root caused by *Plasmodiophora brassicae*, enable an accurate diagnosis in the field.

Legumes such as peanuts and soybeans have root nodules—small swellings on the roots caused by infection with nitrogen-fixing bacteria such as *Rhizobium* spp. These are beneficial and important to the health of these plants. Healthy nodules are usually pink when cut with a knife.

Due to the range of possible symptoms that may be observed in the field, plant pathologists should examine diseased plants carefully and take clear notes to help in making an accurate diagnosis. In addition, plant pathologists should question farmers to get information about the history of the crop and the development of the disease.

A single plant may be affected by several different pathogens, causing a range of disease symptoms.

Diseases thought to be caused by plant viruses should be sent to a laboratory for examination by virologists experienced in identifying viruses. The accurate identification of plant parasitic nematodes, plant pathogenic bacteria and related pathogens also requires expert examination.

In contrast, many fungal pathogens can be identified successfully in the field or in a basic laboratory, provided adequate reference materials are available.

Seek help if you are not confident in determining the cause of a disease. Do not guess—the farmer's income will be affected by your diagnosis and advice.

Plant pathogens are identified as the likely cause of disease in only about half of plant samples sent to diagnostic laboratories. Plants may be affected by other factors such as pesticides or environmental stress.

4.2 Diseases of foliage, flowers or fruit

Many fungal pathogens cause diseases of the foliage (leaves, petioles and stems), flowers or fruit, and usually produce spores from spore-forming structures on the diseased tissue. The spores are carried by wind or splashed by rain onto other plants, which spreads the disease. Fungal pathogens can rapidly build up disease levels and cause epidemics under weather conditions suitable for the specific pathogen.

The presence of spore-forming structures such as pycnidia or perithecia, conidiophores or sporangiophores are the basis for morphological identification of fungal pathogens, and the diagnosis of plant fungal disease.

Use a hand lens to assist with the identification of fungal structures in the field. However, for accurate identification of spores and spore-forming structures, use a microscope in the laboratory.

Many fungal pathogens cause spots (lesions) on the leaves, flowers or fruit. Yellow spots are chlorotic lesions and brown spots are necrotic lesions. Brown spots are called 'necrotic' because the pathogen has caused death, or necrosis, of the tissue. Fungal pathogens that cause lesions on leaves, flowers and fruit can often be isolated and grown on culture media.

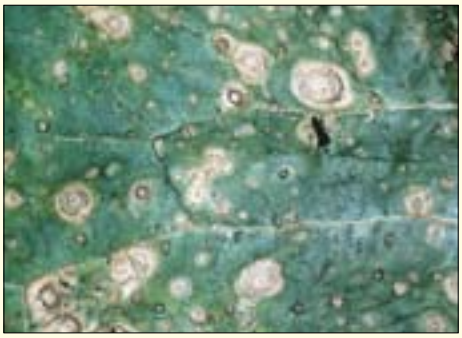
Saprophytic fungi also grow on diseased plant tissues as secondary colonisers. For example, saprophytic species of *Alternaria* and *Pestalotia* commonly grow on diseased leaf tissue and form spores on the tissue. These saprophytic species can lead to an incorrect diagnosis as they are usually visible under the microscope and grow rapidly on the isolation medium.

4.2.1 Spore production on diseased foliage

Fungi may form spores asexually from specialised hyphae called conidiophores. In the species *Stemphylium*, *Botrytis*, *Aspergillus*, *Penicillium*, *Bipolaris*, *Alternaria* and *Cercospora*, conidiophores develop on the diseased tissue from hyphae (Figure 4.1). Some species (e.g. *Septoria*, *Diaporthe*, *Didymella*, *Ascochyta* and *Phoma*) form conidia in specialised structures called pycnidia, others (e.g. *Colletotrichum*, *Cylindrosporium*) in structures called acervuli.

Conidia formed in conidiophores on surface of tissue

Stemphyllium
Botrytis
Aspergillus
Penicillium
Bipolaris
Alternaria
Cercospora



Conidia formed on an acervulus

Colletotrichum
Cylindrosporium

Conidia formed in pycnidia on tissue

Septoria
Diaporthe
Didymella
Ascochyta
Phoma

Figure 4.1 Formation of conidia on foliage by various fungal pathogens

Some fungal pathogens also produce spores from sexual reproductive structures on leaves, stalks, stems or fruit. For example, *Fusarium graminearum* (*Gibberella zeae*) forms ascospores in an ascus within perithecia on mature diseased stalks and cobs of maize. This fungus causes stalk and cob rot of maize.

Perithecia are similar in shape to pycnidia, but pycnidia form conidia and do not have asci.

4.2.2 Obligate foliar fungal and fungal-like pathogens

The downy mildews, powdery mildews and rusts are caused by obligate plant parasites. These pathogens are only able to infect, grow and produce spores in living host tissue—they cannot be isolated and grown in culture—and are usually only able to infect one or two host species or varieties (cultivars). For example, peanut rust can only infect peanuts.

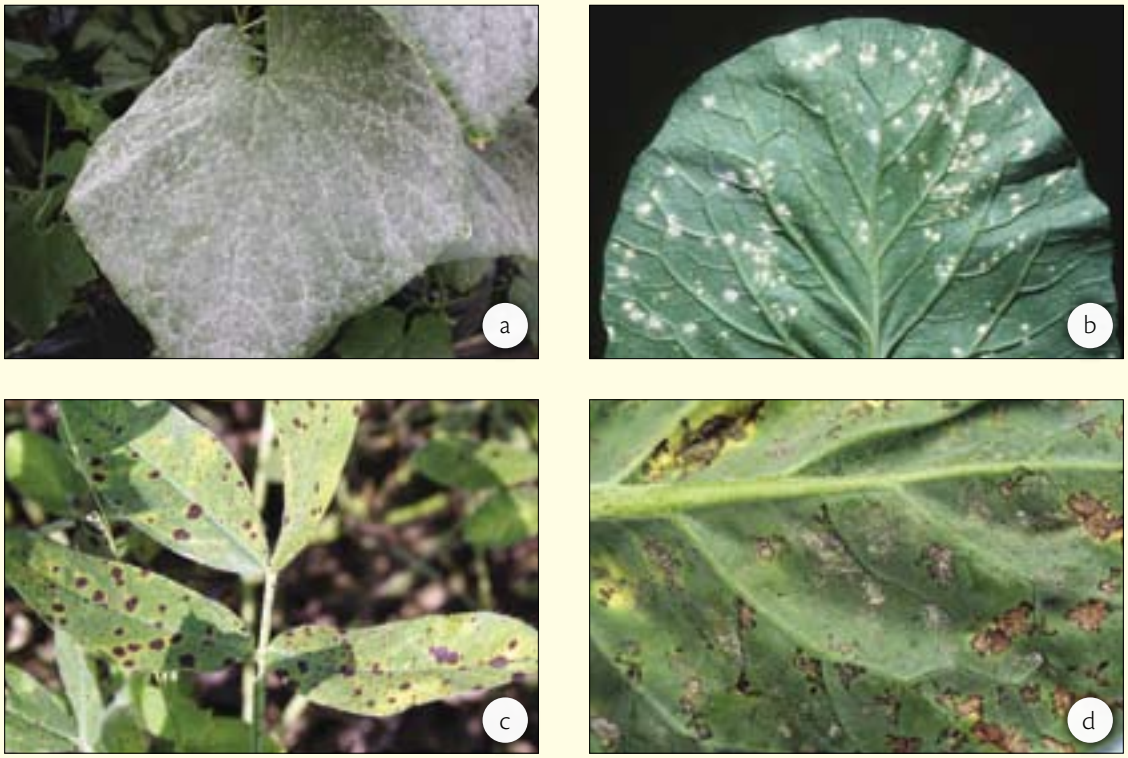


Figure 4.2 Fungal and fungal-like pathogens of the foliage: (a) powdery mildew on a cucurbit, (b) white blister on Brassica sp., (c) Cercospora leaf spot and rust on peanut, (d) downy mildew on cabbage

The symptoms of downy mildews, powdery mildews and rusts are usually obvious (Figure 4.2). They absorb nutrients from living plant cells, which commonly leads to yellowing of the tissue. Photosynthesis in the leaf tissue is reduced, leading to reduced plant growth.

4.2.3 Pathogens that produce sclerotia on infected tissue

In humid conditions, some fungal pathogens produce hyphae and/or sclerotia on infected plant surfaces. Hyphae of some *Rhizoctonia* species grow on infected stem bases and leaves. In Vietnam, some *Rhizoctonia* species produce irregularly shaped brown sclerotia on diseased tissue on maize leaves and cabbages (Figure 4.3a).

Sclerotium rolfsii causes stem base rot on many annual vegetable and field crops in hot wet weather in Vietnam. *S. rolfsii* forms obvious white hyphae (mycelium) on the infected stem base and small round brown sclerotia (Figure 4.3b) from these hyphae.

Sclerotinia sclerotiorum produces white mycelium and large black sclerotia on the stems and foliage of many broad-leafed crops, such as long and short beans, tomatoes, cabbages, potatoes and lettuce (Figure 4.3c).

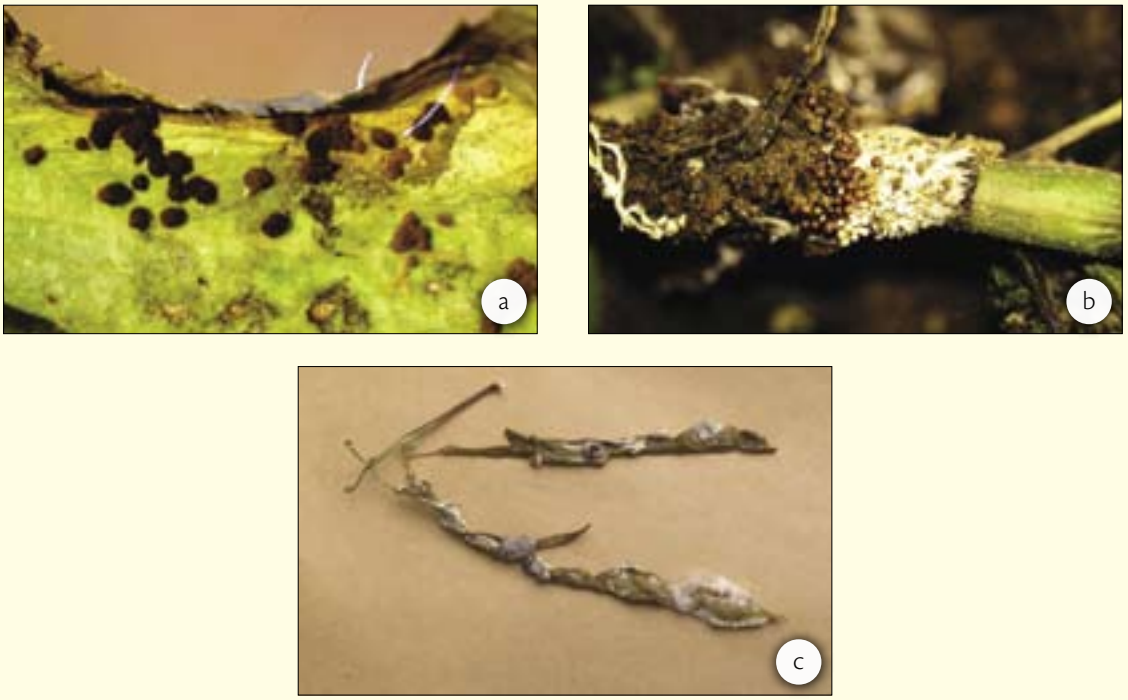


Figure 4.3 Sclerotial formation by (a) *Rhizoctonia solani*, (b) *Sclerotium rolfsii* and (c) *Sclerotinia sclerotiorum*

4.3 Diseases of roots, crown and stem

Fungal pathogens can cause serious diseases of the roots, crown (base of the stem) or the stem. Some fungal pathogens only affect seedlings, killing them before or after emergence; others only affect the feeder rootlets, and some species only affect the stem (e.g. *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*).

Root symptoms may be non-specific. Rots of the feeder rootlets, main roots, crown or stem disrupt the uptake of water and nutrients, causing stunting and yellowing of the leaves, wilting and sometimes death of the plant.

Genera that commonly cause these diseases in Vietnam are *Phytophthora*, *Pythium*, *Fusarium*, *Sclerotinia*, *Sclerotium*, *Rhizoctonia* and *Phoma* (Figure 4.4). One root parasite, *Plasmodiophora brassicae,* which causes club root of crucifers, is an obligate parasite and cannot be grown on culture media. There are also Basidiomycota pathogens of the trunk and roots of perennial tree crops (Shivas and Beasley 2005), which are generally difficult to isolate into pure culture.

Root rot pathogens can be difficult to isolate as there can be many saprophytic fungi and bacteria in diseased root tissues, which will also grow on isolation media—commonly overgrowing pathogens.

4.4 References

Shivas R. and Beasley D. 2005. Management of plant pathogen collections. Australian Government Department of Agriculture, Fisheries and Forestry. At: <http://www.daff.gov.au/planthealth>.

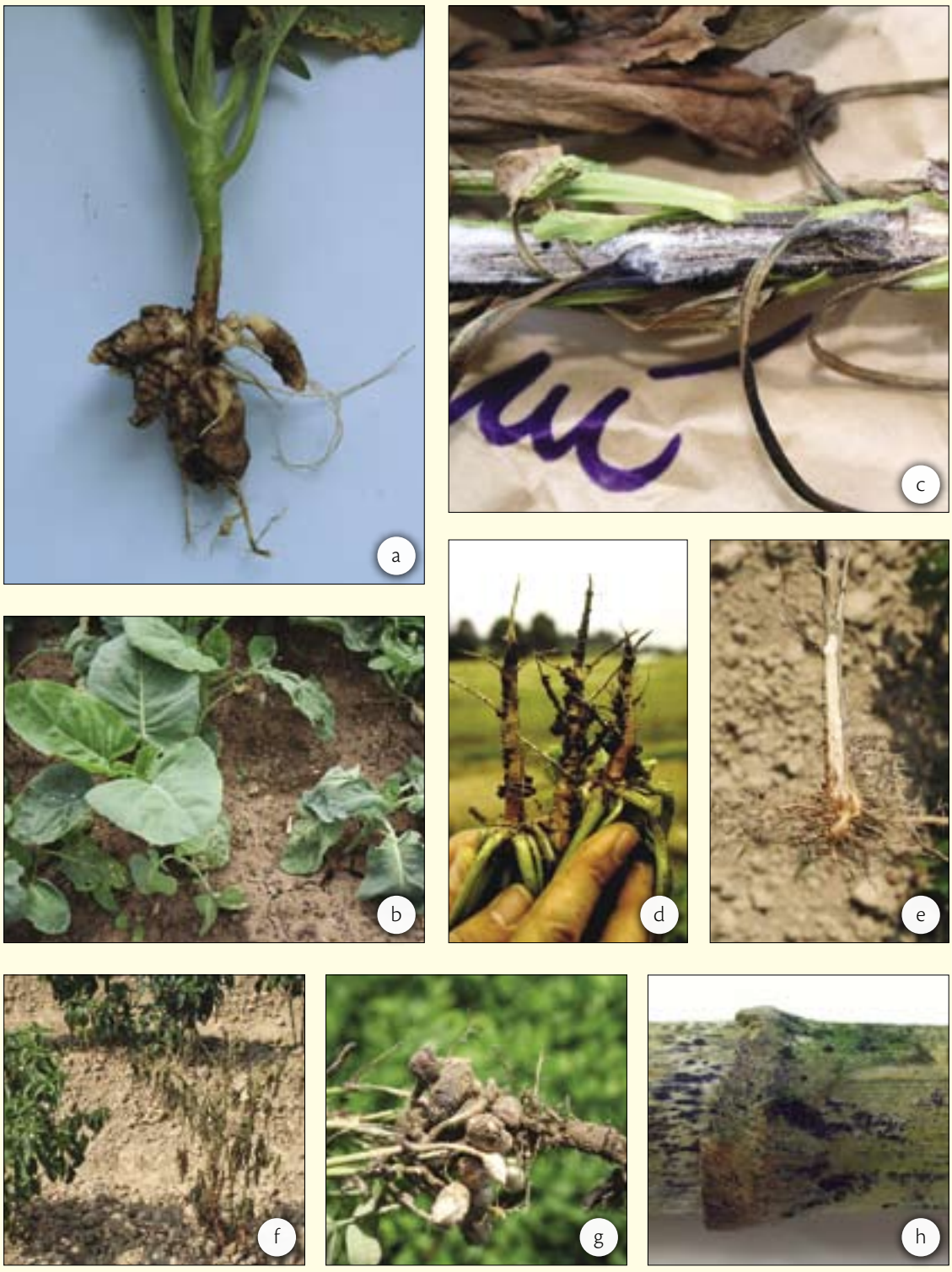


Figure 4.4 Diseases of the crown, roots and stem: (a) club root of crucifers, (b) wilting of crucifers (healthy [left] and diseased [right]) caused by club root (*Plasmodiophora brassicae*), (c) Fusarium wilt of asters (note the production of sporodochia on the stem), (d) spear point caused by *Rhizoctonia* sp., (e) Phytophthora root rot of chilli, (f) Phytophthora root rot of chilli causing severe wilt, (g) Pythium root and pod rot of peanuts, (h) perithecia of *Gibberella zeae* causing stalk rot of maize

5 In the field

Farmers and district staff are usually the first to observe diseases on crops. Plant pathologists may then be asked to identify the disease and the pathogen.

If material is to be sent to a diagnostic laboratory, it is very important that all relevant information is obtained from the farmer or district staff and sent with them (Figure 5.1). District staff can provide information about the disease on a field notes diagnostic sheet (Section 5).

However, it is recommended that the plant pathologist visit the farmer to examine the diseased crop with the help of district staff. The plant pathologist can collect fresh samples for laboratory study and obtain information such as crop variety, management practices, previous crops and climatic data. This information is particularly important in the diagnosis of the disease. A list of field equipment used by plant pathologists in the field is provided in Section 5.1. Samples should be kept cool and transported to the diagnostic laboratory as quickly as possible for detailed examination.

Figure 5.1 Talking with farmers in the field

Disease Surveying: Field Notes

Collector's name: ..... Collection date: .....

Farmer's details:

Name: ..... Phone: .....

Address: .....

Diseased sample:

Type of sample collected: .....

Crop species: ..... Variety/rootstock: .....

Source of seeds/plants: ..... Plant maturity: .....

Symptoms (compare with healthy specimens): .....

.....

.....

Signs (evidence of the pathogen): .....

.....

.....

Field observations:

Percentage affected: ..... Distribution/patterning: .....

Paddock history: .....

Other plant species present: .....

Rainfall, irrigation events: ..... Soil type: .....

Association with terrian (slope of land): .....

Where was the problem first observed: .....

Chemical use (herbicides, insecticides, fungicides): .....

.....

Additional information (insect damage, other diseases): .....

.....

.....

.....

Phiếu điều tra bệnh trên đồng ruộng

Tên mẫu: ..... Ngày lấy mẫu: .....

Thông tin về chủ ruộng:

Tên: ..... Điện thoại: .....

Địa chỉ: .....

Thông tin về mẫu bệnh:

Phân loại mẫu: ví dụ mẫu thân, lá, rễ hay đất: .....

Tên cây trồng:..... Tên giống: .....

Nguồn gốc hạt giống: ..... Tuổi cây: .....

Mô tả triệu chứng bệnh: .....

.....

.....

Những dấu hiệu điển hình chứng tỏ cây bị bệnh: .....

.....

.....

Thông tin quan sát được trên ruộng:

% số cây bị bệnh: ..... sự phân bố của bệnh: .....

Tên các cây trồng trên ruộng trước đó: .....

có luân canh với cây trồng khác không?: .....

lượng mưa và hệ thống tưới tiêu: ..... Loại đất: .....

đất bằng phẳng hay đồi dốc: .....

vị trí phát sinh bệnh đầu tiên: .....

những loại thuốc trừ cỏ, trừ sâu và trừ bệnh đã sử dụng: .....

.....

Sơ qua về tình hình các loại sâu bệnh khác đang tồn tại trên ruộng: .....

.....

.....

.....

5.1 Field equipment for diagnostic studies

It is important to prepare equipment carefully before a field survey. The following equipment is essential for sampling plant material and recording relevant information. Many of the items in this basic diagnostic kit can be obtained from local markets in Vietnam. Box 5.1 is a checklist of field equipment (Figure 5.2).

A notebook is essential for recording disease symptoms and taking notes on the crop and the history of the site. A hand lens or magnifying glass is used to examine fungal structures, such as sclerotia and pycnidia. A digital camera, if available, can be used to record images of symptoms of disease in the field.

Envelopes and several different sizes of paper and plastic bags should be included in the kit for storing plant samples. Critical information should be written on the bags using a permanent marker pen. This should include the name and address of the farmer, the location of crop and the sample number.

Different cutting implements are required for collecting samples:

- a large machete is used to cut large stems (e.g. banana) or woody stems (e.g. cotton), and to dig out entire plant specimens and collect small soil samples
- a small machete is used to cut small hard stems (e.g. chilli)
- two or three small sharp knives are used to cut soft stems (e.g. melon, tomato) to check for stem browning and bacterial ooze, or to collect samples for laboratory use
- secateurs are used to collect large numbers of samples.

Box 5.1 Field equipment checklist

- Notebook and pen
- Digital camera
- Hand lens
- Paper bags, plastic bags and envelopes
- Permanent marker pen
- Small and large machetes
- Knives and secateurs
- Small glass bottle (vial)
- Bottle of clean water
- Squeeze bottle of 70% ethanol
- Ice box and bottles of ice (keep a store in the freezer)
- Drinking water and a hat

This equipment can be transported easily on a motorbike. Digging implements can usually be borrowed from a farmer—they are awkward and dangerous to carry on a motorbike.

A small glass bottle (or vial) is essential for checking for bacterial ooze from the cut stem of a plant suspected of bacterial wilt. Clean water must be used to wash cutting implements and for use in the bacterial ooze test. Cutting implements should be surface sterilised with 70% ethanol before moving to another part of the field or sampling uninfected material.

A polystyrene ice box is essential to keep samples cool during transport between the field and the laboratory. Bottles of ice are ideal for keeping the ice box cool and should be stored in the freezer for this purpose.

It is also very important to take drinking water and a hat on field trips, to protect against the sun and to avoid dehydration.

Do not carry mud between crops on shoes or other clothing or equipment. Mud may contain pathogens and transfer them from diseased crops to healthy crops.

Figure 5.2 Suggested equipment for use in the field

5.2 Conducting a field survey

Step 1

Prepare the field equipment (Section 5.1) and arrange transport.

Step 2

Discuss the disease(s) with farmers and district staff.

Step 3

Walk slowly through the crop and note the types of symptoms present on the plants, the distribution patterns of the disease and any soil or other factors associated with the disease (see disease symptoms in Section 4).

Check carefully for signs of insect damage, the presence of virus vectors, weeds and pesticide damage as you walk through the crop. Talk to farmers about their observations.

Step 4

Carefully remove diseased plants from the soil and examine all parts of the plant for symptoms. Compare the diseased plant with an apparently healthy plant.

Wilting, stunting and leaf yellowing usually indicate a disease of the roots or stem (see Box 5.2). Check the roots carefully for root rot or root lesions, root galls and the proliferation of rootlets from one section of the roots.

Check for fungal structures at the stem base, such as sclerotia of *Sclerotium rolfsii* or *Sclerotinia sclerotiorum*.

Leaf mosaic or mottle, leaf yellowing, leaf curling, leaf roll, stunting or dwarfing of the plant can indicate the presence of a disease caused by a plant virus. Some viruses also cause wilt-like symptoms.

Check leaf spots for the presence of fungal structures, such as pycnidia or acervuli, with a hand lens. Powdery mildew, downy mildew and rust also reproduce on the leaf surface and form distinct spore masses. Downy mildew growth is usually on the underside of the leaf. In contrast, powdery mildews are usually more obvious on the upper side of the leaf.

Leaf spots with a watery, greasy or oily appearance often indicate the presence of a bacterial pathogen (see Box 5.3).

Cut the stem to check for stem browning, which can indicate a fungal or bacterial wilt. Check the cut stem for bacterial ooze by placing it in water (see Box 5.3).

More than one disease may affect different parts of the plant at the same time.

Step 5

Record disease symptoms with diagrams. Look carefully for the presence of fungal structures using the hand lens and take photographs.

Box 5.2 Detecting nematodes

Nematodes can cause non-specific symptoms (stunting, yellowing and wilting) in a range of crop species. The symptoms occur because nematodes reduce the ability of plants to take up water and nutrients through the roots.

Nematode damage can be identified by examining the plant roots closely (see nematode section).

Nematode levels in the soil can be determined using techniques that rely on nematode movement for separation of nematodes from the soil (e.g. Whitehead trays or Baermann funnels), or passive techniques (e.g. sieving).

Parasitic nematodes can be identified by the presence of a piercing stylet. This structure can be observed under a compound microscope or a good dissecting microscope.

There are a number of different genera of nematodes which can cause diseases of crop plants. Common pathogens include *Meloidogyne* spp. and *Pratylenchus* spp.

Step 6

Collect samples for examination and the isolation of potential pathogens in the laboratory. Most plant and soil samples, including samples with roots and soil, should be collected and stored in paper bags. Samples stored in plastic bags tend to 'sweat', encouraging saprophytic bacterial growth. Such growth can interfere with the isolation of the pathogen.

Small leaf samples are best carried in a small plastic box with some paper tissue to 'cushion' the specimen.

Label the samples carefully and store them in an ice box for transport.

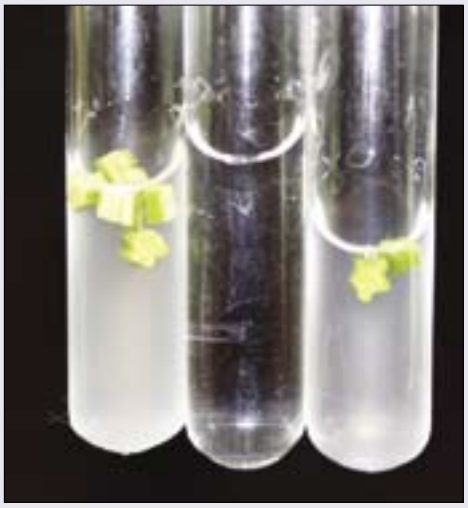
Step 7

Analyse the information collected from farmers, the notes on disease symptoms and the patterns of disease to determine the most likely causes of the disease. Use this analysis to guide laboratory work. Examine samples in the laboratory within a few hours of collection, if possible.

Step 8

Do not enter the laboratory wearing field clothes or shoes. Shower and change into clean clothes before entering the laboratory.

Box 5.3 Detecting bacterial pathogens



Bacterial pathogens can only infect (enter) plants via natural openings, such as those on leaf margins and root tips, or via wound sites, which may be created by insect damage or lateral root emergence.

Once inside the plant, some bacterial pathogens can quickly spread through the vascular system.

The bacterial ooze test provides a quick diagnostic test to determine if a plant has been infected by a bacterial wilt pathogen. This method quickly differentiates between wilt diseases caused by bacteria and those caused by fungi. A short stem section of the diseased plant is cut and placed in a small glass vial with clean water. If ooze emerges from the cut surface and the water solution becomes cloudy after a few minutes, the plant is probably infected by a bacterial pathogen. To be more confident about the outcome of the test, a control for this method can be set up using an asymptomatic stem (a stem from a healthy plant).

6 In the laboratory

In this section, guidelines are provided to assist inexperienced staff to develop skills essential for a diagnostic plant pathologist, including:

- microscope use
- isolation
- subculturing
- purification
- identification
- pathogenicity testing.

With experience, diagnostic staff can modify the procedures to obtain the most efficient and effective results. Techniques for preserving living fungal cultures, recipes for growth media and the principles and methods used in sterilisation are provided in Appendix 3.

6.1 Laboratory examination of the samples

The following procedures are for examining plants with wilting, stunting and foliar disease symptoms in the laboratory. The first step is to examine the whole plant carefully and to compare these observations with observations made in the field.

6.1.1 Wilting and stunting

If the plant is wilted and stunted, the cause is most likely a root or stem rot or vascular wilt disease.

1. **Check for vascular browning.**

   (a) If present, suspect bacterial or fungal wilt. Do bacterial ooze test.

   - If ooze is present, set up streak plates.
   - If no ooze is present, suspect Fusarium or Verticillium wilt. Plate out stem to recover fungus.

   (b) If absent, suspect a fungal or fungal-like stem or root rot or nematodes. (Note: also check for virus symptoms as some viral pathogens cause wilting and stunting.)

2. **Check for sclerotia and fungal mycelium.**

   (a) If present, attempt to isolate *Sclerotinia sclerotiorum*, *S. rolfsii* or *Rhizoctonia* spp. as indicated by the type of sclerotia observed.

   (b) If absent, suspect root rot, nematodes or club root of crucifers.

3. **Examine for evidence of nematodes (root knots or root lesions).**

   (a) If present, extract nematodes to confirm the pathogen and forward to a nematology laboratory for species identification.

   (b) If absent, and there is root rot (browning), attempt to isolate a fungal or fungal-like pathogen from the roots.

6.1.2 Leaf diseases

If the leaves show signs of disease, examine them under a dissecting microscope.

1. **Check for mosaic, mottling, leaf rolling or dwarfing of leaves.**

   (a) If present, suspect a viral disease. Forward the material to a plant virus diagnostic laboratory. (Note: yellowing and spots may also be caused by viruses, for example papaya ring spot virus and tomato spotted wilt virus.)

   (b) If absent, suspect a bacterial or fungal pathogen.

2. **Check for oily leaf spots or blights, or bacterial ooze.**

   (a) If present, suspect a bacterial pathogen. Isolate by streaking a bacterial suspension made from plant sap on King's B medium.

   (b) If absent, suspect a fungal pathogen.

3. Examine under a dissecting microscope for the presence of fungal structures.

With practice, downy mildews, white blister (*Albugo candida*), powdery mildews and rusts can be identified using dissecting and compound microscopes. As these are obligate plant parasites, they cannot be grown on culture media.

If signs of other fungal pathogens are present (hyphae, spores or reproductive bodies), microscope slides should be made for viewing under a compound microscope. Common leaf spot genera that may be identified include *Alternaria*, *Cercospora*, *Stemphylium*, *Septoria* and *Phomopsis*. Sporulation may also be induced by incubating diseased leaves in a moist chamber in the light. Leaves should be examined every day for signs of a fungal pathogen. (Note: saprophytic fungi and bacteria will grow quickly in a moist chamber, which may lead to an incorrect diagnosis.)

It is also recommended that tissue be plated from the margin of leaf spots from freshly collected material onto a low nutrient medium for isolation of the pathogen (see Section 6.3).

6.2 Microscopy

Dissecting and compound microscopes are essential items in a diagnostic laboratory and diagnostic plant pathologists should be familiar with their set up, maintenance and use.

6.2.1 Using a dissecting microscope

A dissecting microscope is used to examine diseased material for the presence of small fungal structures, such as pycnidia, acervuli, sporodochia and perithecia, under low magnification (up to approximately ×100). Using the dissecting microscope, such structures can be easily transferred to a slide preparation for examination under a compound microscope, at higher magnification (up to ×400).

A dissecting microscope is also used for fine work such as the transfer of germinated single spores or hyphal tips during purification of fungal cultures, and the examination of nematodes, which can also be transferred to a slide for examination under a compound microscope. In this way, it can be determined whether nematodes are plant parasitic or non-parasitic. Developing colonies are best examined under a dissecting microscope (Figure 6.1).

Figure 6.1 Examination of colonies under a dissecting microscope

Each dissecting microscope should be fitted with an adjustable mirror that tilts to provide direct and oblique lighting for low-contrast specimens. A built-in light source for illuminating the upper surface of material is ideal, but a separate light source can also be used.

6.2.2 Using a compound microscope

The instructions provided with a compound microscope should be followed carefully to ensure that it is used correctly and to avoid damage.

It is very important that objective lenses are not scratched or touched by agar, fungus or stained preparations. Coverslips (cover glass) are very thin and, if broken, can cut fingers.

Adjusting the compound microscope

1. Place a slide containing a specimen on the stage of the microscope.
2. Turn the lamp on and adjust the transformer to approximately 50% brightness.
3. Bring the specimen into focus with the ×10 objective.
4. Close the field iris diaphragm so that it becomes small.
5. Adjust the condenser height to bring the field iris diaphragm into focus.
6. Turn the two condenser centring knobs until the image of the field iris diaphragm is centred—this is very important!

7. Open the field iris diaphragm until it just disappears from view (i.e. until it is slightly larger than the field of view).
8. Adjust the aperture iris diaphragm to clearly show the specimen. The setting number (numerical aperture) on the aperture iris diaphragm should be approximately 75% of the numerical aperture on the objective being used.
9. Examine the specimen (Figures 6.2 and 6.3).

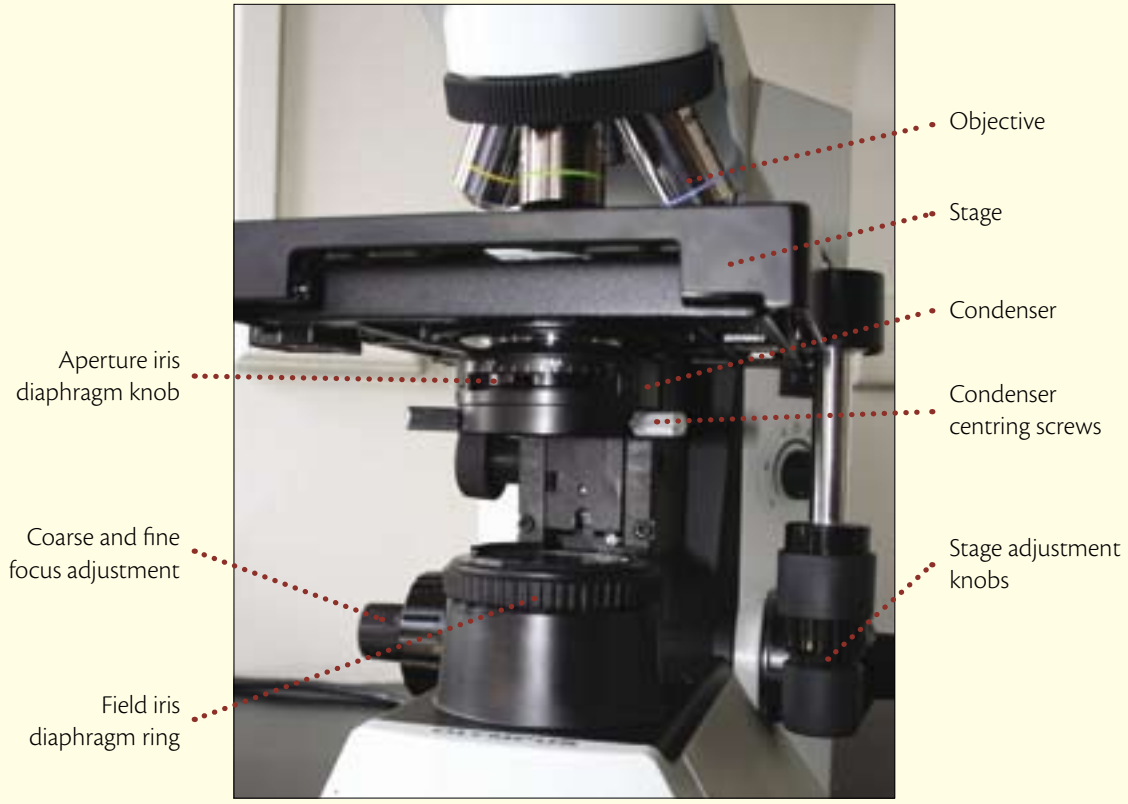
6.2.3 Preparing slides

Specimens such as fungal spores or spore forming structures such as pycnidia, perithecia or cleistothecia can be mounted on slides in water.

Mounting specimens in water

1. Place a small drop of filtered water on a slide.
2. Place material into the water drop, under a dissecting microscope.
4. Place a coverslip with one side touching the slide near one edge of the water drop.
5. Gently lower the other side of the coverslip onto the water drop—this method excludes air bubbles from the preparation.
6. Use a strip of blotting or filter paper to blot excess water at the edge of the coverslip.

Figure 6.2 Examination of fungal spores under a compound microscope



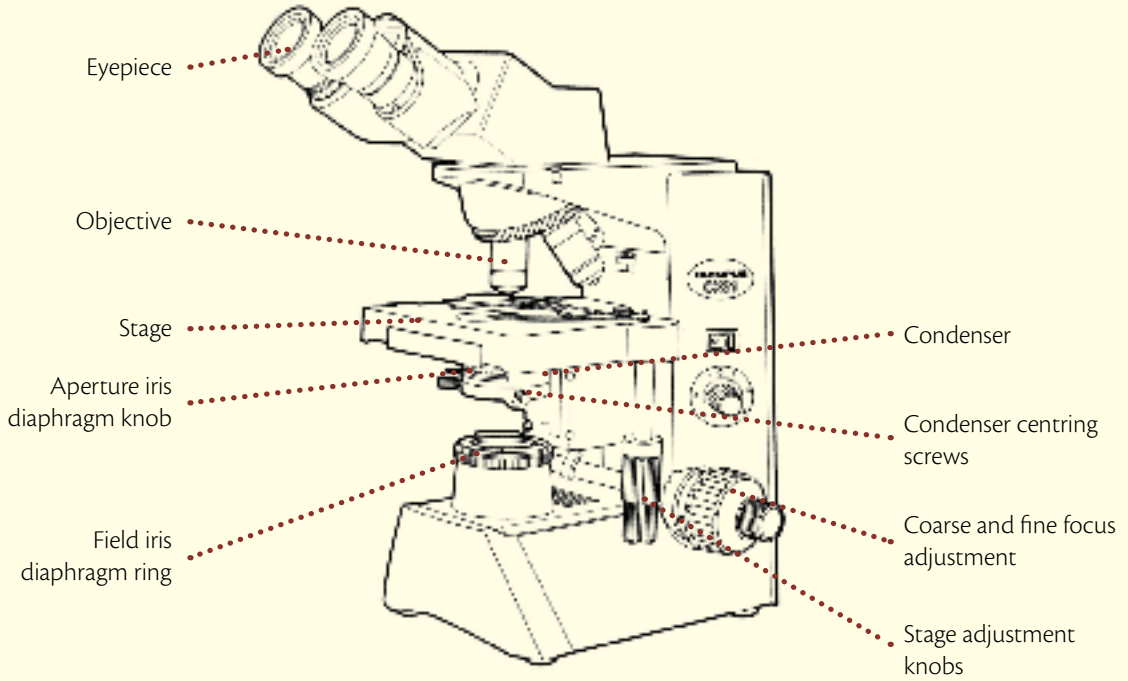


Figure 6.3 Components of a compound microscope

Spores from a diseased plant or culture can be scraped off with a transfer needle and transferred to the water drop.

Larger spore-forming structures should be examined under the dissecting microscope and then placed in the water drop and squashed (flattened) by pressing gently on the coverslip using a flat surface. On re-examination, pycnidia can be identified as containing only spores (conidia), while perithecia and cleistothecia will contain both asci and ascospores.

Structures with soft tissue, such as apothecia of *Sclerotinia sclerotiorum*, should be cut into thin slices (sections) with a wet razor blade or scalpel and transferred to the water drop.

6.3 Isolating fungal pathogens

The following isolation techniques for root, stem and leaf rot fungi do not need to be followed exactly, but can be used as a guide and modified to give the best recovery according to different pathogens and crop situations. It is important to learn and gain experience by experimenting with different methods.

It is recommended that staff participate in laboratory training workshops if they have no previous training in laboratory techniques. It is important to learn how to recognise pathogenic species and distinguish them from common saprophytes.

Practice isolation and subculturing procedures with a range of fungal pathogens.

Isolation techniques may be improved by:

- changing the duration of surface sterilisation of plant material
- removing outer layers of plant material
- making modifications to media (e.g. pH, nutrient and agar concentrations)
- adding antibiotics to the media.

Ethyl alcohol (70%) is a standard surface sterilant for laboratory equipment and diseased material. Sodium hypochlorite may also be used as a surface sterilant, but it becomes ineffective with age and exposure to light.

Always damp dry (blot) the plant tissue on sterile paper before plating.

Select newly diseased tissue for isolation. Do not use older diseased tissue as it will be colonised extensively by saprophytic fungi and bacteria.

The surface of plant tissue harbours many saprophytic fungi and bacteria, which must be killed before the disease-causing pathogen can be isolated. Many of these saprophytes will also grow quickly on isolation medium, so that the pathogen cannot be isolated.

Do not use potato dextrose agar (PDA) or other carbohydrate-rich media for isolation from diseased plant tissues, especially if isolating from roots. Saprophytic fungi and bacteria grow quickly on carbohydrate-rich media and suppress the growth of slower growing fungal pathogens.

The successful isolation of fungi from diseased plants depends on several factors:

- type of diseased tissue (leaves, stems, roots)
- method of surface sterilisation
- plating procedure
- isolation medium
- incubation conditions of isolation plates.

Past experience is an invaluable tool in selecting appropriate isolation procedures. Experience will often provide an indication of which types of fungal pathogens are likely to cause particular symptoms. When in doubt, draw on information from picture databases, accession books and published material.

6.3.1 Isolation from leaves and stems

Isolation from stems is often improved by removing the bark or outer stem tissues before surface sterilisation.

Basic isolation from leaves or stems

1. Wipe the work area with 70% ethyl alcohol.
2. Dip instruments (forceps and knife or scalpel) in 70% ethyl alcohol and flame dry. (Methylated spirits can be substituted for ethyl alcohol.)
3. Rinse leaf or stem tissue in water to remove soil and other debris.

4. Surface sterilise leaf or stem tissue by wiping the leaf surface with soft paper (paper tissue) dipped in 70% ethyl alcohol or by briefly dipping thick leaves in 70% ethyl alcohol for 5 seconds, rinsing in sterile water and damp-drying on sterile paper tissue.
5. Aseptically cut small pieces (approximately 2 × 2 mm) from the margin of the healthy and diseased tissue, and transfer them to a low-nutrient medium (e.g. water agar [WA]) or a selective isolation medium, placing the pieces near the side of the plate.
6. Incubate the plates at approximately 25°C, ideally under lights.
7. Check plates each day, and when fungal colonies develop from the pieces of plant tissue, transfer material from the margins to a medium such as PDA or WA that contains sterile pieces of plant tissue, for example, pieces of green rice stem, carnation leaf or bean pod. (Sterile pieces of plant tissue encourage sporulation, which aids in identification of the pathogen.)
8. Make a final identification using pure cultures grown from a single germinated spore or a hyphal tip. (Techniques for doing this are described in Sections 6.5.1 and 6.5.2.)

The medium used for isolation depends on the fungus suspected to be the cause of the disease. Water agar or one-quarter strength PDA, containing antibiotics if necessary, are the most useful general purpose isolation media. Selective isolation media may be used, such as peptone pentachloronitrobenzene agar (PPA) for *Fusarium* spp. and *Phytophthora* selective medium (PSM) for *Phytophthora* spp.

Saprophytic species of fungi, such as *Alternaria*, *Pestalotia* and *Cladosporium*, commonly colonise dying leaf tissue. The presence of these fungi can make it difficult to isolate pathogenic species of *Alternaria* or other foliar fungal pathogens, such as *Stemphylium* and *Bipolaris*.

Alternative method for isolating from leaf spots

1. Place the leaf or leaf piece on moist paper in a Petri dish in a humid chamber.
2. Incubate at approximately 25 °C under lights to promote sporulation.
3. Examine after 1–2 days under the dissecting microscope to locate spores or spore-forming structures such as pycnidia, acervuli or sporodochia.
4. Pour isolation plates containing WA with a drop of lactic acid (which reduces the pH and suppresses bacterial growth) or with added antibiotics (as used in PPA).
5. Using a sterile transfer needle, transfer the spores to the plates.

6.3.2 Isolation from small, thin roots

Small, thin feeder rootlets and lateral roots absorb nutrients for plant growth and are important to plant health. Pathogens such as *Rhizoctonia*, *Pythium*, *Phytophthora* and *Phoma* commonly cause diseases of these rootlets.

Many saprophytic fungi (e.g. some *Fusarium* spp. and *Trichoderma* spp.) and bacteria colonise the outer cells of the root cortex. Therefore, the isolation of pathogens from rootlets can be difficult.

Do not use severe surface sterilisation of small rootlets as the sterilant may kill all the fungi in the rootlet, including the pathogen.

Isolation from small, thin roots

1. Select diseased rootlets with both healthy (symptomless) and diseased parts, and wash them in three changes of sterile water in a small bottle. Add a small drop of detergent to the first wash.
2. Wipe the work area with 70% ethyl alcohol.
3. Dip instruments (forceps and knife or scalpel) in 70% ethyl alcohol and flame dry. (Methylated spirits can be substituted for ethyl alcohol.)
4. Dip the rootlets briefly in 70% ethyl alcohol, rinse quickly in sterile water and then damp-dry on sterile paper tissue. Alternatively, surface sterilise the rootlets in 1% sodium hypochlorite in 10% ethyl alcohol for 10–15 seconds only, immediately rinse in sterile water and allow to air-dry on sterile paper tissue in a sterile work chamber.
5. Aseptically cut root pieces 1–2 mm in size at the margin of healthy and diseased tissue and transfer onto WA or a selective medium.
6. Press the pieces gently into the surface of the agar to ensure good contact between the entire root segment and the antibiotics in the agar.
7. Incubate at approximately 25 °C and check each day under the dissecting microscope for fungal growth from the root pieces.
8. Subculture each colony onto PDA or WA containing sterile pieces of plant tissue, such as green rice stem pieces.
9. Purify by hyphal tipping (see Section 6.5.1) or by the single germinated spore technique (see Section 6.5.2) before final identification.

Note that it is usual to isolate some saprophytic fungi along with pathogenic fungi from diseased root tissue. With experience, some pathogenic fungi can be recognised on PDA by simple examination. The pathogenicity of isolates must be proven before a decision can be made on the cause of the disease.

6.3.3 Isolation from woody roots and stems

Often fungal root pathogens must be isolated from the main root or stem base of woody plant material. Isolation is generally more successful if stem tissue is plated. Commonly, there are fewer saprophytes in the stem base than in the woody root.

The choice of preparation depends on the amount of lignification (woodiness) of the tissue. Surface sterilisation of softer stems can be as simple as wiping or spraying the stem with 70% ethanol before plating onto media.

Isolation from woody roots and stems

1. Cut off and discard the lateral roots (Figure 6.4).
2. Wash the sample in water with a little detergent to remove soil and other debris.
3. Cut away the outside of the stem or root, an area which is often the source of saprophytes.
4. Remove the lower section of the stem at the soil line. Selection of tissue for isolation will depend on disease severity. Do not attempt to isolate from old diseased tissue. Ideally, plate segments from the margin of healthy and diseased tissue.
5. Spray the sample with 70% alcohol.
6. Flame off excess alcohol, or if stem is soft, allow the alcohol to evaporate.
7. Cut thin segments of stem tissue and plate onto selective or low-nutrient media.

6.3.4 Soil baiting

Soil baiting is an indirect method of isolating *Phytophthora* and *Pythium* species from soil or roots.

Isolation from soil using apples or other fruits as bait

1. Swab the apple with alcohol.
2. Cut a hole approximately 10 mm in diameter through to the core on one side using a sterile cork borer.
3. Pack the hole with soil and cover it with sticky tape to retain the soil.
4. Incubate the apple at room temperature in the light.
5. Isolate fungi after 1–3 days from the margins of the fast-spreading, brown lesions.

This method is not completely selective as fast-growing zygomycetes may also cause similar lesions.

Isolation from flooded soil using leaves or petals as bait

Phytophthora and *Pythium* species can be isolated from soil by floating clean leaves or rose petals over flooded soil. If these species are present in the soil sample, zoospores are produced that move up to and infect the leaf or petal. This method is selective as it favours the isolation of species that produce zoospores.

1. Place up to 100 g of soil in a plastic cup.
2. Cover soil with sterile or filtered water to a depth of 5–10 cm.

Figure 6.4 Technique for isolating plant pathogens from woody tissues: (a) cutting off lateral roots, (b) washing the sample, (c) removing the lower section of the stem at the soil line, (d) spraying the sample with 70% alcohol, (e) allowing the alcohol to evaporate, (f) cutting segments of stem tissue

3. Float pieces of susceptible plant material on top of the water.
4. Incubate the cup for 2–4 days.
5. Isolate fungi after 2–3 days from the margins of lesions that have developed on the bait, using a selective medium (e.g. PSM), after rinsing in sterile water and sterilising the surface.

The bait material can also be the crop species thought to be affected by *Phytophthora* or *Pythium*. Potential baits include chilli leaves, rose petals, citrus leaves, and seedlings of chilli, lupins and soybean. If the bait will not float by itself, it can be suspended from a lid over the container, or from a piece of polystyrene foam or other suitable float.

Isolation from rootlets using host plant as bait

1. Wash diseased rootlets (Figure 6.5).
2. Place rootlets in a plastic cup and fill the cup with sterile or filtered water.
3. Float a leaf from the host plant on the surface of the water.
4. Incubate the cup for 2–4 days.
5. Isolate fungi after 2–3 days from the margins of lesions that have developed on the bait, using a selective medium (e.g. PSM) after rinsing in sterile water and sterilising the surface.

6.3.5 Soil dilution plate method

The soil dilution plate method is used for the isolation of *Fusarium* species from dry soil using PPA. It can be adapted for the isolation of other fungal species using appropriate selective media.

Figure 6.5 Baiting soil for *Phytophthora* using flower petals and leaves

Isolation by the soil dilution plate method from dry soil

1. Air-dry the soil sample, grind it lightly with a mortar and pestle (a marble kitchen mortar and pestle is appropriate) and mix thoroughly.
2. Transfer a 10 g subsample to 100 mL of sterile 0.01% WA in a bottle, to give a dilution of 1:10.
3. Transfer 10 mL to a second bottle containing 90 mL of 0.01% WA to give a dilution of 1:100 and mix well to ensure an even spread of the soil in the solution. Repeat this step to give a 1:1000 dilution, which is usually satisfactory for the isolation of *Fusarium* from vegetable or field crop soils (Figure 6.6).
4. Disperse (spread) 1 mL of soil suspension across the medium in a 90 mm diameter Petri dish:
   - prepare the plates and let them dry for a few days to eliminate water from the surface of the plate
   - carefully pipette 1 mL of soil suspension onto the edge of the medium to one side of the plate
   - hold the plate on a slight slope away from the suspension and gently shake at right angles to the slope, spreading the suspension in a uniform wetting front across the plate.
5. Incubate the isolation plates under lights for 5–7 days until colonies develop.
6. Subculture the colonies and purify them using the single spore technique on PDA, carnation leaf agar (CLA), or WA containing a sterile plant tissue fragment.

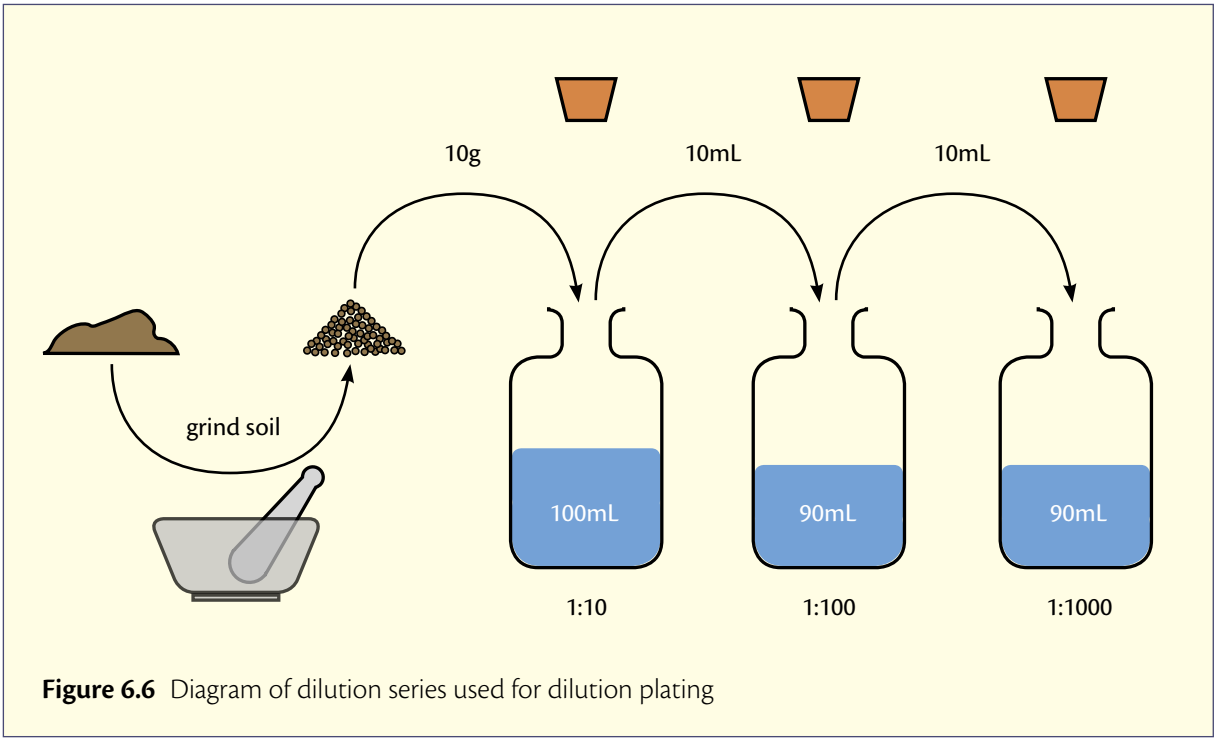


Figure 6.6 Diagram of dilution series used for dilution plating

Although a 1:1000 dilution is usually satisfactory, a dilution should be used that gives 10–30 *Fusarium* colonies per plate (Figure 6.7). As the level of *Fusarium* in soil depends on cropping history and soil type, a different dilution may be required to achieve the desired result.

Figure 6.7 Dilution plate containing *Fusarium* spp. on peptone PCNB agar (ideally the number of colonies should be between 10 and 30)

If the isolation procedure is designed to provide quantitative data, use 3–5 replicate plates per dilution and replicate (use several) soil subsamples. There may be considerable variation between replicate plates and between soil samples.

This technique does not determine the number of spores in the soil, but rather the number of 'propagules' of a species in the soil. Propagules might include conidia, chlamydospores and hyphal fragments in infested plant residues. The number of these colony-forming units (CFU) per gram of soil can then be calculated for each species using the following formula:

Dilution × mean no. colonies of fungal species on isolation plates = CFU/g soil

6.4 Subculturing from isolation plates

Subculturing is the stage between isolation from plant material and the creation of pure cultures. This stage helps to determine which organism has been isolated.

The subculturing process

1. Examine the plates under the dissecting microscope each day and assess the growth of fungal hyphae from the segments of plant tissue.
2. Determine if there is more than one fungal species growing.
3. Subculture when there is approximately 5 mm of hyphal growth from the plant tissue.
4. Cut out a small block of agar (2 × 2 mm) from the margin of each colony and transfer it to PDA or a natural substrate medium (e.g. CLA or green rice stem agar).

There are some fungal diseases where the pathogen is readily isolated and where saprophytes rarely cause problems. For example *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* are readily isolated from the margins of healthy and diseased tissue in green stems.

Isolation from other plant parts can be more difficult. When isolating from diseased roots, generally two or more fungi grow from the root, even on selective isolation media (Figure 6.8). The same problem can occur when attempting to isolate a pathogen from a fungal leaf spot, because saprophytic fungi quickly colonise leaf tissue weakened or killed by the pathogenic organism. It is important to subculture when these colonies are small, as it is easier to subculture before the fast-growing species grow over the slower-growing species.

Therefore, examine isolation plates each day for fungal growth. Check the tissue segment for sporulation, which may give an indication of the identity of the pathogen. However, be aware that the sporulation may be from a saprophyte.

Practice makes perfect! Practice isolating a wide range of fungal pathogens to gain experience and to learn to recognise how common fungal pathogens develop on isolation plates.

Saprophytic fungi are common in diseased tissue and often colonise isolation plates (Figure 6.9). Some, such as *Trichoderma*, interfere with the isolation process. Airborne fungal saprophytes, such as *Penicillium* and *Cladosporium* can also contaminate plates.

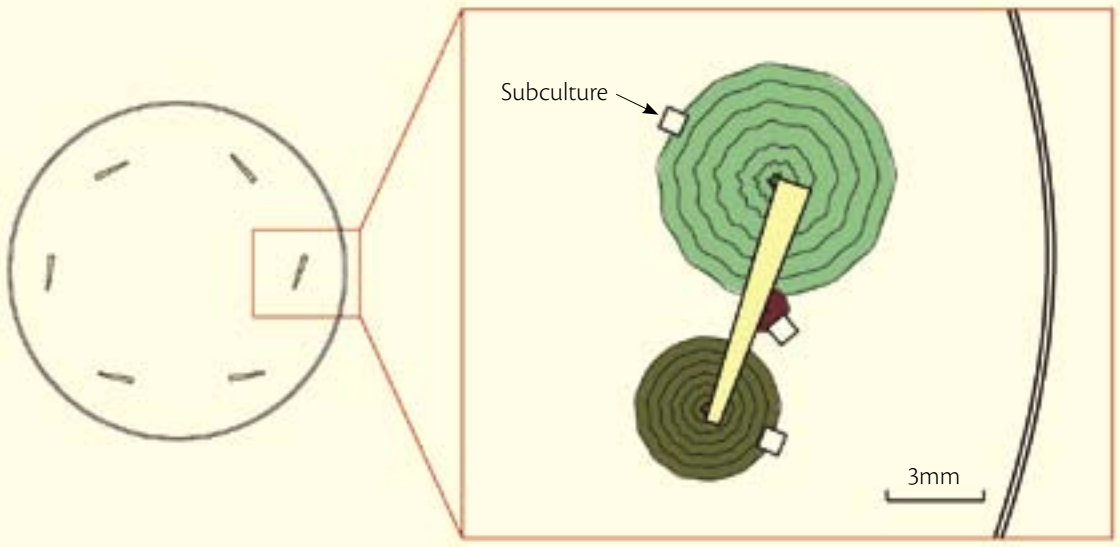


Figure 6.8 Diagram of a root isolation plate showing (inset) multiple fungi growing from the same root section

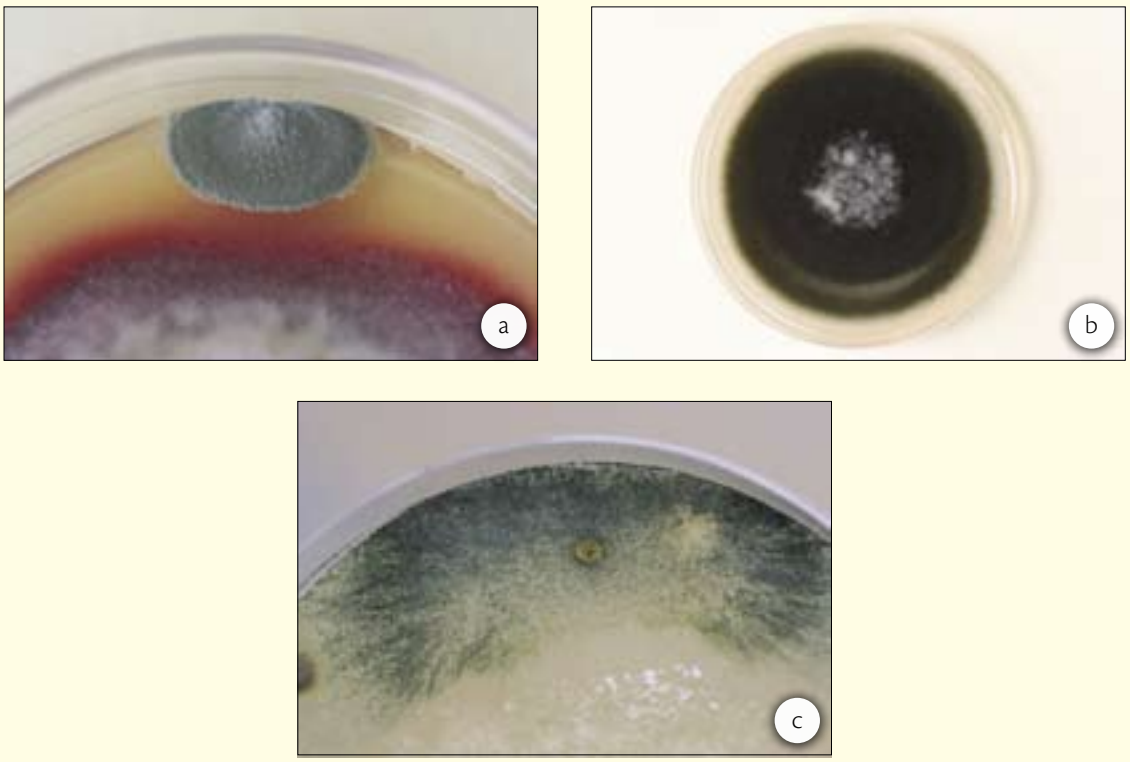


Figure 6.9 Common contaminants found on culture plates: (a) *Penicillium* sp. (airborne contamination), (b) *Cladosporium* sp. (in pure culture), (c) *Trichoderma* sp. (developing from a diseased root segment)

Once subcultures develop into colonies, these can be grouped by colony type and microscopic features for preliminary identification. Well-known saprophytes can be identified and discarded and potential pathogens can be purified by hyphal tip or single spore transfer to appropriate media for subsequent identification.

6.5 Purification of cultures

The final stage in identifying fungal pathogens is the creation of pure cultures. Only a single spore or hyphal tip is transferred to ensure a pure culture is produced.

6.5.1 Single sporing

Single sporing involves the transfer of a single germinated conidium to obtain a pure culture (Figure 6.10). This method is suitable for species of fungal genera that produce spores in culture, for example, *Fusarium*, *Colletotrichum*, *Alternaria*, *Stemphylium*, *Bipolaris*, *Verticillium* and *Phoma*.

Single sporing

1. Sterilise transfer needle.

2 & 3. Create a spore suspension by removing a small amount of surface mycelium with conidia or a small scraping of sporodochia from *Fusarium* spp. and place in 10 mL of sterile water in a test tube.

4. Shake the suspension to disperse the spores and check the spore concentration by holding the tube against the light or by examining a drop of the suspension under a dissecting microscope. Avoid high concentrations of spores. With experience, the concentration can be assessed visually in the test tube.

5. Dilute with sterile water if needed.

6. Pour the spore suspension onto a Petri dish containing a thin layer of water agar.

7. Pour out excess water. This leaves some spores on the agar.

8. Store the plate on its side for 18 hours until the spores germinate.

9. Examine the Petri dish under a dissecting microscope with a light source underneath. (Adjust the mirror on the light source carefully to obtain a good contrast between the agar and the conidia and germ tubes.)

10. Remove a single germinating spore using a flat transfer needle (Figure 6.11) and transfer it to a new medium (Appendix 1 describes how to make a flat transfer needle).

Sterilize transfer needle

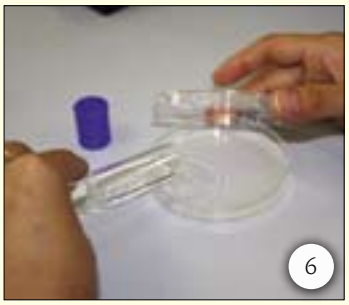
Scrape spores from colony edge

Add spores to sterile water

Check spore concentration

Dilute if necessary

Pour spore suspension onto thin water agar plate



Pour off excess water

Store plates on their side for 18 hours

Aseptically cut out a single germinated spore

Transfer to a new agar medium

Figure 6.10 Steps in the single sporing process

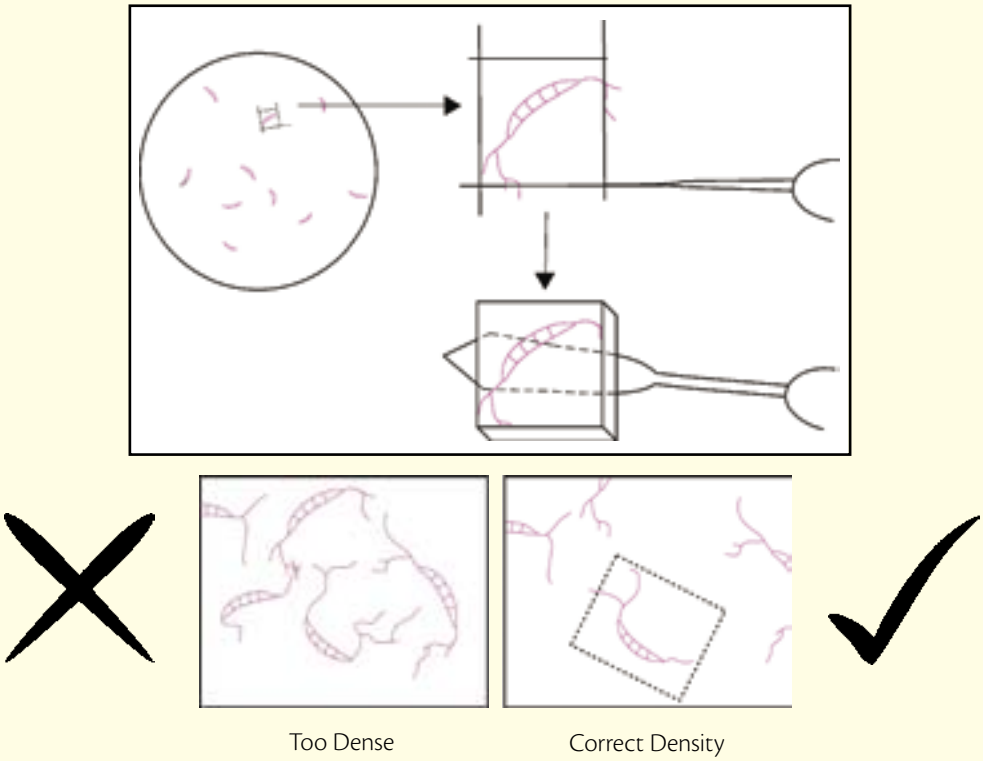


Figure 6.11 The single sporing procedure, showing correct selection of an individual spore

6.5.2 Hyphal tip transfer

Hyphal tipping involves the transfer of a single hyphal tip to obtain a pure culture. This method is suitable for species of fungal genera such as *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotium* and *Sclerotinia*.

Hyphal tip transfer

1. Pour a plate of water agar so that it is shallow on one side of the plate (Figure 6.12).
2. Inoculate on the side of the plate where the agar is deeper with a small agar block taken from a subculture isolation plate.
3. Place the plate under a dissecting microscope and focus on the hyphae at the growing margin of the colony. (The hyphae will grow sparsely across the shallow region of the agar.)
4. Adjust the light source (mirror) to obtain a good contrast between the medium and the hyphae.
5. Aseptically transfer a small block of agar containing a single hyphal tip to a plate of suitable agar medium, using a flat transfer needle.

Transfer only a single hyphal tip to ensure a pure culture.

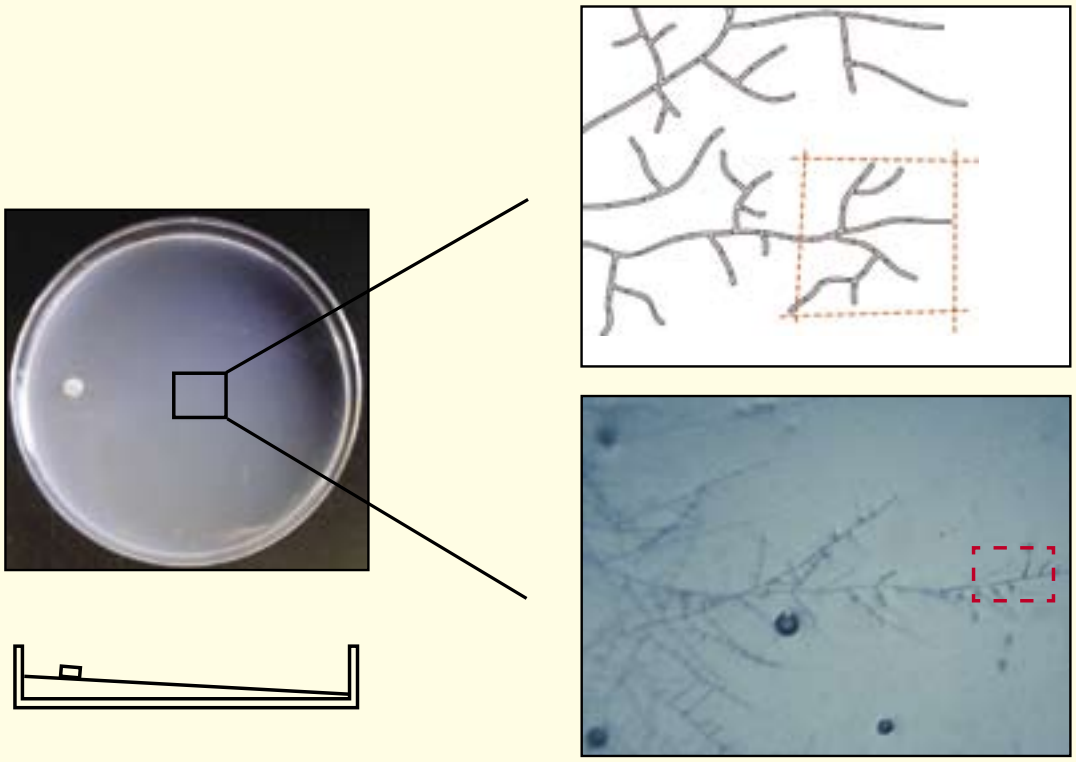
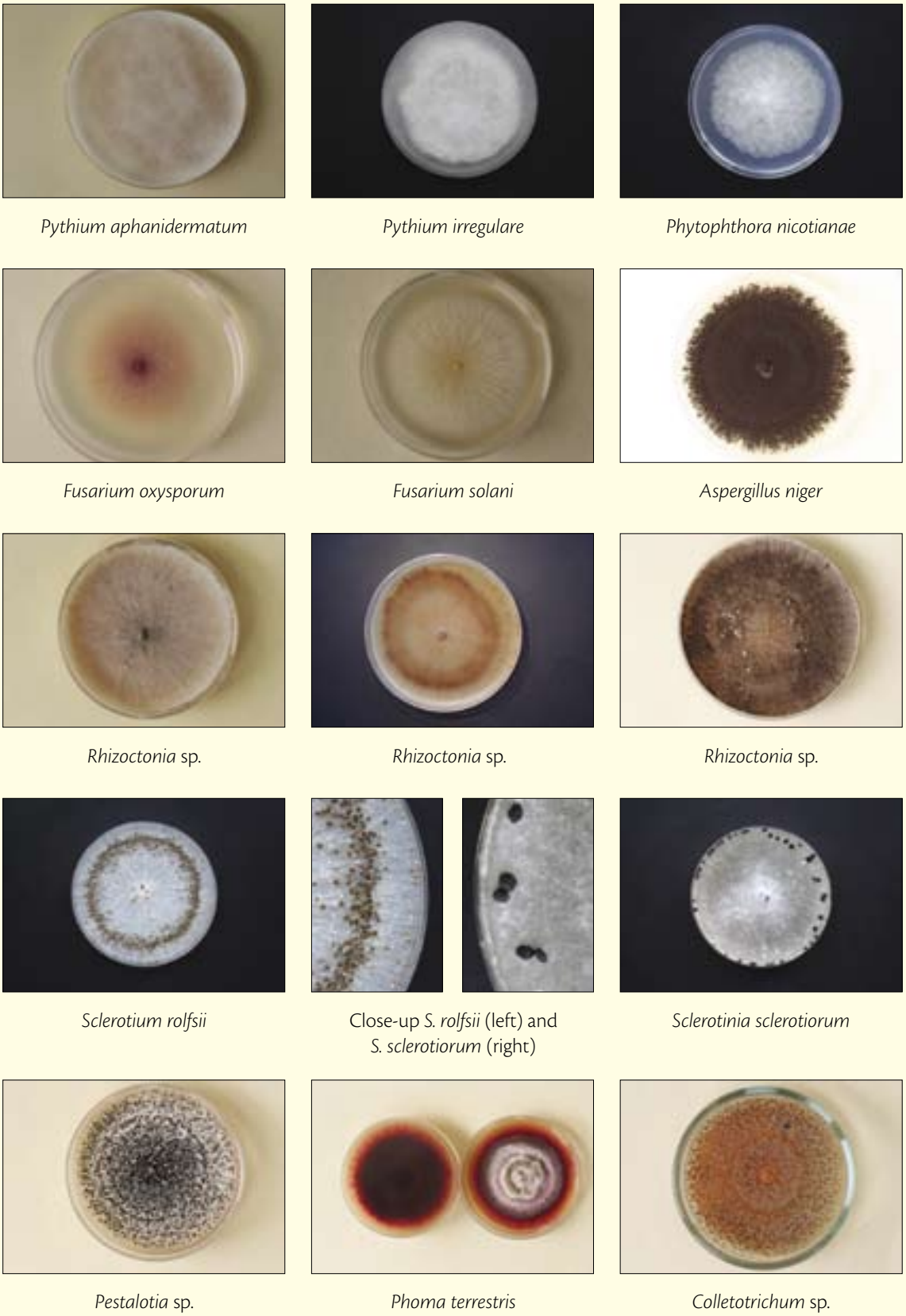


Figure 6.12 Hyphal tip transfer, example of tip removal from a sloped water agar plate of *Rhizoctonia* sp.

6.6 Recognising pure cultures

There can be considerable variation in colony morphology and pigmentation within a species. Diagnosis becomes easier as staff learn to recognise colonies of common fungal species on growth media (Figure 6.13). Cultures may then be sorted easily by eye into potential pathogenic species and probable saprophytic species. This can reduce the number of pure cultures that need to be prepared, saving resources and time.



Pythium aphanidermatum | *Pythium irregulare* | *Phytophthora nicotianae*

Fusarium oxysporum | *Fusarium solani* | *Aspergillus niger*

Rhizoctonia sp. | *Rhizoctonia* sp. | *Rhizoctonia* sp.

Sclerotium rolfsii | Close-up *S. rolfsii* (left) and *S. sclerotiorum* (right) | *Sclerotinia sclerotiorum*

Pestalotia sp. | *Phoma terrestris* | *Colletotrichum* sp.

Figure 6.13 Colonies of common fungal pathogens on potato dextrose agar

Final identification of pure cultures should be based on:

- micromorphological features (e.g. asexual and sexual fruiting bodies)
- spore morphology or morphology of sclerotia
- mode of formation of spores (e.g. nature of the conidiogenous cell and the presence or absence of chains of condia).

Some important plant pathogens do not produce spores and can only be distinguished by the presence and morphology of sclerotia (e.g. *Sclerotinia* spp., *Sclerotium* spp. and some species of *Rhizoctonia*). If asexual or sexual fruiting bodies, spores or sclerotia are not present, identification to species level can be difficult.

6.7 Identification of fungal pathogens

There are over 10,000 species of fungal and fungal-like plant pathogens. These pathogens cause a wide range of diseases, including leaf spots and blights, stem rots, cankers, root rots, wilt diseases and dieback, seedling diseases, fruit, head and grain rots, galls, rusts, smuts and mildews.

The accurate identification of a pathogen is the critical first step in the development of control measures and integrated disease management. If the name of the pathogen is known, information on its biology, epidemiology and potential control measures can be obtained from relevant publications, or via the internet.

Accurate identification is essential in selecting an appropriate fungicide for chemical control. For example, some fungicides that control downy mildews are not effective against powdery mildews.

The identification of fungal pathogens is based initially on morphological features, such as spores and spore-forming structures. Microscopic examination (see Section 6.2) is generally required to enable identification of the pathogen and diagnosis of the disease. Keys and guides to morphological identification will assist with this process, and there are also excellent illustrations of many common fungal pathogens in Agrios (2005). Other books and manuals should be kept as resources in diagnostic laboratories, and fortunately many scientific journal publications on taxonomy and identification can be accessed via the internet.

Obligate fungal parasites only grow on living host tissue (e.g. downy mildews, powdery mildews and rusts) and cannot be isolated into pure culture on agar media. Therefore, morphological identification depends on careful examination of the spores and spore-forming structures on the diseased tissue.

Many other fungal pathogens can be isolated and grown as pure cultures on artificial media under standard conditions. Spore forming structures and spores of these pathogens can be examined in culture for identification purposes. For example, most fungal pathogens that cause leaf diseases produce spore forming structures—perithecia, pycnidia, acervuli, sporangiophores or conidiophores—and these are readily examined microscopically.

Vegetative compatibility is another technique that can be used to identify strains. Sexual compatibility studies and molecular techniques can provide an understanding of genetic variation, and may be used to distinguish species that are morphologically similar. However, these techniques require more experience and resources than are available in small diagnostic laboratories.

6.8 References

Agrios G.N. 2005. Plant pathology, 5th edition. Elsevier Academic Press: San Diego, California.

7 Fungal taxonomy and plant pathogens

The following section provides a brief introduction to key features of the fungi and fungal taxonomy. The taxonomic system is the basis for learning to identify fungal pathogens and for understanding their biology.

Prepare a wall chart summarising the main taxonomic groups with examples of common fungi isolated in your laboratory.

7.1 Key features of fungi and fungal-like organisms

Fungal and fungal-like pathogens are heterotrophic—they need an external source of nutrients for growth, development and reproduction. An understanding of other key features of these organisms can assist in their identification:

- Hyphae—thread-like strands with a filmentous growth habit—are a common feature in most fungi. The hyphae colonise (grow through) substrates so that the organism can obtain nutrients. Plant pathogenic species colonise plants through the host surface, sometimes through direct penetration of intact plant surfaces. Saprophytic fungi tend to penetrate and colonise diseased plant tissue, senescing (dying) plants and plant residues. These fungi are major decomposers of organic matter in soil.
- Hyphal cell walls—true fungi have cell walls composed mainly of glucans and chitin, whereas fungal-like organisms have cell walls composed of cellulose and glycans.

- Septate hyphae—true fungi have cross-walls within the hyphae, whereas fungal-like organisms do not. This can aid in the differentiation of these two groups under microscopic examination.
- Motile spores—true fungi do not have motile spores, with the exception of the Chytrids. Motile zoospores (asexually produced spores) are common in many species in the Oomycota (e.g. *Pythium* and *Phytophthora*) and some downy mildews. Zoospores enable dispersal through water in soil and on plant surfaces.
- Wind-dispersed spores—many species of true fungi produce asexual or sexual spores for dispersal in the wind. This is a common feature of foliar fungal pathogens. However some spores are adapted to splash dispersal.
- Survival structures—thick walled spores (e.g. oospores and chlamydospores), sclerotia and multicellular reproductive structures (e.g. pycnidia and perithecia) are important in the disease cycle. During unfavourable environmental conditions or in the absence of a suitable plant host or other substrate, these organisms persist in such specialised survival structures.

7.2 Classification of plant pathogenic fungi

The classification of the fungi has changed significantly over the past 15 years, following phylogenetic analyses using molecular techniques. One approach to modern classification is summarised below. It generally follows the system in Agrios (2005) and includes some representative plant pathogens, common saprophytes and mycorrhizal species.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Protozoa						
	Plasmodiophoromycota (endoparasitic slime moulds)					
		Plasmodiophoromycetes				
			Plasmodiophorales (obligate parasites)			
				Plasmodiophoraceae		
					Plasmodiophora	
						brassicae (causes club root of crucifers)

Kingdom	Phylum	Class	Order	Family	Genus	Species
Fungal-like organisms						
Chromista						
	Oomycota (filamentous organisms that produce non-septate hyphae, asexual motile zoospores with flagella from sporangia, as well as oospores through sexual reproduction; cell walls composed mainly of glycans and cellulose)					
		Oomycetes				
			Peronosporales			
				Pythiaceae		
					Pythium	
					Phytophthora	
				Peronosporaceae (form wind-borne sporangia on sporangiophores, obligate parasites)		
					Peronospora	
					Pseudoperonospora	
					Peronosclerospora	
				Albuginaceae (white blister diseases)		
					Albugo	
						candida (white blister of crucifers)
True fungi						
Fungi (normally produce hyphae, cell walls contain mainly glucans and chitin)						
	Chytridiomycota (produce zoospores)					
		Chytridiomycetes				
			Chytridiales			
				Olpidiaceae		
					Olpidium	
						brassicae (parasitic on cabbage roots and can transmit some plant viruses)

Kingdom	Phylum	Class	Order	Family	Genus	Species
	Zygomycota (produce wind-borne asexual spores in sporangia, no zoospores)					
		Zygomyecetes				
			Mucorales			
				Mucoraceae		
					Rhizopus	
					Choanephora	
						cucurbitarum (causes soft rot of squash)
			Glomales (fungi which develop vesicular-arbuscular mycorrhizae with roots)			
	Ascomycota[1] (sexual reporduction involves the formation of 8 ascospores in a sac-like ascus in or on an ascocarp, many species also produce spores called conidia, asexually)					
	Filamentous Ascomycetes					
		Plectomycetes	Erysiphales (powdery mildews, asci in cleistothecia)			
		Pyrenomycetes (species producing ascospores in perithecia)				
					Gibberella	
						zeae
					Ceratocystis	
					Glomerella	
					Diaporthe	
		Loculoascomycetes (form ascospores in double-walled asci in the locule of an ascostroma)				
					Mycosphaerella	
					Pleospora	
		Discomycetes (produce ascospores in asci in a disc-shaped structure called an apothecium)				
					Monilinia	
					Sclerotinia	
						sclerotiorum

1 The arrangement of classes within the phylum Ascomycota has recently been changed to reflect advances in taxonomy. Traditional classes have been retained here as they are commonly known in Vietnam. See literature for more information.

Kingdom	Phylum	Class	Order	Family	Genus	Species
		Deuteromycetes (fungi which have no known sexual state or the sexual state is rare, produce conidia asexually)				
					Penicillium	
					Aspergillus	
					Oidium	
					Trichoderma	
					Verticillium	
					Fusarium	
					Colletotrichum	
					Cercospora	
					Septoria	
					Alternaria	
					Stemphylium	
					Cladosporium	
					Botrytis	
					Monilia	
					Rhizoctonia	
					Sclerotium	
	Basidiomycota (basidiomycetes, produce basidiospores sexually on a basidium, many species form basidia in or on a basidiocarp)					
		Basidiomycetes				
			Ustilaginales (smut fungi)			
			Uredinales (rust fungi, obligate parasites)			
			Agaricales (mushrooms, some are root pathogens especially of trees, many are mycorrhizal)			
			(Several other orders of the Basidiomycotina also include some plant pathogens)			

7.3 References

Agrios G.N. 2005. Plant pathology, 5th edition. Elsevier Academic Press: San Diego, California.

8 Pathogenicity testing

To test pathogenicity, susceptible plant species are grown under controlled conditions and inoculated with a suspected pathogenic organism. Pathogenicity tests can provide information to:

- confirm an isolated organism as a plant pathogen using Koch's postulates (Box 8.1)
- determine the host range of a pathogen
- measure the virulence of different isolates of a pathogen.

When choosing healthy plants to inoculate for a pathogenicity test to confirm Koch's postulates, it is important to use the same cultivar (variety) from which the pathogen was isolated. The symptoms expressed will then be as close as possible to those seen in the original disease—cultivars can differ significantly in susceptibility to a pathogen.

Box 8.1 Steps to perform Koch's postulates

1. Describe the symptoms expressed by the diseased crop plants.
2. Isolate the suspected pathogen—the same cultures should be isolated from plants with similar symptoms
3. Obtain a pure culture and use it to inoculate healthy plant material.
4. Observe the symptoms expressed by the inoculated plants—symptoms should be the same as those observed originally in the crop plants.
5. Re-isolate the pathogen from the newly diseased material—the culture should be the same as the original purified culture.

Factors that need to be considered in pathogenicity testing include:

- temperature
- too little or too much water
- nutrient toxicities or deficiencies
- unrealistic inoculum loading of the soil (either too little or too much)
- general growing conditions.

If all tests and plant combinations have associated controls (no treatment) to compare with the treated (inoculated) pots, the effects of these factors can be measured and accounted for. Controls can also provide a means of comparison and can highlight experimental flaws if present.

Always use controls (plants given no treatment) in pathogenicity tests.

8.1 Techniques of pathogenicity testing

An important part of disease diagnosis is the reproduction of a disease during a pathogenicity test to allow the completion of Koch's postulates. Diseases may be reproduced by inoculating the pathogen onto the plant surface, in which case the infection mechanisms of the pathogen operate, or by introducing the pathogen directly into the plant. The technique selected will depend on the pathogen being tested (Table 8.1).

Table 8.1 Techniques of plant pathogenicity testing

Technique	Appropriate for
Stem inoculation	*Sclerotinia*, *Sclerotium* and fungal and bacterial wilt pathogens
Foliar inoculation (and moist chamber)	*Septoria*, *Colletotrichum*
Soil inoculation	
Admixed	*Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*
Thin layer	*Sclerotium*, *Rhizoctonia*
Spore suspension (with and without mechanical damage)	Fusarium and bacterial wilts

High levels of moisture facilitate the infection and spread of many diseases. Mist sprays or humid chambers (made from plastic bags covering pots) can create a moist environment and significantly increase the success rate of pathogenicity tests. Pots in moist chambers or with plastic bag covers should not be placed in direct sunlight.

8.1.1 Stem and foliar infection

The stem and foliar infection technique is a simple test that does not require the production of inoculum in a flask (Figure 8.1). Symptoms are produced quickly, but the plant tissue is pierced with a sharp implement, which does not simulate the natural infection process.

Two plants should be grown per pot—one inoculated and the other used as a control for comparison. This method can also be used successfully to infect other plant parts, such as flowers and fruit.

Stem inoculation

1. Pierce the lower stem of the treatment plant with a sterile inoculating needle or hypodermic needle and place a small piece of agar from a pure culture of the pathogen onto the wound site (or inject a small volume of spore suspension into the stem using a hypodermic syringe and needle).
2. Pierce the lower stem of the control plant with the sterile inoculating needle (or with the hypodermic needle), but do not treat with the inoculum.
3. Wrap parafilm or plastic wrap over the wounds or injection sites.
4. Water the soil each day.
5. Examine and compare the inoculated plants with the uninoculated plants. Observe and record symptoms and compare these with symptoms observed in the field.

Foliar inoculation

1. Spray the foliage of the treatment plant with a spore suspension (or place a drop of spore suspension on several leaves).
2. Spray the control plant with sterile water (or place drops of sterile water on several leaves).
3. Incubate the pot in a moist chamber or a plastic bag in a greenhouse, avoiding direct sunlight.
4. Examine and compare the inoculated plants with the uninoculated plants. Observe and record symptoms and compare these with symptoms observed in the field.

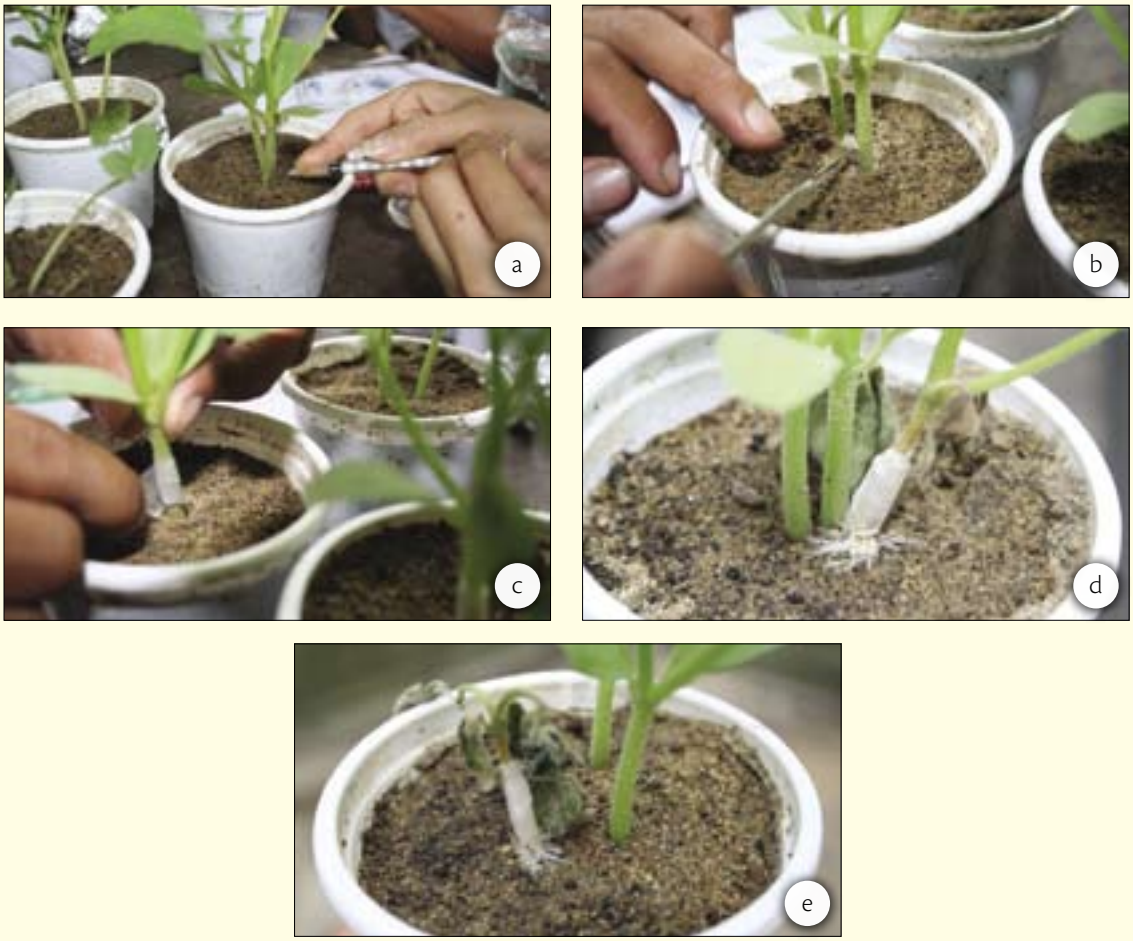


Figure 8.1 Stem inoculation technique for pathogenicity testing: (a) piercing the lower stem, (b) transferring the pure culture to the wound site, (c) wrapping the wound site in plastic, (d) mycelium developing on soil surface from diseased stem, (e) an inoculated plant (left) and an uninoculated control (right)

8.1.2 Soil inoculation

Soil can be inoculated directly using a spore suspension made from a pure agar culture or from a culture grown in flasks (Figure 8.2). A fungal spore or bacterial suspension can be added post-emergence so that the root system is drenched by the suspension. This method is used as a quick initial test of pathogenicity.

A more natural infection process is provided by the admixed or thin layer techniques. Both of these techniques require the production of inoculum on a natural substrate, such as millet seed and rice hulls. Growth of cultures on these substrates in a flask takes 2–3 weeks. A standard amount of inoculum is used for both techniques. However because the inoculum is placed in the soil at the same time as planting, plants may contract the disease at the seedling stage—this can cause misleading results if the aim is to produce disease in older plants.

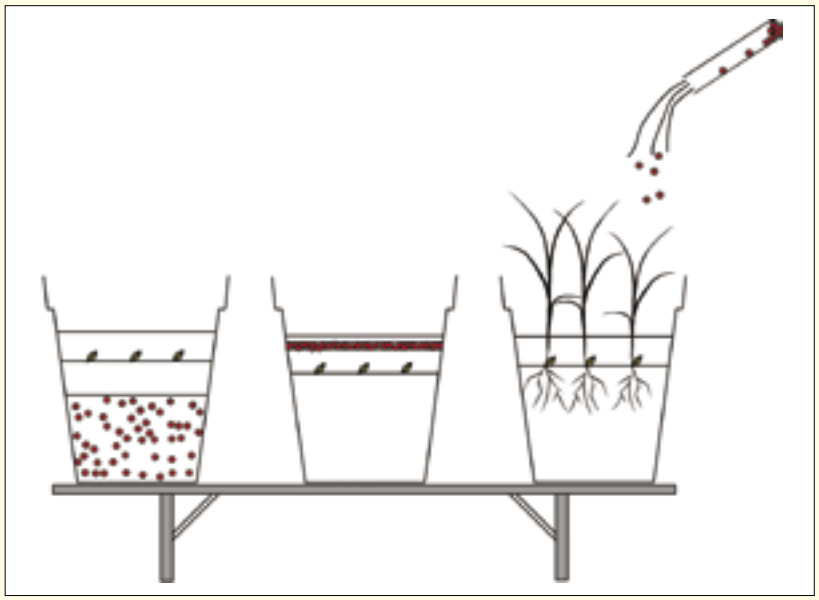


Figure 8.2 Different methods for inoculating soil to produce disease in the glasshouse

8.2 Preparation of inoculum for pathogenicity testing

8.2.1 Spore suspension

Inoculum for preparing spore suspensions can be grown on water agar containing sterile seeds or stem or leaf pieces, on carnation leaf agar, or on half-strength potato dextrose agar. Simply scrape the fungal spores and hyphae from the colony and transfer to sterile water. This spore suspension can be poured onto the soil.

8.2.2 Millet seed/rice hull medium (50:50 by volume)

1. Soak millet seed and rice hulls overnight in water in a refrigerator, to allow the mixture to absorb water.
2. Pour the water away.
3. Transfer approximately 150 mL of medium to a 250 mL conical flask (Figures 8.3–8.5).
4. Roll a tight fitting cotton wool plug, cover it with muslin, and insert it into the opening of the conical flask.

5. Cover the opening of the flask with a layer of aluminium foil and autoclave. (This keeps the neck area sterile before inoculation and the cotton wool plug dry during autoclaving.)
6. Allow the flasks to cool.
7. Inoculate the flasks using mycelial plugs or a spore suspension, making sure that the cotton plug remains sterile, in a laminar flow cabinet.
8. Incubate at approximately 25 °C for 2 weeks under alternating light and dark conditions to allow complete colonisation of the substrate.
9. Shake the flasks 2–3 days after inoculation to ensure an even distribution of the pathogen throughout the substrate.

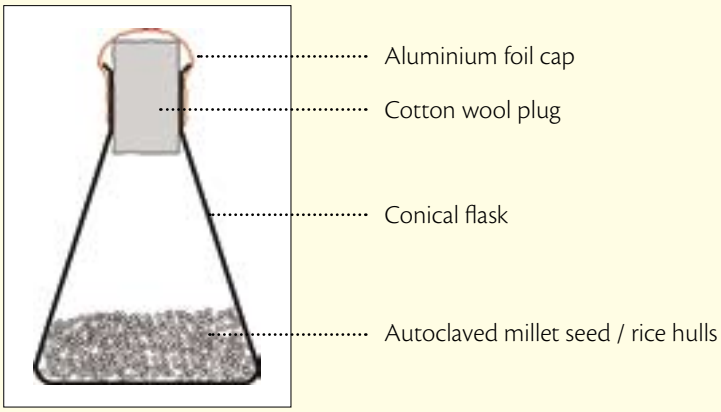


Figure 8.3 An inoculum flask

Use 'fresh' (recently isolated) cultures to prepare inoculum. Cultures that have been subcultured repeatedly on high-nutrient media often have reduced virulence.

Figure 8.4 Preparation of millet seed/rice hull medium in flasks

Figure 8.5 Preparation of millet seed/rice hull medium for pathogenicity testing: (a) millet seed and rice hulls that have been soaked in distilled water for 24 hours, (b) thorough mixing of inoculum medium components, (c and d) transfer of medium to conical flasks using a makeshift funnel, (e) flask plugged with cotton wool wrapped in muslin, (f) flask covered with aluminium foil ready for autoclaving

9 Integrated disease management

The control of the majority of plant diseases involves using a number of complementary control measures. This strategy (program) is called integrated disease management (IDM). The development of an IDM program is based on a thorough knowledge of the disease cycles of the diseases affecting a crop or crops, as well as the host range of each pathogen.

In summary, for each pathogen it is essential to have knowledge of:

- how the pathogen survives in the absence of a susceptible host
- how the pathogen infects the host
- how the pathogen is dispersed (spreads) within and between crops
- how farming practices and environmental factors affect survival, infection and dispersal
- the host range of the pathogen.

It is also essential for the plant pathologist to have a thorough understanding of the farming system. Some farming systems involve only one crop, as with perennial or plantation crops: coffee, cashew, durian, pineapple and banana. Disease management in such systems is focused on only one crop and its associated diseases.

In contrast, in mixed farming systems one farmer may grow a number of crops each year, such as a range of vegetable crops together with paddy rice or maize. Many pathogens that survive in soil affect a wide range of hosts. Therefore, an IDM program for a mixed farming system involves the management of diseases on a range of crops.

The main IDM strategies (Figure 9.1) are:

- crop rotation
- crop management
  - good drainage
  - flooding (paddy rice)
- pathogen-free transplants, seed, rhizomes, tubers etc.
- quarantine
- resistant or tolerant cultivars
- resistant rootstocks (grafting)
- fungicides
- hygiene (sanitation).

9.1 Crop rotation

Crop rotation is an important component of IDM in mixed farming systems such as vegetables and field crops.

Rotation is a key strategy for minimising the amount of pathogens that survive in soil.

It is important to understand the host ranges of the pathogens before a rotation program is recommended. In Vietnam, many vegetable crops are susceptible to bacterial wilt (*Ralstonia solanacearum*). Therefore, an IDM program for a vegetable and field crop farming system should include a rotation with crops resistant to bacterial wilt.

Maize, rice, tropical grasses, cabbages and mustard are examples of crops that are resistant to bacterial wilt. These can be recommended for rotations to minimise the disease. An example of rotation program to reduce disease is chilli—maize—beans—bitter melon. An example of a rotation program that will lead to severe bacterial wilt is: chilli—tomato—eggplant—bitter melon.

Many weeds act as alternative hosts for important crop pathogens (e.g. *Ageratum conyzoides*). Weeds also can host insects which are virus vectors. Susceptible weeds should be controlled during the rotation.

Many pathogens that survive in soil affect particular plant families. For example, bacterial wilt affects most crops in the *Solanaceae* including tomato, chilli and eggplant, which should not be grown in succession. *Sclerotinia sclerotiorum* affects many legumes (such as soybeans, short beans and long beans), as well as lettuce, tomato and potato. These crops should not be grown in succession in regions with cool wet winters, such as northern and central Vietnam.

Rotation is not effective in controlling pathogens that are wind dispersed over long distances, such as leaf blights, mildews and rusts.

9.2 Crop management

Changes in crop management practices can often help to reduce disease. For example, planting dates can be altered to avoid cold wet periods, which favour many seedling diseases. Irrigation can be managed to avoid stress on the crops and to minimise soil saturation and the movement of pathogens in the water between farmer plots.

Crop nutrition is important as healthy plants with vigorous root systems can tolerate some pathogens. Organic fertiliser (especially chicken manure) may suppress some fungal pathogens in the soil (e.g. *Phytophthora*).

Organic residues on the soil surface, such as rice hulls, may increase some diseases; for example, *Sclerotium rolfsii* can be more severe if residues are present on the soil surface. However, organic residues and organic fertiliser improve soil structure, which leads to more vigorous root systems. Recent studies in Australia (Stirling and Eden 2007) indicate that sugar cane residue, mulch and other amendments can significantly reduce inoculum levels of root knot nematode (*Meloidogyne incognia*) in soil. It is usually necessary to add a nitrogen source such as ammonium nitrate with mulches to avoid nitrogen deficiency.

9.2.1 Good drainage

Wet soil favours root diseases caused by pathogens that survive in soil. In particular, wet soil favours seedling diseases and root rot caused by *Pythium* and *Phytophthora*, which produce motile (swimming) zoospores. Thus, good drainage is a key control measure in IDM programs for Pythium and Phytophthora diseases. Good drainage usually involves using raised planting beds at least 30 cm high and removing weeds from drainage furrows (Figure 9.2).

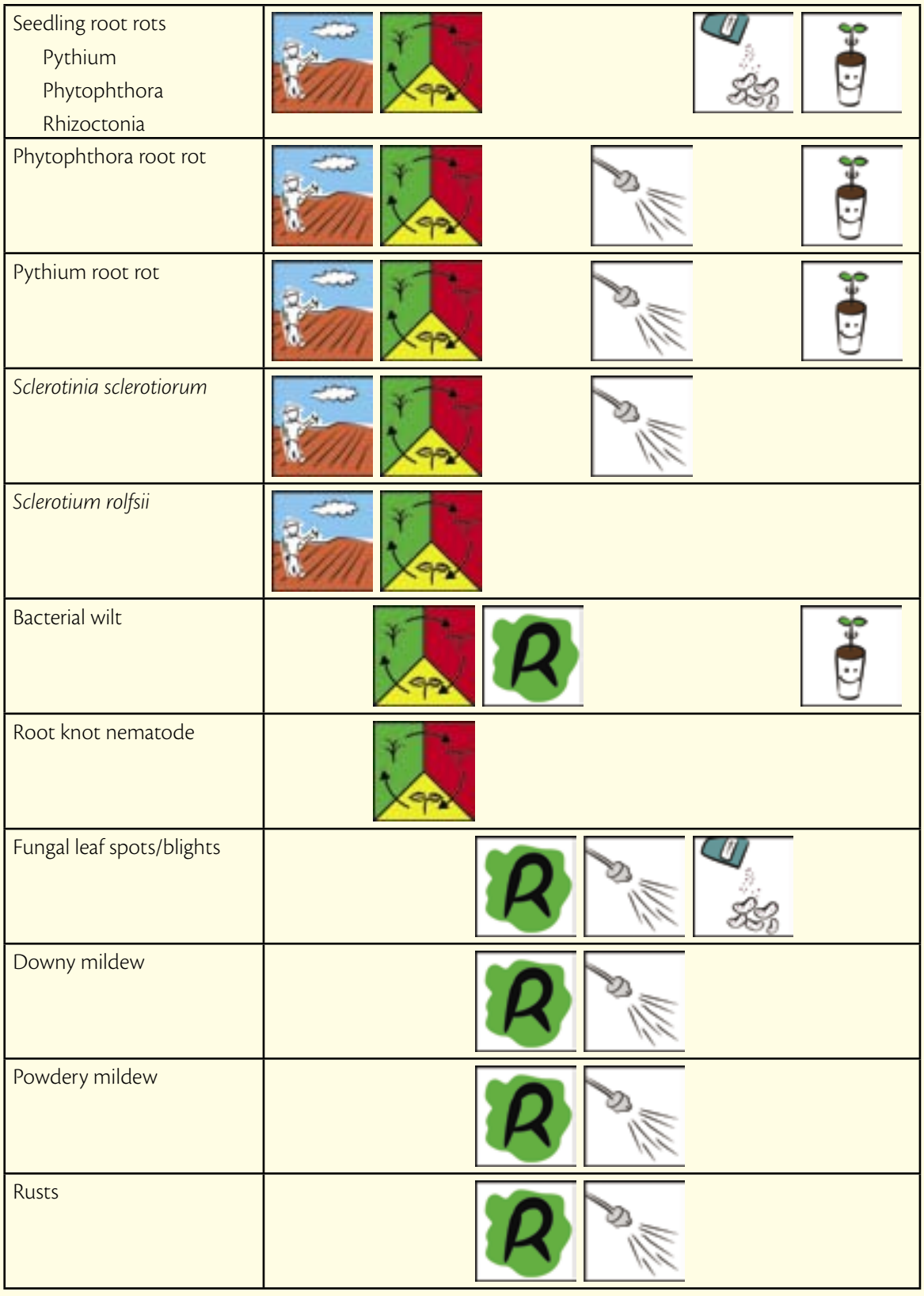


Figure 9.1 Diagrammatic summary of appropriate control measures for common groups of diseases

Hygiene

Resistance

Crop management

Healthy transplants

Seed treatment (dressings)

Quarantine

Fungicides

Crop rotation

Figure 9.2 Chipping weeds from a drainage furrow to improve drainage in a black pepper crop affected by Phytophthora root rot

9.2.2 Flooding

Flooding during paddy rice production will reduce the levels of some pathogens that survive in soil. For example, Mrs Dang Luu Hoa and colleagues (pers. comm.) demonstrated that two successive paddy rice crops eliminated sclerotia of *Sclerotium rolfsii*. Even one rice crop caused a significant reduction in sclerotia. A decline in paddy rice production could lead to an increase in some pathogens which survive in soil.

9.3 Pathogen-free transplants, seed, and other planting material

It is important to use pathogen-free seed and transplants. In our experience in Vietnam, seedling transplants are commonly infected or contaminated with pathogens which survive in soil. These pathogens can then contaminate the field and spread the pathogen to new areas.

If seed is contaminated, it should be treated with a fungicide recommended for seed treatment of that crop. Some fungicides will affect germination, so it is best to use pathogen-free seed if it is available. Many pathogens are carried in rhizomes, tubers and bulbs. It is important to avoid using such planting material. Provincial (Plant Protection Sub-department) staff and district staff may need to help farmers develop special programs for producing pathogen-free planting material. This is a major priority for many crops established from such planting material (e.g. ginger and potatoes).

9.4 Quarantine

Quarantine measures are valuable for excluding exotic pathogens from a country or region. These measures are difficult to apply in Vietnam because of its long land border with China, Laos and Cambodia. Many foliar pathogens and insect vectors can simply cross such a border in the wind. However, it could be beneficial for Vietnam to strengthen quarantine measures at a national level for importations of seed and other planting materials. At the local level, plant protection staff should clean shoes carefully between surveys of diseased and healthy crops (see hygiene section).

9.5 Resistant or tolerant cultivars

Resistant cultivars provide a valuable strategy for disease control. These should be recommended strongly by provincial and district staff whenever they are available for a particular disease.

9.6 Grafting to resistant rootstock

The grafting of desirable but susceptible scions (stems) onto resistant rootstocks is a valuable method for preventing diseases caused by pathogens which survive in soil. For example, many cucurbits are susceptible to *Fusarium* wilt and/or *Pythium*. These diseases can be avoided by grafting the susceptible cucurbits onto resistant pumpkin rootstocks. This is an old practice in Vietnam and other parts of Asia.

This practice can also be applied to fruit trees. For example, *Phytophthora*-susceptible citrus varieties can be grafted onto the resistant 'trifoliata' (*Poncirus trifoliata*) rootstocks. Care must be taken to assess the impact of the rootstock on the performance of the scion.

9.7 Fungicides

Fungicides are commonly used as foliar sprays to control leaf and fruit diseases. However, they can also be used on seed to control seed-borne pathogens or to protect emerging seedlings from disease. In addition, they can be used as soil drenches in seedling beds or with high value fruit tree crops.

Identify fungal diseases correctly before selecting a fungicide. Different fungal pathogens require different fungicides, so taxonomy is important! For example, the downy mildews require quite different fungicides to the powdery mildews.

Epidemics of foliar fungal pathogens, such as leaf spots, rusts and mildews, increase quickly under favourable conditions of leaf wetness and temperature. These pathogens produce abundant spores, which spread easily by wind and/or rain splash within and between crops.

It is essential to monitor the weather and predict when a foliar disease is likely to develop. That way, fungicide can be first applied when the fungus is at very low levels. This gives the most effective control.

It is very difficult to control a foliar fungal disease when it is well established. Fungal pathogens can develop resistance to some fungicides, rendering them ineffective. It is important to minimise the risk of the development of resistant strains by minimising the number of sprays per season of a fungicide. This is achieved by:

- spraying before the disease is obvious
- rotating protectant and specific fungicides
- applying fungicides at the recommended rates and at a uniform distance.

Be sure the fungicide is effective against the disease. Buy fungicides from reliable companies and shops.

9.8 Hygiene

Strict hygiene (sanitation) practices are particularly important in plastic/green house production of valuable vegetable and flower crops. Strict hygiene practices are also essential in nurseries where seedlings are produced for transplanting to the field or greenhouse.

Hygiene practices include:

- maintenance of pathogen-free soil
- use of pathogen-free seed or planting material
- disinfection of benches and planting pots
- disinfection of equipment
- use of disposable overshoes and disinfectant footbaths to prevent staff introducing pathogens on footwear (Figure 9.3)
- regular checking for plants affected by diseases surviving in the soil
- removal and burning of diseased plants
- removal of contaminated soil.

Disinfect shoes thoroughly after inspecting a crop affected by a pathogen that can survive in soil. Do not inspect healthy crops wearing shoes contaminated with infested soil.

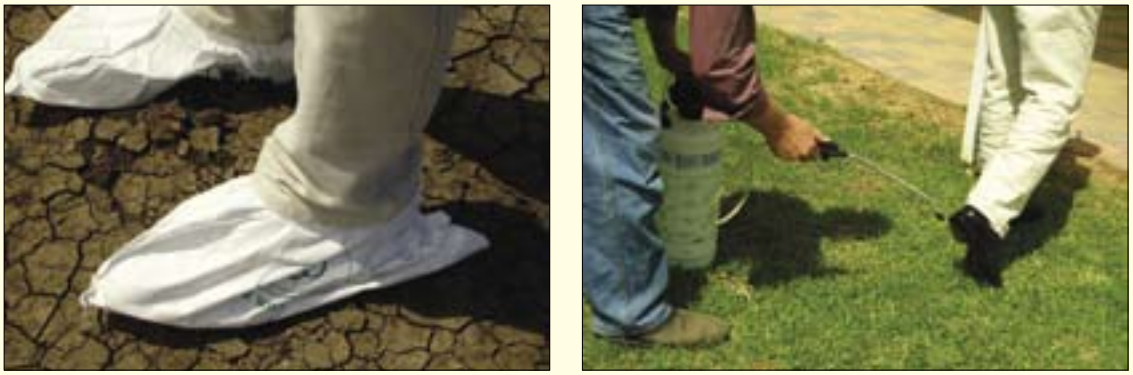


Figure 9.3 Measures for preventing transfer of contaminated soil on footwear: disposable synthetic overshoes (left) and disinfecting shoes after inspecting a crop affected by a pathogen which survives in soil (right)

9.9 References

Stirling G.R. and Eden L.M. 2007. The impact of organic amendments and mulch on root-knot nematode and Pythium root rot of capsicum. Presented at the Australasian Plant Pathology Society Conference, Adelaide, 24–27 September 2007.

10 Root and stem rot diseases caused by pathogens that survive in soil

Root and stem rot diseases caused by pathogens which survive in soil are responsible for serious losses in crop yield in Vietnam. The intensive nature of cropping in Vietnam's delta regions, movement of pathogens in irrigation water, poor drainage, contaminated planting material and the tropical climate favour these diseases.

The pathogens responsible for these diseases cause non-specific symptoms, namely stunting, yellowing of leaves, wilting and plant death. Note that these symptoms can also be caused by some other pathogens as well as stem boring insects, curl grubs which feed on the roots, and unfavourable soil conditions.

These diseases are caused by a number of common pathogens, including fungal and bacterial pathogens and plant parasitic nematodes.

The pathogens listed in Table 10.1 have the following key features:

- they survive in soil for long periods in the absence of a host, and inoculum levels in soil increase slowly over several years (crop cycles)
- they all have a wide host range, except formae speciales of *Fusarium oxysporum*
- they can be spread in:
  - irrigation water
  - soil carried on animals and humans
  - contaminated planting material (potato tubers, ginger rhizomes, seedling transplants)
- they are not usually dispersed by wind.

Bacterial wilt pathogens can also be carried on seed.

Table 10.1 Features of common crop pathogens that survive in soil in Vietnam

Pathogen	Diseases	Host range	Survival (overseasoning)	Comments
Pythium species[a] (e.g. *P. aphanidermatum*[a], *P. myriotilum*[a], *P. spinosum*[a])	Seedling death, rootlet rots, root rots	Wide	Oospores in soil	Zoospores dispersed in soil water and water splash
Phytophthora palmivora[a]	Wide range of root, stem, leaf and fruit diseases of tree crops	Wide	Chlamydospores, hyphae in residues and possibly oospores in soil	Zoospores dispersed in soil water and water splash
Phytophthora capsici[a]	Foot rot (quick wilt) of black pepper, root rot of chilli and other diseases	Wide	Chlamydospores, hyphae in residues and possibly oospores in soil	Zoospores dispersed in soil water and water splash
Phytophthora nicotianae[a]	Heart rot of pineapple and other diseases	Wide	Chlamydospores, hyphae in residues and possibly oospores in soil	Chlamydospores in soil, zoospores dispersed in soil water and water splash
Fusarium oxysporum, f. sp. *lycopersici*[a]	Fusarium wilt	Tomato	Chlamydospores in soil, also infects non-host roots	Stem vascular browning
Fusarium oxysporum, f. sp. *pisi*[a]	Fusarium wilt	Peas	Chlamydospores in soil, also infects non-host roots	Stem vascular browning
Fusarium oxysporum, f. sp. *cubense*[a]	Fusarium wilt	Banana	Chlamydospores in soil, also infects non-hosts; in planting material	Stem vascular browning
Sclerotinia sclerotiorum	Stem, head and pod rots	Wide	Large black sclerotia in soil	Sclerotia are diagnostic in field
Sclerotium rolfsii	Stem base rot	Wide	Small brown round sclerotia in soil	Sclerotia are diagnostic in field

Pathogen	Diseases	Host range	Survival (overseasoning)	Comments
Rhizoctonia sp.[a]	Seedling death, root, stem, stalk and head rots	Wide	Sclerotia or distinctive hyphae in residue in soil	Sclerotia diagnostic for some species in the field; right-angled hyphal branching in culture
Verticillium albo-atrum[ab]	Verticillium wilt	Wide	Hyphae in residue	Stem vascular browning
Verticillium dahliae[ab]	Verticillium wilt	Wide	Microsclerotia in soil, hyphae in residue	Stem vascular browning
Ralstonia solanacearum[a]	Bacterial wilt	Wide	Bacteria in soil, crop residues and propagating material	Stem browning and bacterial ooze are diagnostic features in field
Meloidogyne	Root knot nematode	Wide	Dormant nematodes in soil	Females live in root galls (knots)—a diagnostic feature
Root lesion nematodes[a]	Root lesions and plant stunting	Wide	Dormant nematodes in soil	Small lesions on roots are visible with a hand lens
Plasmodiophora brassicae	Club root of crucifers	*Brassica* and *Raphanus* species	Resting spores in soil	Club root symptoms diagnosable in field; add lime to soil for control

a The accurate diagnosis of these pathogens depends on isolation or extraction in the laboratory and subsequent identification. Pathogenicity tests are essential to prove that they are the primary pathogen in the local hosts, unless tested previously in Vietnam.

b These species have not been officially recorded in Vietnam.

These pathogens are often overlooked because they are difficult to identify (see Box 10.1)—the majority of them can only be identified accurately in the laboratory.

Two or more of these pathogens may simultaneously affect a crop in the intensive vegetable farming areas in Vietnam. For example, a chilli crop can be affected by bacterial wilt, Phytophthora root rot and basal stem rot. Stem boring insects may also be present. All these problems cause the same symptoms (wilting and death).

Ideally plants with root and stem rot diseases should be examined in the laboratory within a few hours of collection, while 'fresh'. Thus it is important to locate basic diagnostic laboratories in provincial sub-departments of plant protection, close to farming areas.

The national diagnostic laboratories such as the Plant Protection Research Institute in Hanoi can identify cultures, specimens, plant viruses, nematodes and bacterial pathogens.

Box 10.1 Diagnosis tip: distinguishing vascular wilts from root and stem rots

It can sometimes be difficult to determine the cause of non-specific symptoms such as stunting, yellowing and wilting. Vascular wilt diseases and root and stem rot diseases commonly cause these symptoms. This diagram shows how these diseases can be distinguished.

Stem (vascular) browning + bacterial ooze*	→	Bacterial wilt
Stem (vascular) browning + no bacterial ooze	→	Fusarium wilt or Verticillum wilt
No stem (vascular) browning + no bacterial ooze	→	Root and stem rot pathogens (fungal and fungal-like) or Plant parasitic nematodes or Club root

Note: Bacterial ooze may not be observed in early stages of infection by *Ralstonia solanacearum*.

Fusarium wilt can be confused with bacterial wilt and Verticillium wilt (at present, an exotic disease to Vietnam). They cause similar symptoms and all cause vascular (stem) browning. However, plants affected by bacterial wilt are usually characterised by the presence of bacterial ooze. If there is no sign of ooze then check for *F. oxysporum* and *Verticillium* species by isolation. Formae speciales of *F. oxysporum* can be readily distinguished from *Verticillium albo-atrum* and *V. dahliae* in pure culture. Colonies of *Verticillium* grow slowly compared to colonies of *F. oxysporum*.

Always pathogenicity test *Fusarium* isolates from roots before assuming that they are pathogenic.

Fusarium species mainly cause wilt diseases and bulb and tuber rots of flower and vegetable crops. They are not common root rot pathogens. However, saprophytic strains of *F. oxysporum* and *F. solani* are very common colonisers of root tissue affected by other pathogens, and are easily isolated on non-selective media.

10.1 *Sclerotinia sclerotiorum*

Table 10.2 provides information about *Sclerotinia sclerotiorum*, a fungus that causes Sclerotinia rot of stems, heads, fruit and flowers.

Table 10.2 Characteristics of *Sclerotinia sclerotiorum*

Key symptoms	Wet rot of plant tissue
Diagnostic signs	Presence of white mycelium and large irregularly shaped black sclerotia.
Host range	Affects a wide range of dicotyledonous (broadleaved) crops including tomato and potato, lettuce, soybeans, peanuts, short beans, long beans, cabbage, broccoli, cauliflower and cucurbits.
Weather	Requires cool wet weather.
Overseasoning	Sclerotia survive in soil for long periods. Under mild wet conditions sclerotia germinate to produce apothecia. The apothecia produce ascospores which infect the plant.
Infection	Produces ascospores from apothecia. Ascospores infect plant usually at leaf axils. Old flower petals assist the pathogen in the infection process.
Control	Use rotation to crops such as maize and cotton, avoid dense plant canopies (these lead to high humidity within the crop and favour infection).
Isolation	1. Surface sterilise diseased stem by dipping in 70% ethyl alcohol and drying on sterile paper tissue (facial tissues or good toilet paper can also be used). 2. Cut sections from the margins of healthy and diseased tissue and aseptically transfer them to potato dextrose agar. 3. Purify by hyphal tip method. The fungus can also be isolated from sclerotia: 1. Surface sterilise the sclerotia for 1 minute in 70% ethyl alcohol. 2. Wash in sterile water and air dry. 3. Cut sclerotia into halves. 4. Plate the pieces on potato dextrose agar with the cut side facing down on the agar.

Figure 10.1 illustrates the disease cycle of *Sclerotinia sclerotiorum* and Figure 10.2 is a series of images showing the effect of *Sclerotinia sclerotiorum* on a variety of crop plants, as well as sclerotia and apothecia.

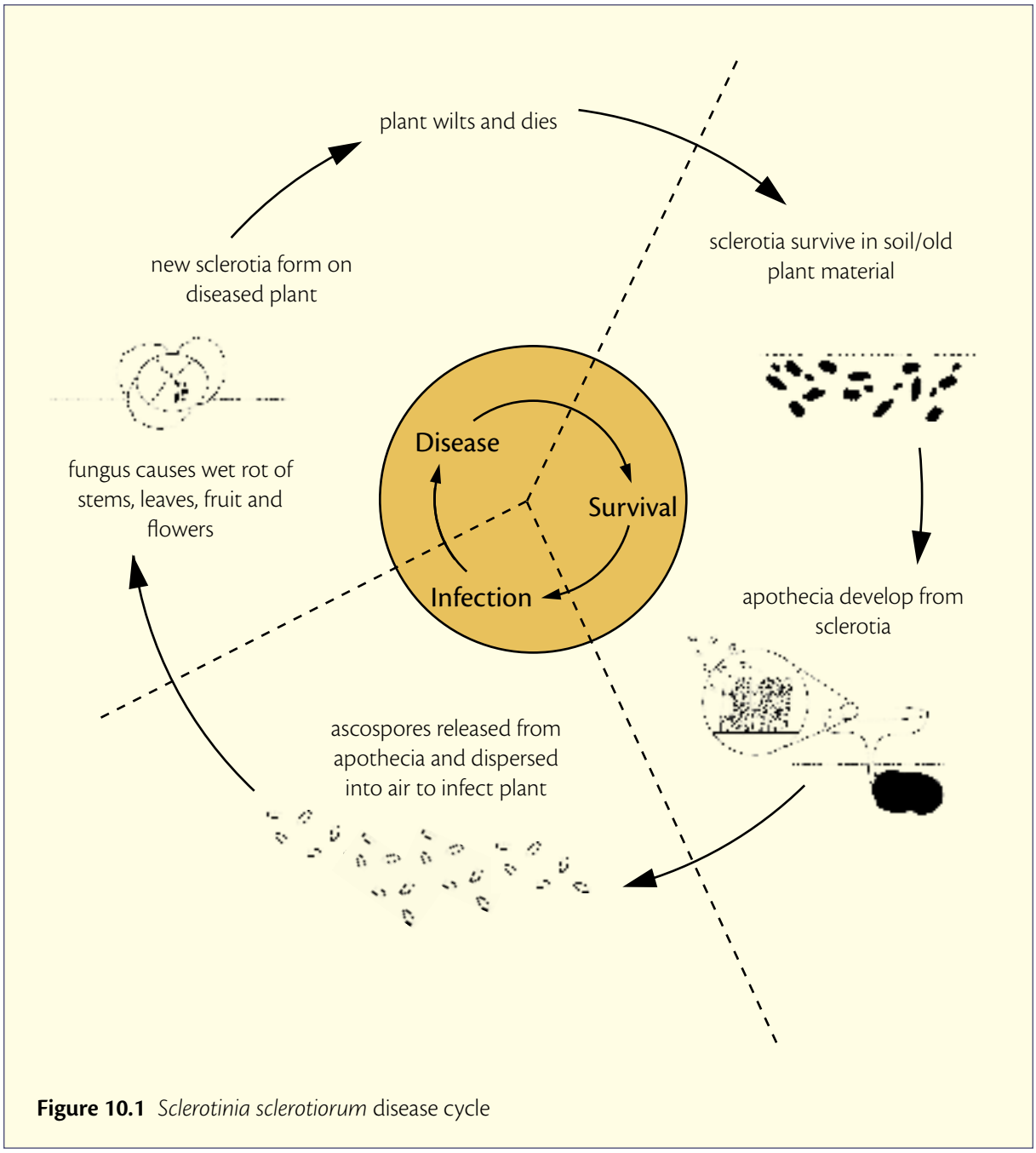


Figure 10.1 *Sclerotinia sclerotiorum* disease cycle

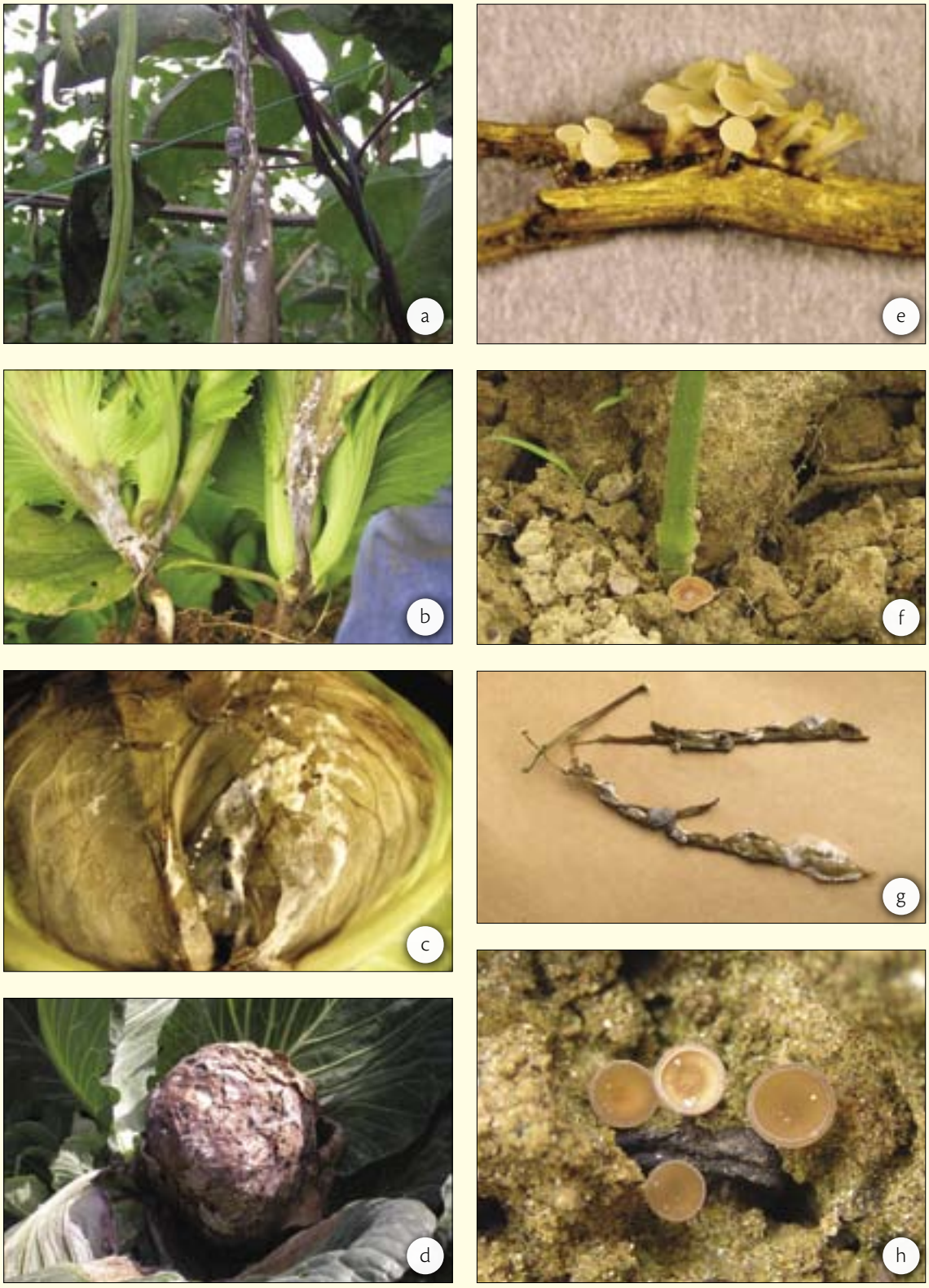


Figure 10.2 *Sclerotinia sclerotiorum* affecting: (a) long beans, (b) lettuce, (c) cabbage (wet rot), (d) cabbage; (e) apothecia from sclerotia in soybean residue; (f) apothecium next to short bean; (g) long bean (sclerotia produced on bean); (h) germinated sclerotium producing apothecia

10.2 *Sclerotium rolfsii*

Table 10.3 provides information about *Sclerotium rolfsii*, a fungus that causes basal rot of stems.

Table 10.3 Characteristics of *Sclerotium rolfsii*

Key symptoms	Causes rot of the stem base, wilting and death of the diseased plant.
Diagnostic signs	White fungal mycelium and small round brown sclerotia are formed on the surface of the diseased stem base. Obvious white hyphal growth is produced as disease spreads from infected to healthy plants.
Host range	Wide host range includes tomato, chilli, cucurbits, beans, carrots and onions. Commonly infects plants affected by other pathogens.
Weather	Most severe in warm to hot, wet or humid conditions.
Overseasoning	Survives in soil for long periods as sclerotia.
Infection	Infects through the base of the stem from hyphae from sclerotia. Infection can be more severe where plant residues are on the soil surface. Hyphal runners (mycelium) can grow several centimetres over the soil surface from diseased plants or tissue to infect nearby plants.
Control	Crop rotation. Flooding during two successive paddy rice crops will kill all sclerotia in the soil.
Isolation	Can be isolated on potato dextrose agar from surface sterilised stem tissue, cut from the margin of the diseased and healthy tissue. *S. rolfsii* cultures can also be isolated from sclerotia: 1. Surface sterilise sclerotia in 70% ethyl alcohol for 1 minute. 2. Wash in sterile water and air dry. 3. Cut in half and plate the pieces on potato dextrose agar with the cut surfaces on the agar.

Figure 10.3 shows characteristics of *Sclerotium rolfsii*.

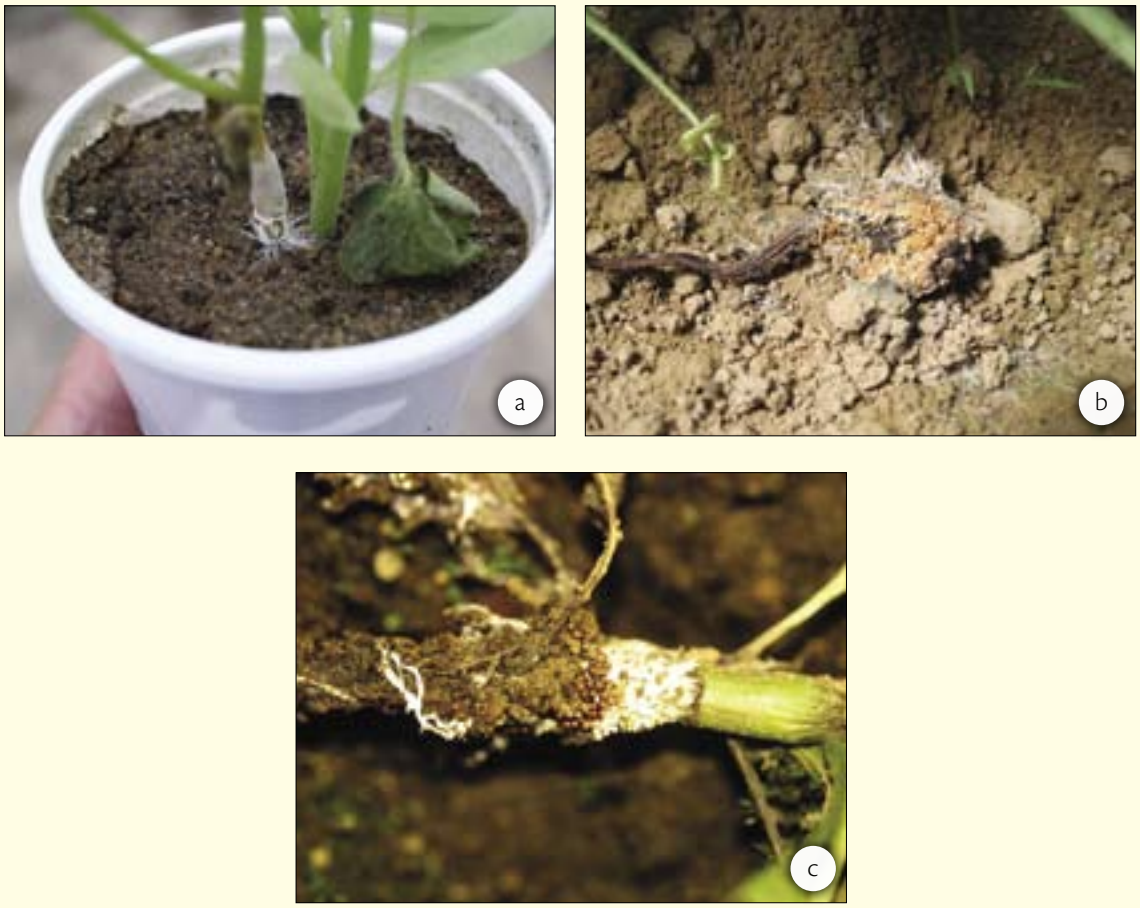


Figure 10.3 *Sclerotium rolfsii*: (a) in pathogenicity test (note hyphal runners), (b) on decaying watermelon, (c) basal rot with the formation of brown spherical sclerotia

10.3 *Rhizoctonia* species

There are many *Rhizoctonia* species and strains in Vietnam. These species are quite variable in their distribution and host range. Morphological identification to species level is difficult.

A variety of diseases are caused by *Rhizoctonia* species in Vietnam (Figure 10.4). Some species grow on plant stem and leaf surfaces in warm, wet or humid conditions causing infection and disease of these plant parts. For example, one *Rhizoctonia* species infects maize leaves and causes distinctive patterning (Figure 10.4d). It is thought that the same species, or a similar species, causes head rot of cabbage. These fungi may produce irregular brown sclerotia on diseased plant surfaces. *Rhizoctonia oryzae* causes sheath blight of rice, a well-known disease.

Figure 10.4 Examples of Rhizoctonia diseases: (a) spear point symptoms on diseased roots, (b) Rhizoctonia sheath blight on rice, (c) sclerotia of *Rhizoctonia* on diseased cabbage, (d) Rhizoctonia disease on maize hull

Rhizoctonia species also cause collar rot of seedlings such as beans, cabbage, peanuts and cotton. Collar rot is caused by infection at the soil surface and can kill seedlings.

Rhizoctonia root rot develops from infection of the growing tip of small lateral roots. The fungus then progressively grows from the root tip and may cause rot of the main root. *Rhizoctonia* infection of a rootlet often results in the 'spear-point' symptom of roots (Figure 10.4a).

Table 10.4 shows characteristics of *Rhizoctonia* species, which are fungi that cause a variety of diseases on a range of crop plants.

Table 10.4 Characteristics of *Rhizoctonia* species

Key symptoms	Symptoms depend on the species and strain of the fungus and the host plant, and may include collar rot of seedlings, wilting, seedling death, rootlet rot and root rot. Rhizoctonia head rot in cabbages causes black necrotic areas on leaves. Sheath blight in rice and tiger stripe or blotch in maize cause irregular chlorotic or bleached areas.
Diagnostic signs	Diagnosis usually depends on isolation and identification of the fungus in pure culture. Distinctive irregular brown sclerotia are formed by some species on diseased host tissues.
Host range	Variable, depending on species and strain of the fungus.
Weather	Diseases of leaves, stems and heads favoured by warm to hot, wet weather. Seedling diseases and root rot are more severe in plants affected by unfavourable conditions. For example, bean seedlings are more susceptible to collar rot in cold weather, which slows germination and emergence.
Overseasoning	*Rhizoctonia* species survive in soil as sclerotia or as hyphae in host plant residues.
Infection	*Rhizoctonia* hyphae in infested residue directly infect plant tissues and some form special infection structures. Sclerotia germinate to produce hyphae which then infect the plant.
Control	Seedling blight (collar rot) can be minimised by seed treatment with fungicides such as quintozene (pentachloronitrobenzene), and by altering planting dates to when soil temperatures and moisture favour rapid germination and emergence. The effectiveness of crop rotation depends on the host range of the particular *Rhizoctonia* species being managed.
Isolation	Can be readily isolated from rice sheath blight, maize leaf blotch and cabbage head rot using surface sterilised tissue, plated on water agar and subcultured onto potato dextrose agar containing antibiotics. Isolation from roots or rootlets with root rot is more difficult: 1. Wash roots free of soil. 2. Surface sterilise for 5 seconds in 70% ethyl alcohol. 3. Rinse in sterile water and damp-dry on sterile paper tissue. 4. Plate small (1–2 mm long) root segments from the margin of healthy and diseased root tissue onto water agar. 5. Subculture to potato dextrose agar. *Rhizoctonia* can be distinguished from *Pythium* and *Phytophthora* on water agar by right-angle hyphal branching and presence of wide septate hyphae. Sclerotia may form in culture, especially on rice straw agar.

10.4 *Phytophthora* and *Pythium*

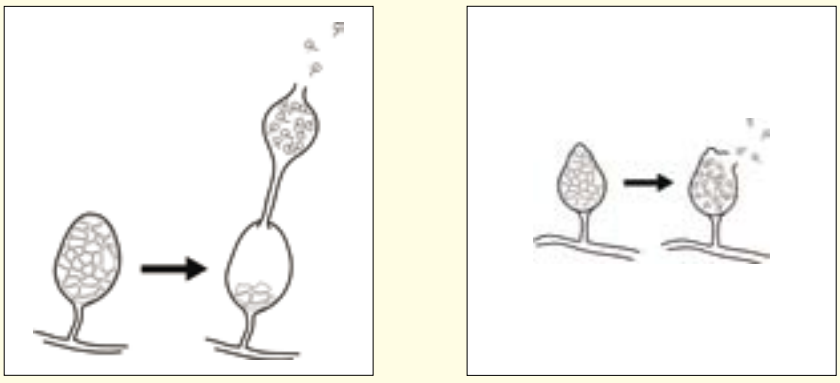
The genera *Phytophthora* and *Pythium* belong to the class Oomycetes within the Kingdom Chromista. Thus, they are not true fungi but fungal-like organisms. These genera produce non-septate hyphae, a key feature which distinguishes them from genera of true fungi.

10.4.1 Asexual reproduction

Asexual reproduction results in structures called sporangia, which give rise to zoospores. These zoospores are motile and have a key role in the disease cycle, particularly in the dispersal of these organisms in wet soil or on plant surfaces. The formation of motile zoospores also distinguishes *Phytophthora* and *Pythium* from genera of true fungi. Zoospores enable the rapid spread of disease from infected plants.

The sporangia of *Pythium* are formed at the end of hyphae or within hyphae, and are either rounded (globose/spherical) or filamentous (like a swollen hypha). A discharge tube is formed by the sporangium of *Pythium,* with a very thin-walled vesicle formed at the end of the discharge tube (Figure 10.5). Cytoplasm flows from the sporangium through the discharge tube to the vesicle. Zoospores then develop in the vesicle and are released when the vesicle ruptures (splits open).

In contrast, *Phytophthora* species form obvious regularly shaped sporangia on a sporangiophore. Zoospores form in the sporangia and are released directly from the sporangium. Some species such as *P. infestans* and *P. palmivora* form deciduous sporangia which can be aerially dispersed.



Pythium *Phytophthora*

Figure 10.5 Sporangium of *Pythium* illustrating zoospore release through a vesicle (left), and zoospore release directly from *Phytophthora* sporangium (right)

Some species of *Phytophthora,* such as *P. cinnamomi,* form asexually produced chlamydospores in culture. These act as survival spores in soil.

10.4.2 Sexual reproduction

Sexual reproduction involves the formation of oogonia (considered 'female') and antheridia (considered 'male'). Following fertilisation the oosphere ('female' gamete) within the oogonium develops into a thick-walled oospore. The oospore is a survival spore and has a key role in the disease cycle. Oogonia of *Pythium* may have smooth walls or horn-like ornamentation. The oogonia of *Phytophthora* are smooth walled.

Sterols are essential for oogonial production. Therefore, these fungi should be cultured on PCA (potato carrot agar) as carrot extract contains sterols. The PCA should contain some sediment from the carrot extract.

Some species of *Pythium* are heterothallic; however, many of the common pathogens are homothallic and form the sexual structures in a pure culture from a hyphal tip. Sexual reproduction in a homothallic species only requires one strain. Sexual reproduction in a heterothallic species requires two strains of opposite mating types.

Approximately 50% of *Phytophthora* species are heterothallic and require two mating type strains (A1 and A2) for sexual reproduction to occur. Figure 10.6 illustrates sexual reproduction in *Pythium*—reproduction is by a similar process in *Phytophthora*.

10.4.3 Identifying and differentiating *Phytophthora* and *Pythium*

Cultures (colonies) of many species of *Phytophthora* and *Pythium* appear quite similar on artificial media. Accurate identification of these species can be based on the morphology of the sporangia and the morphology and arrangement of the oogonia and antheridia. The presence or absence of chlamydospores can assist with identification, as can the nature of hyphae in some species of *Phytophthora*.

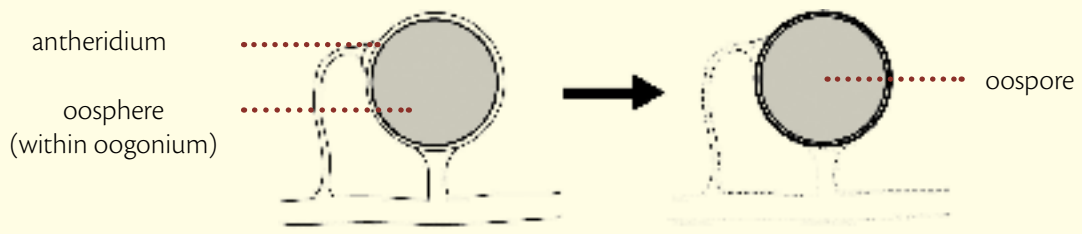


Figure 10.6 Diagram illustrating sexual reproduction in *Pythium,* involving contact between an antheridium and an oogonium to form an oospore

Pythium species usually produce abundant fluffy white mycelium on potato dextrose agar (PDA), filling the culture plate (Figure 10.7). Some *Pythium* species have a very high growth rate, and may cover a large (90 mm) PDA plate in less than 2 days. In contrast, *Phytophthora* species usually grow more slowly producing less abundant white mycelium. However, this is not a reliable criterion for separating the two genera.

Figure 10.7 *Pythium* sp. (left) and *Phytophthora* sp. (right), showing the characteristic faster growth and aerial mycelium on the *Pythium* plate

Pythium species usually produce sporangia and zoospores on water agar (WA) or PCA after flooding with water. A low-temperature shock (5–10 °C for approximately 2 hours) may assist sporangial production in *Pythium*. Some homothallic *Pythium* species also produce the oospores on WA. However, some cultures of homothallic *Pythium* species grown on sterile rice leaf pieces in sterile water in a Petri dish have produced abundant oogonia and antheridia at room temperature.

Refer to published descriptions of *Pythium* species to assist with identification to species level and forward cultures to reference laboratories to confirm identification.

Isolates of some common *Pythium* species in Vietnam will also produce sporangia and zoospores in rice-leaf water culture.

Some *Phytophthora* species will produce sporangia on *Phytophthora* selective medium (PSM), a selective isolation medium, if exposed to the light. Some species will also produce sporangia on PCA, which is readily prepared in the laboratory.

Sporangial production can also be stimulated by transferring 1 cm^2 blocks of cultures on PSM or PCA to sterile water in a Petri dish and incubating in the light for 2 days.

Many excellent texts have been produced and should be referred to for more in-depth information. Refer to *Phytophthora Diseases Worldwide* by Erwin and Ribeiro (1996) for detailed descriptions of the sporangia of *Phytophthora* species to assist in identification. For further assistance with *Phytophthora* identification also refer to the booklet *Practical Guide to Detection and Identification of* Phytophthora by Drenth and Sendall (2001).

The majority of common plant pathogenic *Phytophthora* species in Vietnam are heterothallic, namely *P. capsici*, *P. palmivora*, *P. nicotianae*, *P. infestans*, *P. cinnamomi* and *P. colocasiae*. Note that *P. heveae* is homothallic, and sexual reproduction in *P. citrophthora* is rare.

In heterothallic species, it is necessary to cross strains of opposite mating type for sexual reproduction. This may not be feasible in a provincial diagnostic laboratory.

The mode of formation and morphology of sporangia of *Phytophthora* provide a practical guide to the identification of the most important species in Vietnam if a reliable text such as Erwin and Ribeiro (1996) is available.

Zoospores are normally formed in a vesicle at the end of the discharge tube in *Pythium*. In contrast, zoospores are normally formed in the sporangium of *Phytophthora* species. This is a reliable difference for separating the two genera.

10.4.4 Oomycete disease cycle—*Phytophthora* and *Pythium*

Figure 10.8 is a diagrammatic representation of the oomycete disease cycle and Figure 10.9 shows features of *Pythium* species and sporangia of a *Phytophthora* sp.

10.4.5 *Pythium* species

Pythium species belong to the class Oomycetes. They are not true fungi as this class is in the kingdom Chromista.

Motile spores called zoospores are important spores formed by *Pythium* (and *Phytophthora*) species, and distinguish these fungi from the true fungi in the kingdom Fungi (Mycota). The asexually produced zoospores enable these fungi to disperse in wet soil and irrigation water. Figure 10.10 shows diseases of peanuts caused by *Pythium*.

Pythium species can cause seedling death, but rarely cause death of older plants. However, they can cause severe feeder rootlet rots and disrupt the uptake of nutrients, which causes stunting, slight yellowing and yield loss.

Table 10.5 provides information about *Pythium* species, which are oomycetes that cause various fungal-like diseases on a range of crop plants.

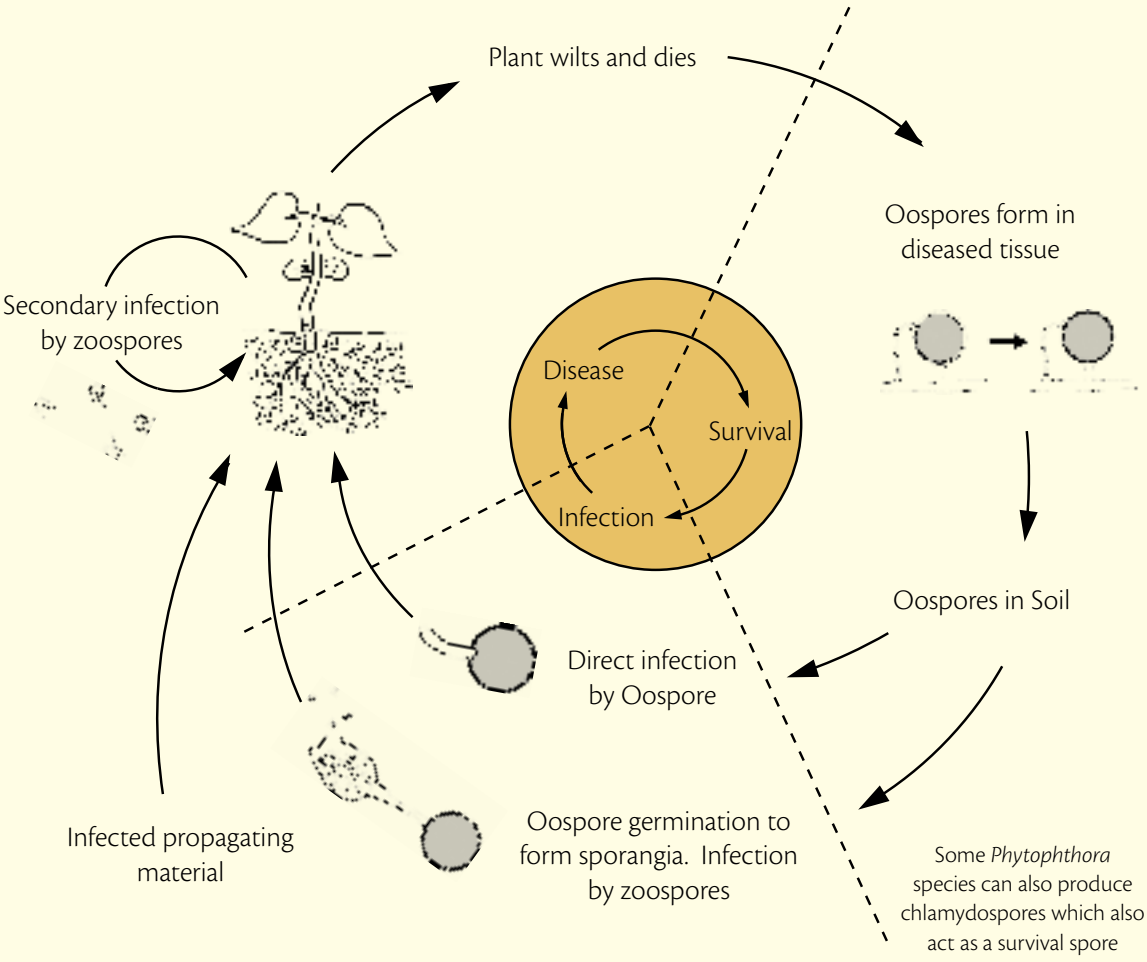


Figure 10.8 Simplified disease cycle of an oomycete plant pathogen

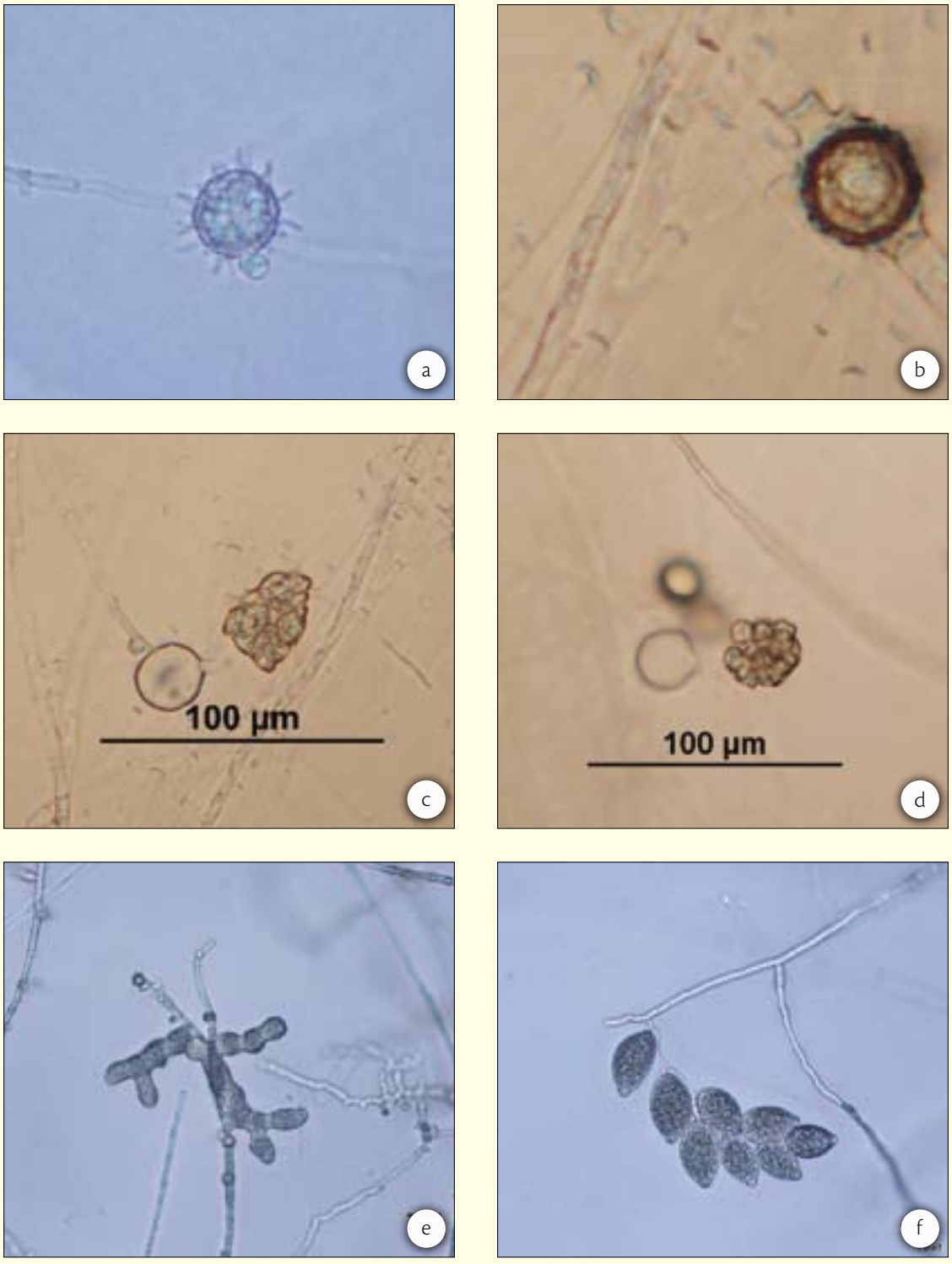


Figure 10.9 (a) Oogonium of *Pythium spinosum* showing attached lobe of an antheridium, (b) mature oospore of *P. mamillatum*, (c) sporangium of *P. mamillatum* showing discharge tube and vesicle containing developing zoospores, (d) sporangium of *P. irregulare* showing mature zoospores in thin walled vesicle prior to release, (e) digitate sporangia in *P. myriotilum*, (f) distinct sporangiophore and sporangia of *Phytophthora* sp.

Table 10.5 Characteristics of *Pythium* species

Diseases	*Pythium* species cause seedling blights and death (damping-off diseases), and cause feeder rootlet rot of mature plants. They also cause rot of potato tubers, carrots and other storage organs. Pythium root and pod rot is a major disease of peanuts.
Key symptoms	In seedlings, the typical symptoms are wilting and death caused by root rot (browning) of the young rootlets and stem. *Pythium* species can also infect the feeder rootlets, causing stunting, and yellowing of the leaves of older plants. As infected plants mature, *Pythium* species can colonise and cause root rot of the main roots or taproot. *Pythium* species can also cause pod rot in peanuts.
Diagnostic signs	There are no diagnostic signs indicative of *Pythium*. It is necessary to isolate and identify the fungus in culture for accurate identification of the pathogen.
Host range	Most *Pythium* species have a wide host range.
Weather	Wet soil favours infection of plants by *Pythium* zoospores and the dispersal of zoospores through the soil. Environmental and soil conditions which inhibit root growth increase the risk of seedling blight and feeder rootlet rot.
Overseasoning	*Pythium* species survive as oospores produced though sexual reproduction. Under favourable conditions, these thick-walled spores germinate and initiate rootlet infection.
Infection	In wet soil, zoospores are attracted to the rootlet tip, where they produce germ tubes (young hyphae) that penetrate the rootlet tip and initiate rootlet rot.
Control	Seeds can be treated with fungicide, and seedling roots can be treated with fungicide by dipping prior to transplanting. Crop rotation is an important measure for reducing the incidence of Pythium root rots. It is essential to use pathogen-free transplants.
Isolation	*Pythium* species can be isolated from diseased rootlets: 1. Dip briefly in 70% ethyl alcohol and wash in sterile water. 2. Damp-dry on paper tissue. 3. Plate on water agar. Water agar is commonly used for isolation, as *Pythium* species quickly colonise the agar—most *Pythium* species have high growth rates. White dense fine mycelium is formed in culture on PDA. Many species can colonise a large PDA plate in less than 48 hours. Purify *Pythium* cultures by hyphal tipping. *Pythium* species usually produce abundant sporangia and oospores in rice-leaf water cultures.

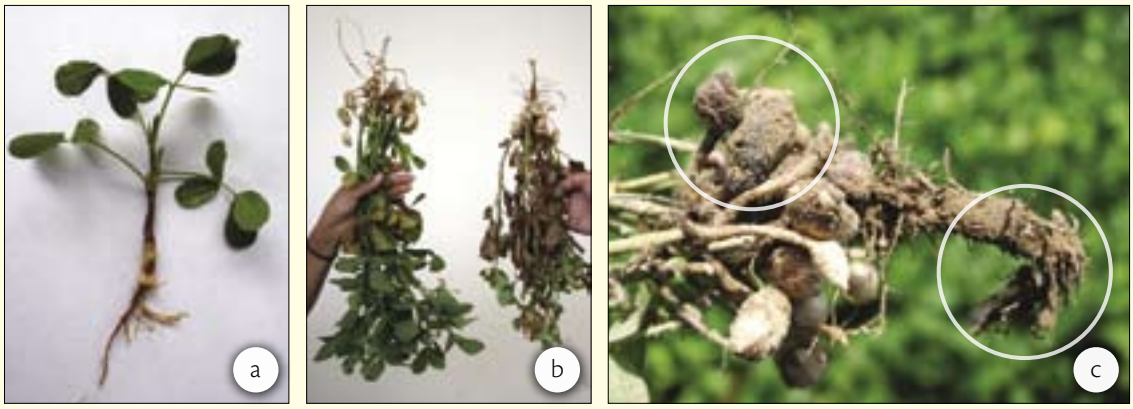


Figure 10.10 Pythium diseases on peanuts: (a) Pythium rootlet rot and stem rot of peanut seedling grown under very wet conditions, (b) comparison of two mature peanut plants, healthy plant (left), stunted plant with severe Pythium root rot (right), (c) severe Pythium pod and tap root rot of peanuts

10.4.6 *Phytophthora* species

Phytophthora, like *Pythium*, produces zoospores and is an oomycete—not a true fungus. Therefore, control of *Phytophthora* and *Pythium* pathogens differs from control of the diseases caused by true fungi and different fungicides are used.

Phytophthora diseases of tree, vegetable and other crops cause significant economic losses throughout South-East Asia. The isolation and identification of *Phytophthora* species and the use of integrated disease management are discussed in detail in the publications listed in the bookshelf section. Table 10.6 provides information about *Phytophthora*, an oomycete that causes a wide range of fungal-like diseases on many different crop plants in Vietnam. Some of these diseases are shown in Figure 10.11.

Table 10.6 Characteristics of *Phytophthora* species

Diseases	*Phytophthora* species cause a very wide range of diseases in Vietnam in fruit trees and vegetable, field and industrial crops. Diseases include root rot; trunk canker and fruit rot of durian; root rot of chilli; heart rot of pineapple; foot rot (quick wilt) of black pepper; late blight of potato and tomato; root, stem and fruit rot of paw paw; dieback of rubber and other tree crops.
Key symptoms	Infected trees die back from the top of the tree and may show root rot as well as canker symptoms on the trunk near the soil surface. Vegetable crops affected by root rot, such as chilli, become stunted and wilt. Plants usually die soon after severe wilt symptoms occur.

Diagnostic signs	Diagnosis requires the isolation and identification of the pathogen. Wilting is also caused by other root and stem pathogens.
Infection	The mode of infection depends on the species. However, oospores, sporangia and zoospores can incite infection of various plant parts. Rain splash dispersal of spores onto foliar plant parts can lead to infection of stems, leaves and fruit, depending on the species of *Phytophthora* and the host. Crawling and flying insects may also carry the fungus from the soil to the upper plant parts.
Host range	The host range of *Phytophthora* species depends on the particular species. Some species such as *P. palmivora* have a wide host range, whereas other species such as *P. infestans* have a narrow host range.
Overseasoning	The pathogens overseason as oospores and/or chlamydospores in soil, and can be transported in diseased propagating material or contaminated soil or farm implements.
Weather	Phytophthora diseases are favoured by wet conditions. High rainfall in tropical regions promotes splash dispersal of zoospores and other inocula. Zoospores also move in water in irrigation furrows and channels. Many *Phytophthora* species are favoured by hot wet conditions. In contrast, some species, such as *P. infestans* (late blight), are favoured by cool wet conditions.
Control	Successful control of Phytophthora diseases usually involves a number of control measures: • good drainage • use of disease-free planting material • exclusion of *Phytophthora* from non-infested areas • use of chicken manure as fertiliser to suppress activity of the pathogen in the soil • injection of trees with phosphonate • drenching of seedling roots at transplanting to reduce seedling death.
Isolation	*Phytophthora* species can be isolated readily from diseased foliar plant parts, such as pineapple leaves, using selective isolation media. For the method, see the protocol for isolation from pineapple heart rot samples, as pictorially described in the pineapple heart rot case study (Section 3.1). Isolation from diseased roots can be much more difficult. This is because there are many saprophytic fungi and bacteria growing in the diseased root tissues. A protocol for isolation of root pathogens can be found in Section 6.3.2. Baiting is recommended for the isolation of *Phytophthora* from small roots and soil. For more information on this technique refer to the section on baiting of pathogens from roots and soil (Section 6.3.4).

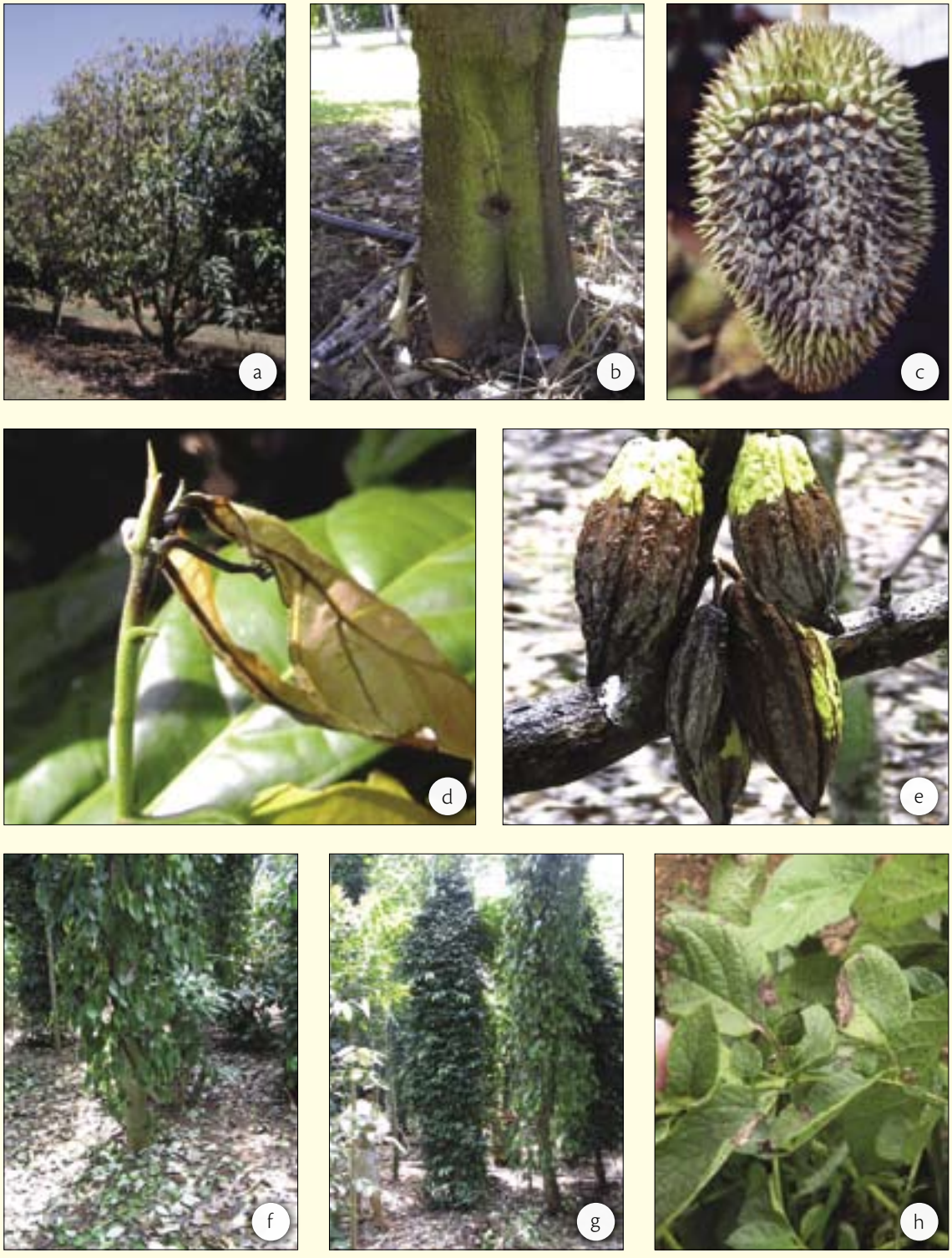


Figure 10.11 Diseases caused by *Phytophthora palmivora* on durian: (a) tree yellowing, (b) canker on trunk, (c) fruit rot. Diseases caused by *P. palmivora* on cocoa: (d) seedling blight, (e) black pod symptoms. Root rot (quick wilt) of black pepper caused by *P. capsici*: (f) leaf drop, (g) wilting. Disease caused by *P. infestans*: (h) late blight of potato.
Pictures (a) to (e) supplied by David Guest, (f) and (g) supplied by N. V. Truong.

10.5 *Fusarium* species

10.5.1 Introduction

The genus *Fusarium* includes many species that cause plant diseases, such as vascular wilts, root, stalk and cob rots, collar rot of seedlings, and rots of tubers, bulbs and corms. Some of the pathogenic species also produce mycotoxins that contaminate grain (see Mycotoxigenic *Fusarium* species, Section 12.3).

Many other *Fusarium* species are saprophytes which occur commonly in soil. The saprophytic species commonly colonise diseased roots and stems. These saprophytic species grow quickly on isolation media and can be readily isolated from diseased root and stem material, making it difficult to isolate the pathogen or pathogens that caused the disease. Therefore, it is important to test *Fusarium* isolates from diseased roots for pathogenicity to the plant. This is an important part of the diagnostic process, and is one of the reasons why the diagnosis of a root disease can be difficult. For example, *Fusarium oxysporum* includes many important pathogens (formae speciales) that cause vascular wilt diseases and some root rots. However, *F. oxysporum* also includes many saprophytic strains which colonise diseased roots after the pathogen has killed root tissues. Some of these saprophytic species may also colonise the outer cells of the roots as endophytes, causing no damage.

Saprophytic species of *Fusarium* in roots grow rapidly on PDA and are often assumed incorrectly to be pathogens.

Do not use PDA for isolating fungal pathogens from roots.

10.5.2 *Fusarium* pathogens in Vietnam

Fusarium vascular wilt diseases are important problems in Vietnam and these are discussed in detail later in this section. The wilts are caused by formae speciales of *F. oxysporum*. Some strains of *F. oxysporum* can also cause rots of melons and potato tubers damaged by insect pests or harvesting tools.

Cob rots in maize, caused mainly by *F. graminearum* and *F. verticillioides*, are becoming increasingly important in Vietnam. Both species produce mycotoxins which contaminate the grain (see *Fusarium* mycotoxins section in Section 12).

Some strains of *Fusarium solani* cause collar rot of legume seedlings such as peas and beans, and root rot of older plants. Other strains can affect the collar region of trees, such as lychee weakened by environmental stress or other diseases.

Fusarium decemcellulare has been isolated occasionally from branch cankers on longan in northern Vietnam (L. Burgess, unpublished data) and from coffee in Dac Lac province (Dr Tran Kim Loang, pers. comm.).

This list is not exhaustive and many other species probably occur in Vietnam. Figure 10.12 shows some of the diseases caused by *Fusarium* species.

Table 10.7 lists some formae speciales of *Fusarium oxysporum*, which causes vascular wilts.

Figure 10.12 Diseases caused by *Fusarium* species: (a) *Fusarium oxysporum* f. sp. *pisi* causing wilt on snowpeas, (b) *F. oxysporum* f. sp. *zingiberi* sporodochia on ginger rhizome, (c) stem browning caused by *F. oxysporum*, (d) Perithecia of *F. graminearum* on maize stalk.
Pictures (a) and (c) supplied by Ameera Yousiph.

Table 10.7 *Fusarium oxysporum* (vascular wilts)

Diseases	Fusarium vascular wilt diseases are caused by formae speciales of *F. oxysporum*. Each forma specialis can usually only cause wilt on one plant host species. There are more than 100 Fusarium vascular wilt diseases world-wide. In Vietnam, Fusarium vascular wilt disease of bananas is one of the well-known and important wilt diseases. Some Fusarium vascular wilt pathogens and their associated diseases in Vietnam are:	
	F. oxysporum f. sp. *cubense*	Fusarium wilt of banana (Panama disease)
	F. oxysporum f. sp. *lycopersici*	Fusarium wilt of tomato
	F. oxysporum f. sp. *pisi*	Fusarium wilt of peas
	F. oxysporum f. sp. *niveum*	Fusarium wilt of watermelon
	F. oxysporum f. sp. *callistephi*	Fusarium wilt of asters
	F. oxysporum f. sp. *zingiberi*	Fusarium wilt of ginger
	F. oxysporum f. sp. *dianthi*	Fusarium wilt of carnations
	There is a need to survey Fusarium wilt diseases in Vietnam as there are many important exotic Fusarium wilt diseases that have not been reported, to the authors' knowledge (e.g. Fusarium wilt of cabbage). Such diseases would be best excluded by good quarantine regulations.	
Symptoms	Early symptoms include leaf yellowing, slight wilting during the day and stunting. In hot conditions diseased plants such as tomato and peas will die within a few days. Diseased bananas usually die slowly, taking 1–2 months.	

Yellowing, wilting and stunting are general symptoms of many diseases of the root and stems.

Browning of the internal stem (vascular) tissue is a key symptom of pathogens which cause vascular wilt disease, including Fusarium wilt pathogens.

Figure 10.13 shows some of the Fusarium wilts that occur in Vietnam.

Table 10.8 provides information about formae speciales of *Fusarium oxysporum*, fungi that cause vascular wilt diseases on crop plants.

Figure 10.13 Fusarium wilt of banana caused by *F. oxysporum* f. sp. *cubense*: (a) severe wilt symptoms, (b) stem-splitting symptom, (c) vascular browning. Fusarium wilt of asters caused by *F. oxysporum* f. sp. *callistephi*: (d) severe wilt causing death, (e) wilted stem with abundant white sporodochia on the surface. Fusarium wilt of snowpeas caused by *F. oxysporum* f. sp. *pisi*: (f) field symptoms of wilt (note patches of dead plants), (g) vascular browning in wilted stem. Picture (f) supplied by Ameera Yousiph.

Table 10.8 Characteristics of Fusarium wilts

Diagnostic signs	**Banana** Initially, infected plants develop yellowing on leaf margins, then leaves droop and wilt. Later in disease development stem cracking becomes obvious and plants die. Stem browning is an obvious symptom of infection. Note that banana stem borer can also cause similar symptoms of leaf yellowing and wilting. **Tomato** The first symptoms are usually leaf yellowing followed by wilting and within a few days plant death. Browning of the outer part of the stem (vascular symptoms) is usually obvious. **Cucurbits** Infected plants may wilt and die suddenly in hot weather, especially late in the season when plants have many fruit. Yellowing occurs in some varieties under cooler, less stressful conditions. Stem and root browning may not be obvious until severe wilting occurs.
Host range	A forma specialis usually causes vascular wilt in only a single host species. For example, *F. oxysporum* f. sp. *niveum* causes wilt of watermelon.
Weather	Fusarium vascular wilt diseases are usually more severe in warm, wet conditions.
Overseasoning	Fusarium wilt pathogens persist/survive as chlamydospores in soil for long periods. Chlamydospores are round, one-celled spores with thick resistant cell walls, formed in diseased tissue. Fusarium wilt pathogens can also colonise the root cortex of some non-host plants, including both weed and crop plants. Chlamydospores form in the cortex when the plant dies. Thus non-host crops must be tested before being recommended for use in rotation to control Fusarium wilt.
Infection	Hyphae and germinating chlamydospores in diseased plant residues and soil infect young rootlets and enter the xylem vessels. The pathogen then colonises the xylem, growing up the vascular system in the stem. Colonisation causes the plant to react, producing brown phenolic compounds and tyloses. These compounds cause browning of the vascular tissue, an obvious sign of wilt disease in cut stems. Blocking of the xylem decreases water movement, causing the infected plant to wilt and die. Fusarium wilt diseases are commonly associated with root knot nematodes. The *Fusarium* infects through wound sites made by the nematode.

Control	Fusarium wilt diseases are difficult to control as the chlamydospores persist for long periods in soil. Rotation to resistant crops involving a minimum of 2 years break between susceptible crops can assist in reducing inoculum levels. However, these fungi can persist (survive) by infecting the root cortex of some symptomless, non-host crops. This highlights the need for research into the biology of the fungus in each country to determine the role of non-host crops and the length of survival of chlamydospores in soil. Resistant crop varieties are available against some Fusarium wilt pathogens. However a resistant variety may not be resistant to all races of the particular forma specialis. There are no effective fungicide treatments.
Isolation	Fusarium wilt pathogens can be readily isolated from infected stem tissue (Section 6.3.1), using a *Fusarium*-selective medium (PPA) or WA. Isolation should be from stems with early wilt symptoms.

10.5.3 Fusarium wilt isolation

The following technique is for isolating *Fusarium* species from crop plants:

1. Select a 4 cm piece of stem from at least 20 cm above the soil surface.
2. Wash the stem in tap water and surface sterilise in 70% ethyl alcohol for 1 minute.
3. Dry on sterile paper tissue or flame dry if the stem is thick.
4. Aseptically cut the stem piece into 1–2 mm thick sections.
5. Plate sections on isolation medium (WA or PPA). A fungal colony will develop from each section in 2–3 days.
6. Subculture onto carnation leaf piece medium or rice stem medium and grow under lights.
7. Purify using the single spore technique (Section 6.5.2) and grow pure cultures on CLA or green rice stem medium, and PDA under lights.

Identify the pathogen on CLA or green rice stem medium using the following key features:

- oval microconidia formed in false heads on short monophialides
- medium-length banana-shaped macroconidia with foot cells in sporodochia on leaf pieces
- chlamydospores (formed after 2–3 weeks)

Do not try to identify *Fusarium* species using conidia from PDA cultures.

On PDA *F. oxysporum* produces:

- a range of pigments produced by the colonies in agar, from none to purple to violet
- mycelium white to purple.

10.5.4 *Fusarium oxysporum* and *Fusarium solani*—key morphological features for identification

Isolates of *Fusarium oxysporum* and *F. solani* can be difficult to distinguish by inexperienced researchers (Table 10.9 and Figures 10.14–10.16). *F. oxysporum* mainly causes vascular wilt diseases, while *F. solani* mainly causes collar and root rots.

It is important to remember that non-pathogenic (saprophytic) isolates of these species are commonly isolated from healthy and diseased roots. Before making conclusions about their role in disease, pathogenicity testing is required.

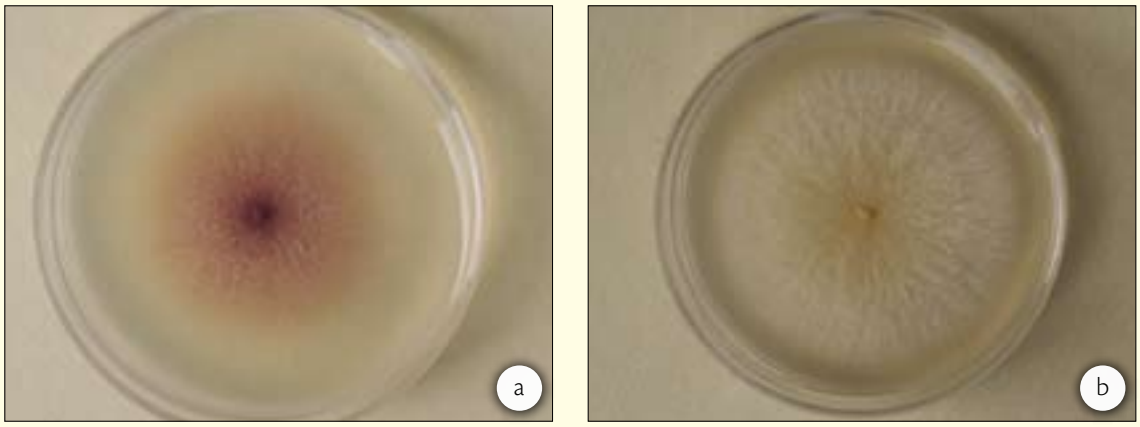


Figure 10.14 Four-day-old cultures of *Fusarium oxysporum* (left) and *F. solani* (right), in 60 mm Petri dishes on potato dextrose agar

Occasionally pure cultures of *F. solani* in Vietnam will produce orange perithecia, a product of sexual reproduction.

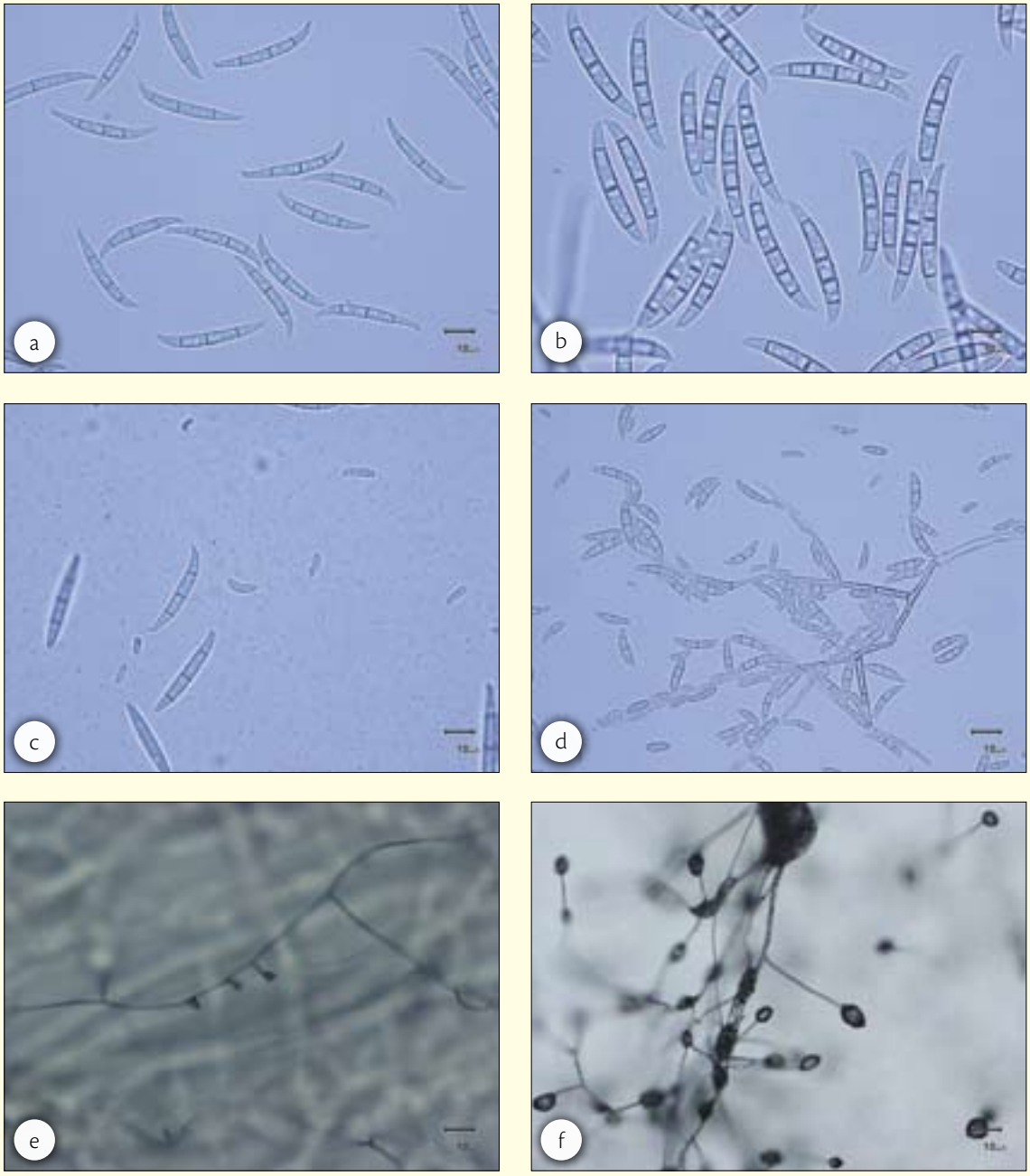


Figure 10.15 Differentiating between *Fusarium oxysporum* (left) and *F. solani* (right): (a) and (b) macroconidia, (c) and (d) microconidia and some macroconidia, (e) and (f) microconidia in false heads on phialides (note the short phialide in *F. oxysporum* and the long phialide typical of *F. solani*)

Table 10.9 Hints for differentiating between *Fusarium oxysporum* and *Fusarium solani*

	F. oxysporum	*F. solani*
On PDA	Colonies produce violet to purple pigment in agar and mycelium	Colonies are white to cream in colour, some have a slight green or blue pigmentation
On CLA (or green rice stem agar)	Macroconidia in sporodochia are slender and of medium length	Macroconidia in sporodochia are wide relative to length and larger than in *F. oxysporum*
	Microconidia are small, usually non-septate and are formed in false heads on very short phialides	Microconidia are large, often 1–3 septate and are formed in false heads on very long phialides or branched conidiophores

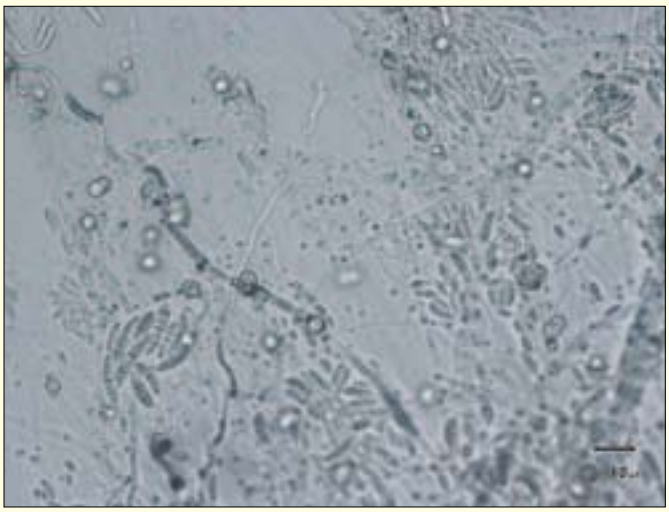


Figure 10.16 Chlamydospores of *Fusarium solani* in culture on carnation leaf agar (CLA) (*F. oxysporum* chlamydospores look the same)

10.6 *Verticillium albo-atrum* and *V. dahliae*— exotic fungal wilt pathogens

The fungal wilt pathogens *Verticillium albo-atrum* and *V. dahliae* have not been reported in Vietnam and *V. albo-atrum* is included in the checklist of quarantine diseases. These pathogens cause similar key symptoms (Figure 10.17).

Table 10.10 provides information about *Verticillium albo-atrum* and *V. dahliae*, which are fungal wilt pathogens currently exotic to Vietnam.

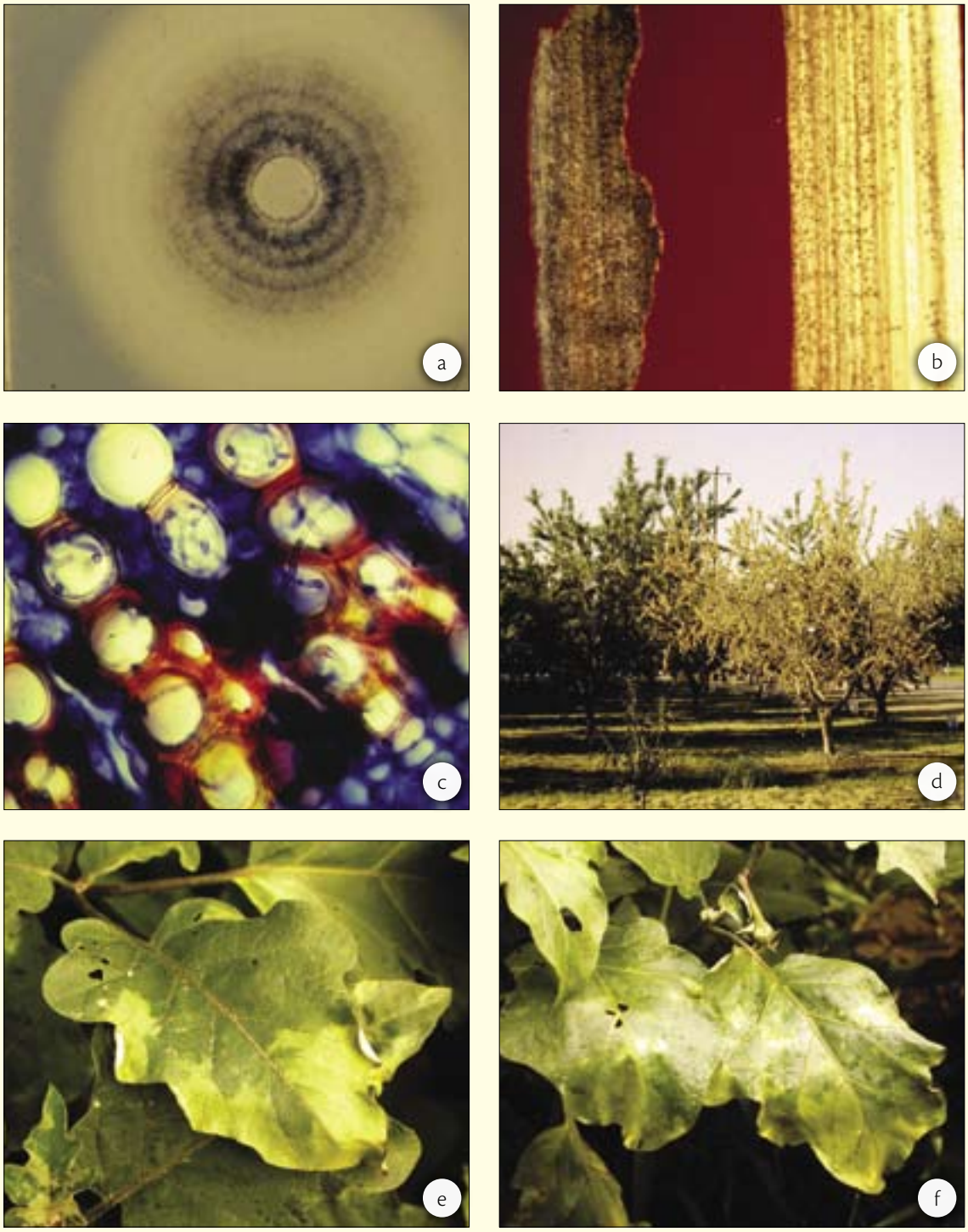


Figure 10.17 *Verticillium dahliae*: (a) culture on potato dextrose agar (cultures grow slowly), (b) microsclerotia on old cotton stem, (c) hyphae in infected xylem vessels, (d) wilted pistachio tree affected by *V. dahliae*, (e) and (f) wilted leaves of eggplant infected by *V. dahliae*

Table 10.10 Characteristics of *Verticillium albo-atrum* and *V. dahliae*

Key symptoms	Symptoms include yellowing, wilting and vein browning of the leaves. Browning of the vascular tissues is usually present in the stem.
Diagnostic signs	Wilting and vascular browning of the stem. Isolation and identification of the fungus is essential for accurate diagnosis.
Infection	These fungi infect through the feeder rootlets and enter the xylem. They then grow through (colonise) the xylem in the stem, petiole and leaves. Growth of the fungi in the stem causes stem browning and reduces the uptake of water, causing wilting and plant death.
Host range	Both pathogens have a wide host range, causing vascular wilt of many broad-leafed (dicotyledonous) plants including tomato, potato, cotton, cucurbits, strawberry and some temperate fruit crops such as almonds and walnuts.
Overseasoning	*Verticillium albo-atrum* survives as hyphae in host residues. *V. dahliae* survives as microsclerotia in host residues and soil, and as hyphae in host residues.
Weather	Both pathogens are more common in temperate regions of the world. In Vietnam, the north west mountainous regions and Dac Lac region would be most suitable for these pathogens. They could also establish in the central and northern regions, which experience low winter temperatures.
Control	Crop rotation is effective if resistant crops are available. The pathogens' wide host range limits choice of crops in vegetable growing areas. Resistant varieties are available for some crops. Pathogen-free cuttings and rootstocks are essential for crops such as strawberries. Susceptible weed hosts should be controlled.
Isolation	*V. albo-atrum* and *V. dahliae* are slow growing in culture. They can be difficult to isolate. If possible, isolate from stems or petioles of plants with early symptoms of stem browning. 1. Surface sterilise stem sections for 1 minute in 70% ethyl alcohol. 2. Dry on paper tissue. 3. Cut discs of stem tissue and plate on water agar or green rice stem piece agar (water agar containing small pieces of sterilised green rice stem). 4. Subculture, purify by single spore technique and grow on PDA and green rice stem piece agar. *V. dahliae* will produce black microsclerotia in culture, especially on pieces of straw or sterile host root fragments. **These species will produce their typical verticillate conidiophore from the infected xylem vessels of the stem pieces plated on WA or green rice stem agar.**

10.7 Plant parasitic nematodes

Plant parasitic nematodes are small non-segmented round worms. Plant parasitic and non-plant parasitic nematodes are found in soil. The presence of a stylet (mouth spear) (Figure 10.18a) is a key feature of plant parasitic nematodes. The stylet can be seen clearly using a compound microscope, and it can also be seen under a good dissecting microscope at higher magnification. Note that there are some species with stylets that feed on fungal hyphae in soil but do not affect plants.

Figure 10.18 Nematodes: (a) plant parasitic with piercing stylet (mouth spear), (b) non-plant parasitic with no stylet

Plant parasitic nematodes cause a wide range of diseases in Vietnam (Nguyen 2003). The most common diseases in Vietnam are the root knot nematode diseases caused by *Meloidogyne* species and root lesion nematode diseases caused by *Pratylenchus* and other species (Figure 10.19).

Cyst nematodes infect roots, causing root proliferation (a cluster of small roots). The female cyst is obvious, being attached to the outside of the root at the point of root proliferation.

Plant parasitic nematodes commonly affect roots and reduce water and nutrient uptake. They normally cause stunting and poor yield and sometimes cause obvious yellowing. Severe infection by nematodes sometimes causes wilting and plant collapse under stress conditions. This can be seen most commonly from root knot nematode infection of tomato plants.

Root knot nematode can be diagnosed in the field by obvious root galls (Figure 10.20).

Figure 10.19 Damage to a plant root system caused by: (a) root knot nematode, (b) root lesion nematode, both diseases resulting in stunting and yellowing

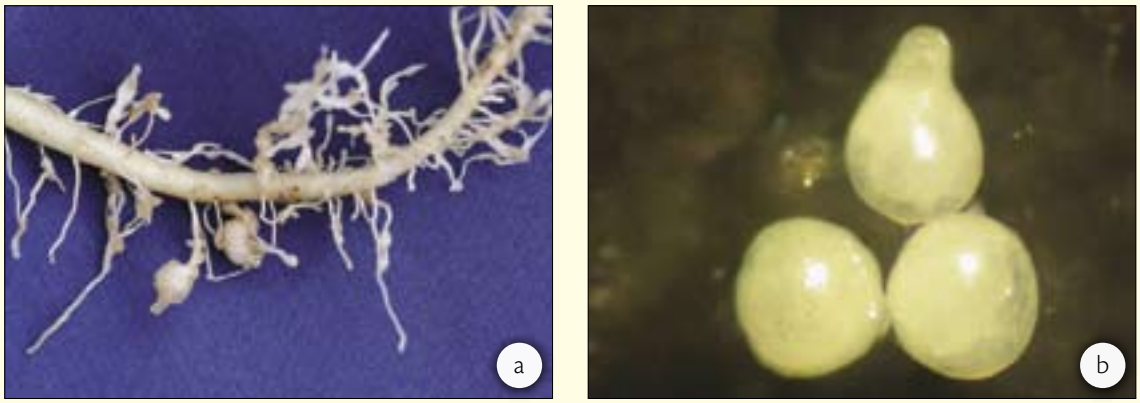


Figure 10.20 Root knot nematode symptoms: (a) swollen root (knot) symptoms, (b) female nematodes found within root knots (galls)

Root lesion nematode damage can be difficult to see on small roots in the field, but lesions can be detected with a magnifying glass (hand lens). They are more obvious using a dissecting microscope. If root lesion nematodes are suspected, they can be seen under the microscope more easily if infected rootlets are stained. Nematodes which enter the root to feed can be extracted using the techniques described for extracting nematodes from soil (see Section 10.7.1).

Hosts resistant to a particular nematode species are not affected by the nematode and prevent multiplication (reproduction) of the plant parasitic nematodes. These hosts help reduce nematode inoculum in soil.

Tolerant hosts are not affected by a particular species of plant parasitic nematode, but allow the nematode to multiply. These hosts maintain or increase nematode inoculum in soil. It is important when recommending control measures to understand this difference in host–nematode relationships.

Nematodes cannot swim. They move in soil or plant roots by 'wriggling' with a snake-like movement and pressing against the soil particles or plant tissue. They can be carried in moving irrigation water, but in still water they sink to the bottom. They can move in all directions through wet soil in search of host roots.

Nematodes can survive in the absence of the host in a dormant state. In dry periods they move down deeper in the soil profile.

The majority of plant parasitic nematodes affect a wide range of hosts, but the degree of susceptibility varies depending on the host plant.

10.7.1 Nematode extraction from soil and small roots.

Nematodes cannot swim in water. This feature is the basis for the simple extraction techniques described below (Figure 10.21).

Baerman funnel technique

This Baerman funnel technique involves the use of the equipment shown in Figure 10.22.

1. Take a small subsample from a composite, well-mixed soil sample from the root zones of 10 plants.
2. Pour the subsample gently into the water in a glass or plastic funnel containing paper tissue, which is supported by a plastic sieve with small (1 mm) mesh. Do not tear the tissue.

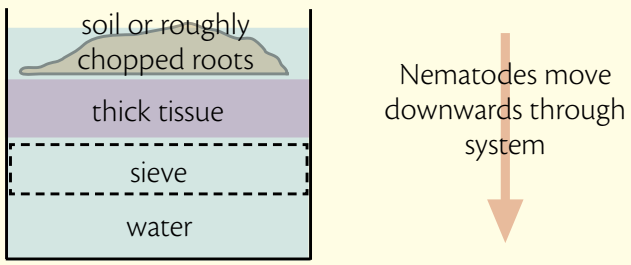


Figure 10.21 Schematic illustration of common procedures for extracting nematodes from roots or soil

3. After 24 hours release 5 mL of water from the tube into a small Petri plate or counting plate. The nematodes will have moved from the wet soil down through the tissue into the water. As they cannot swim, nematodes sink down the tube and collect at the base of the plastic tube.
4. Spread the suspension of nematodes across the plate and examine under a dissecting microscope at highest magnification, or transfer to a glass slide for examination under a compound microscope using an eye-dropper or a fine hair glued to a thin length of plastic or wood.

Plant parasitic nematodes can be identified by the presence of a stylet.

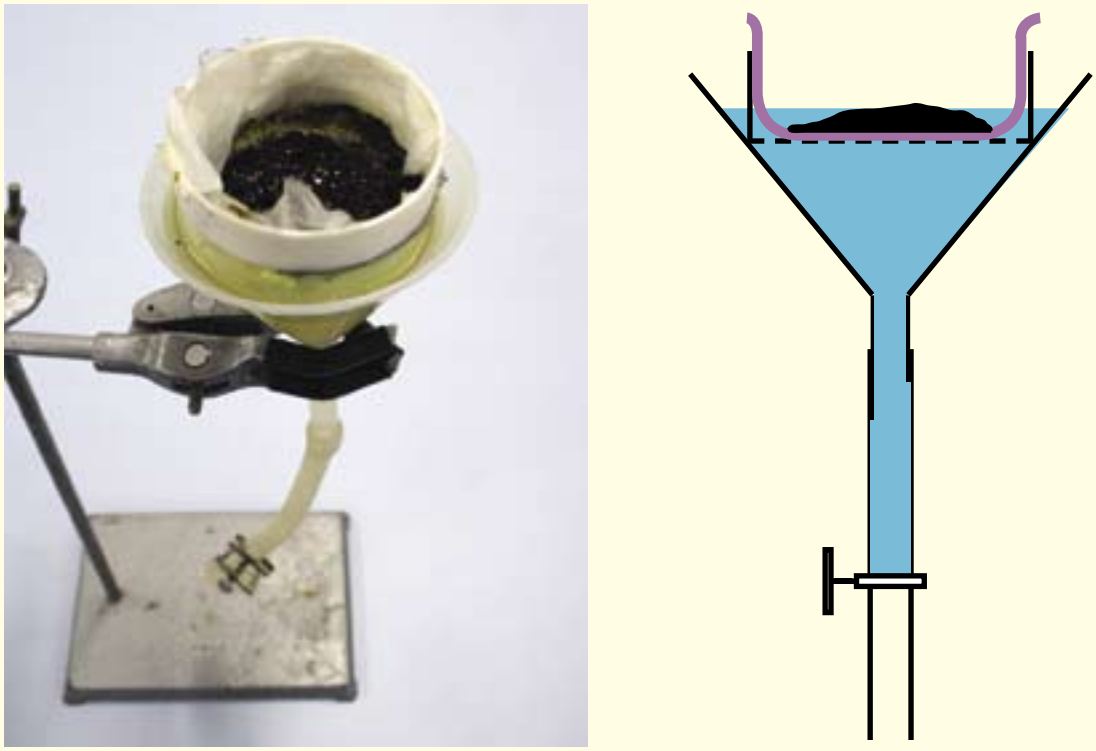


Figure 10.22 Baerman funnel apparatus for nematode extraction

Whitehead tray technique

The Whitehead tray technique is also commonly used for soil or root samples and involves the use of equipment shown in Figure 10.23.

1. Line a kitchen sieve with a large thick paper tissue and place the sieve in a bowl.
2. Add water to a depth of approximately 2 cm above the sieve.
3. Gently place soil or root material in the water on the sieve. Do not tear the tissue. The nematodes collect in the water below the tissue.

4. After 24 hours, pour the water into a glass beaker or jar and allow the nematodes to settle to the bottom of the jar.
5. Pipette the water from the bottom of the jar into a small Petri plate for examination under the dissecting microscope.

Both plant parasitic and non-plant parasitic nematodes are likely to be present.

These techniques are designed primarily for diagnostic use. However, with experience and the use of standard sampling techniques and replicated samples they can also provide useful quantitative data on nematode numbers. The Whitehead tray is the most appropriate technique for quantitative studies.

This system can be made from sieves in containers which are readily available in Vietnam in markets and shops.

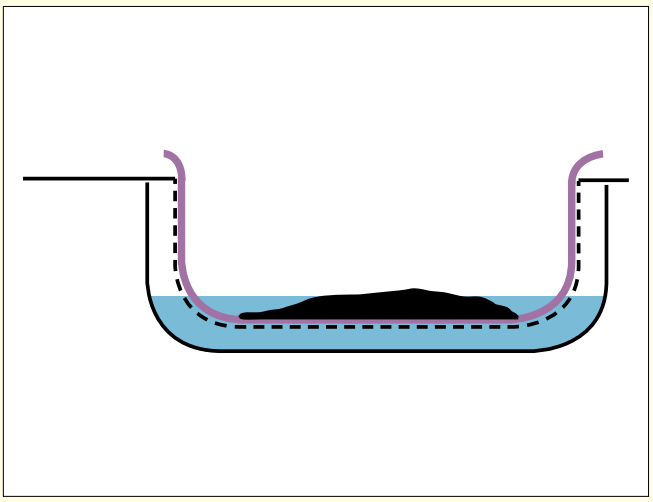


Figure 10.23 Whitehead tray apparatus for nematode extraction

10.8 Diseases caused by bacterial pathogens

Many bacterial species cause diseases of plants, while others cause diseases of humans and animals. The majority of bacteria are saprophytic and occur in soil and organic matter as decomposers.

Bacterial plant pathogens are small prokaryotic organisms that can be seen with the ×100 objective of the compound microscope. They are easier to see using appropriate stains. They are variable in size and shape; some species have flagella and are motile. Most bacterial plant pathogens can be isolated and grown on appropriate media.

A bacterial cell reproduces by simple division into two cells. Multiplication can be quite rapid under optimal conditions.

Bacterial diseases are common in tropical regions. There are a wide range of diseases caused by bacterial plant pathogens, including bacterial wilts, leaf spots, leaf blights, galls and cankers (Figure 10.24). Some species also cause serious soft rots of fruits and vegetables before and after harvest.

Common bacterial plant pathogens in Vietnam include the genera *Ralstonia*, *Xanthomonas*, *Pseudomonas* and *Erwinia*. Some pathogens are carried on seed, others on infected plant material.

Bacterial ooze is an indicator of the presence of a bacterial pathogen in diseased tissue. Bacterial pathogens may produce ooze on leaf spots under wet conditions and from the vascular tissue of stems of plants with bacterial wilt.

10.8.1 Bacterial wilt

Bacterial wilt caused by *Ralstonia solanacearum* is a serious disease of many vegetable and other crops in Vietnam. In Quang Nam province, for example, bacterial wilt occurs in tomato, chilli, eggplant, bitter melon, tobacco and some other crops and weeds. This wide host range makes it difficult to control by rotation. This bacterium survives for long periods in infected host residues in soil. *R. solanacearum* can be disseminated in infected planting material such as potato and ginger, in seedlings and in soil attached to farm tools and animals.

Bacterial wilt can be diagnosed in wilted plants, either in the field or in the laboratory, by the presence of stem browning in the vascular tissue and bacterial ooze. If a cut stem is placed in water, white strands of ooze stream into the water.

Note that there are other bacterial pathogens that cause wilt diseases.

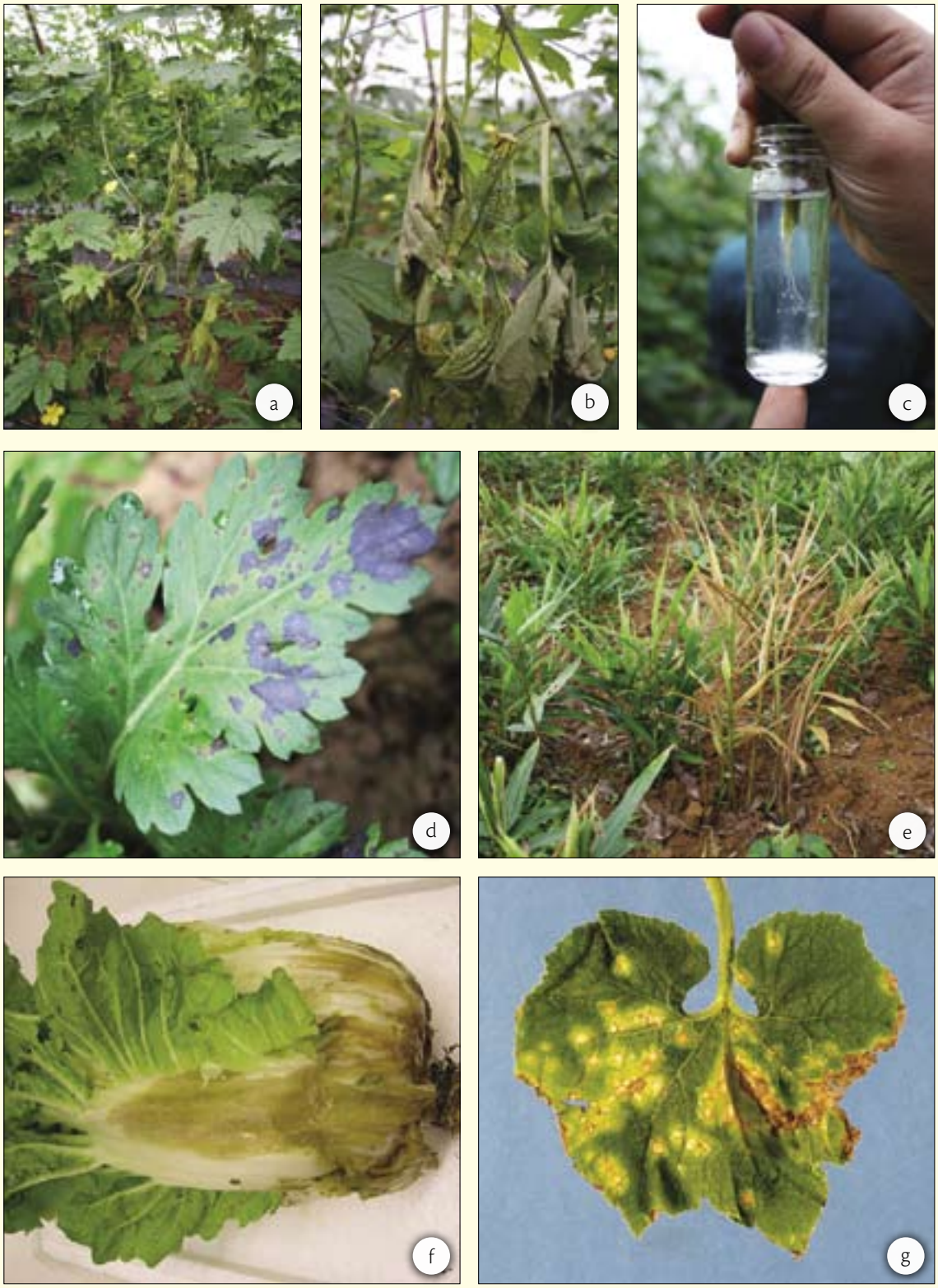


Figure 10.24 Diseases caused by bacterial pathogens: (a–c) Bacterial wilt of bitter melon, (d) bacterial leaf blight, (e) *Ralstonia solanacearum* causing quick wilt of ginger, (f) bacterial soft rot of chinese cabbage caused by *Erwinia aroideae*, (g) *Pseudomonas syringae* on cucurbit leaf

Control measures include rotation to crops such as maize and rice, the use of disease-free planting material (seedlings and cuttings) and removal and burning of diseased plants. Resistant varieties of peanuts and some other crops are available. Bacterial wilt of some susceptible crop cultivars is controlled by grafting onto resistant rootstocks.

10.8.2 Isolation of bacterial plant pathogens

Bacterial wilt and bacterial leaf spots and blights are common in Vietnam. Many of these pathogens can be isolated and purified in a basic laboratory. Pure cultures can then be tested for pathogenicity using Koch's postulates (see Box 8.1). If cultures are pathogenic they can be sent to a bacteriology laboratory for identification. Precise identification of species is best done in a specialist laboratory.

King's B medium is recommended for isolation of the common bacterial plant pathogens.

Isolation procedure for *Ralstonia solanacearum*, the cause of bacterial wilt

1. Cut a 2–3 cm section of the stem of the wilted plant, after checking for bacterial ooze (Figure 10.25a).
2. Surface sterilise by wiping the section with a tissue with 70% ethyl alcohol, or by dipping the section in 70% ethyl alcohol and flaming (Figure 10.25b).
3. Cut the section into three pieces with a sterile knife or scalpel (Figure 10.25c).
4. Transfer pieces to 10 mL of sterile water in a test tube (Figure 10.25d) and leave until the bacterial ooze turns the water a milky colour (Figure 10.25e).
5. Flame a transfer loop and let it cool (Figure 10.25f).
6. Dip the transfer loop into the liquid containing the bacterial ooze (Figure 10.25g).
7. Streak a plate of King's B medium by touching the agar near one side and gently making 3–4 streaks on the agar (Figure 10.25h).
8. Flame the loop again and let it cool.
9. Gently streak the loop 3–4 times, making sure the loop crosses the previously made streaks (Figure 10.26).
10. Repeat steps 8 and 9 one more time, and add a final zig-zag streak.
11. Incubate the plate for 2 days at approximately 25–30 °C (Figure 10.27).
12. Examine the plate to look for small single colonies on the third or fourth set of streaks (Large colonies that develop within 24 hours are not *R. solanacearum*.)
13. Aseptically transfer one small colony and streak on King's B medium.
14. Incubate for 2 days.

15. Subculture from a single colony on the new plate to a small McCartney bottle or test tube slope of King's B medium. This should be a pure culture and can be used for a pathogenicity test (see, for example, the ginger wilt case study in Section 3.1].

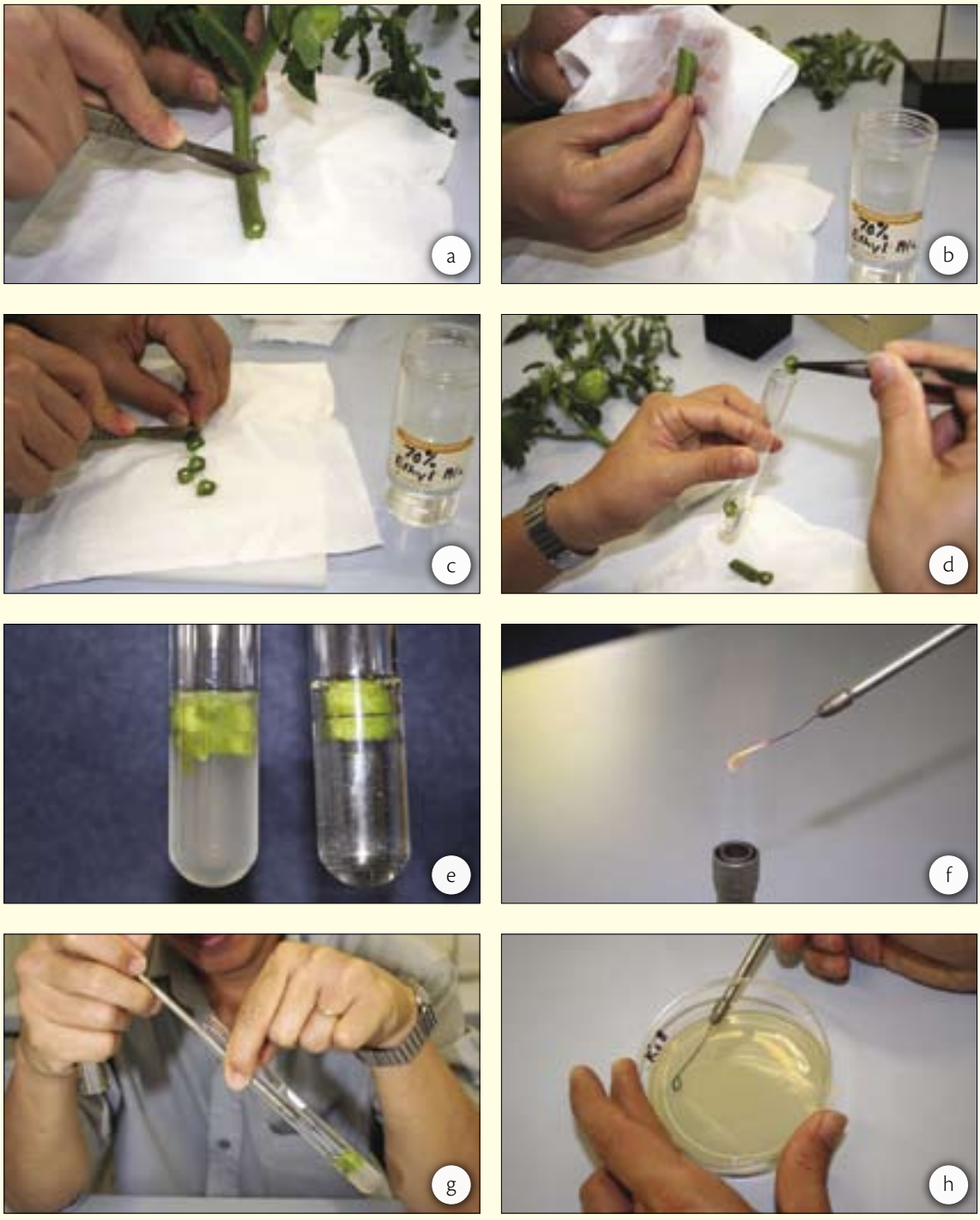


Figure 10.25 Technique for isolating *Ralstonia solanacearum* from an infected stem

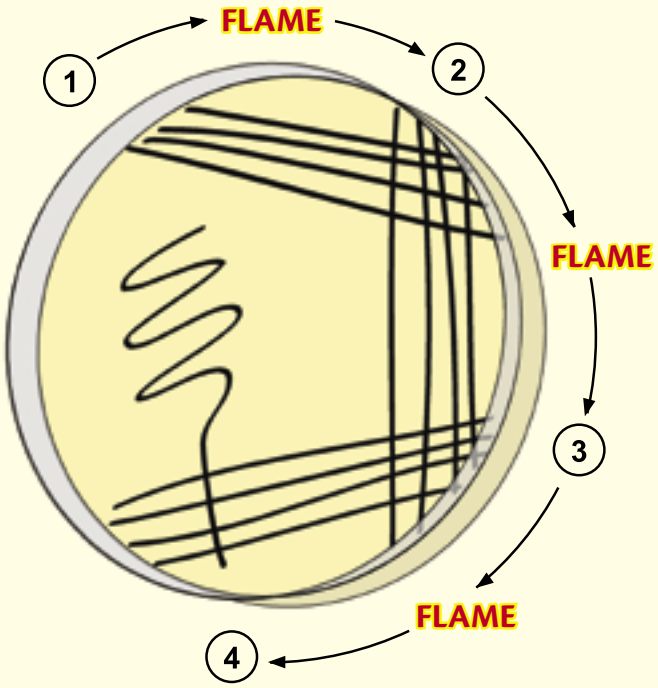


Figure 10.26 Diagram of bacterial streak plating, showing order of streaking and flaming between each step

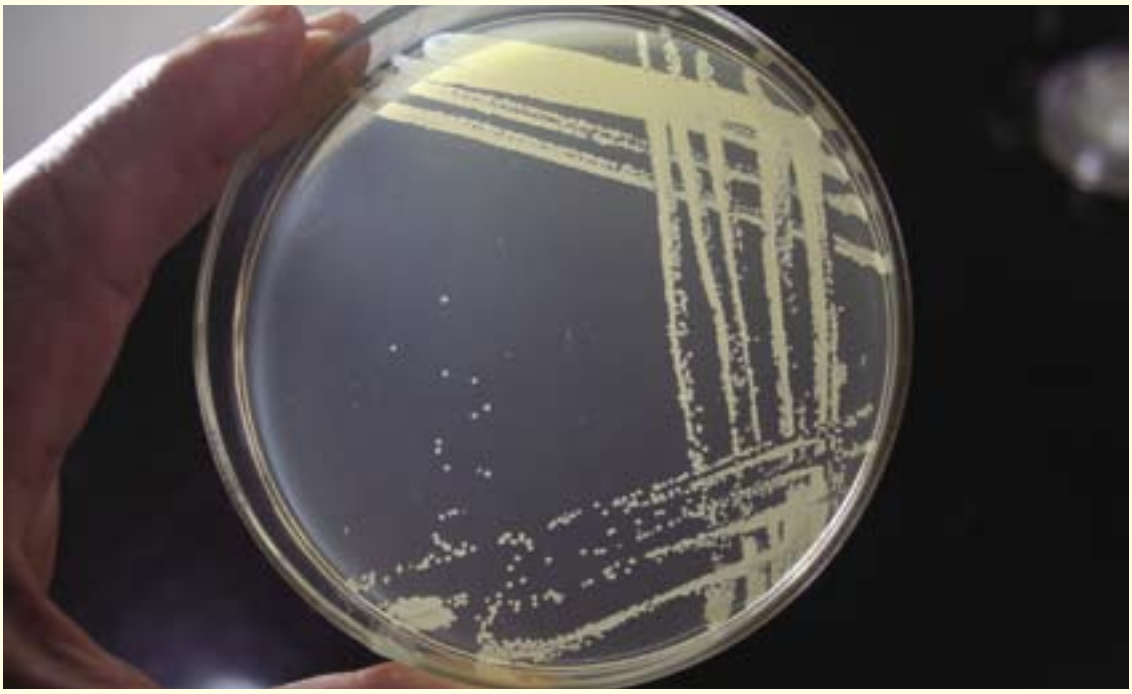


Figure 10.27 Bacterial streak plate after 2 days growth at 25 °C

Isolation of bacteria from leaf spots and blights

1. Surface sterilise a glass slide and place a drop of sterile water on the slide.
2. Gently surface sterilise the leaf with a tissue moistened with 70% ethyl alcohol.
3. Aseptically cut a small section of the leaf spot or blight, including a vein, and transfer to the sterile water drop on the slide. Check the section with a compound microscope (×10 objective). It is common to see bacterial ooze flowing from the cut vein if the disease is caused by a bacterial pathogen.
4. Cut (macerate) the section to release the bacteria into the water drop. Leave for 3–5 minutes to allow the bacteria adequate time to release into the water drop.
5. Place a sterile transfer loop in the water drop and streak on King's B medium as described previously.
6. Prepare a pure culture as described previously, perform a pathogenicity test, and send a pure culture to a bacteriologist for precise identification, if necessary.
7. To test for pathogenicity, spray a leaf with a bacterial suspension in sterile water and incubate in a large plastic bag at high humidity. Do not place in sunlight or the plastic bag will get too hot and prevent infection.

Isolation of bacteria from roots or rhizomes

The isolation of bacteria from roots and rhizomes is essentially the same as that from leaf spots and blights (Figure 10.28). However, the amount of surface sterilisation will vary depending on the thickness of the roots and the pathogen being isolated. It is recommended that the outer tissues of roots or rhizomes be removed by cutting or scraping before surface sterilisation and isolation.

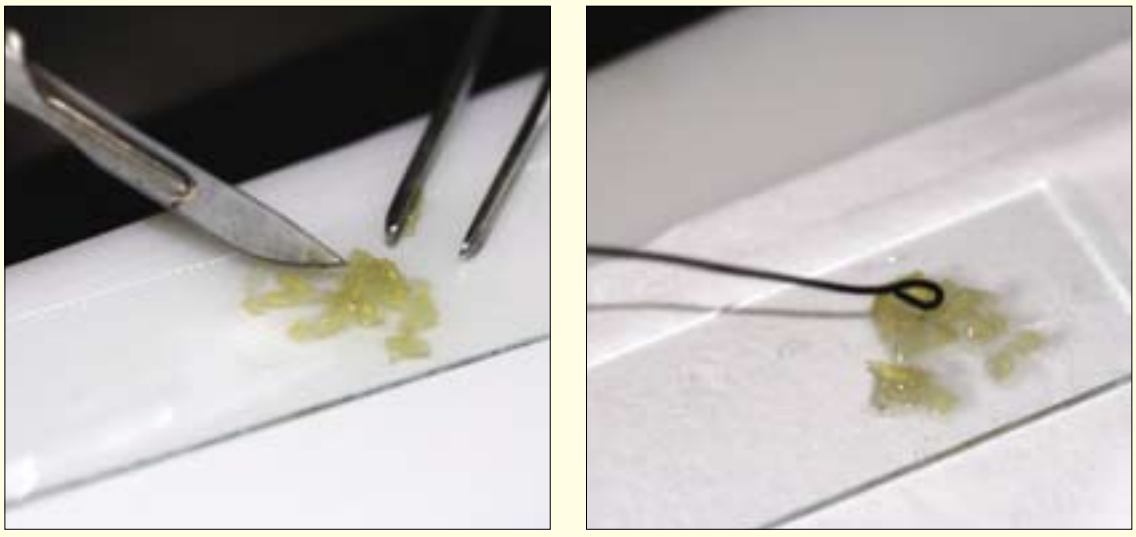


Figure 10.28 Maceration of roots or rhizome for use in bacterial streak plating

10.9 Diseases caused by plant viruses

An in-depth discussion on plant viruses is beyond the scope of this manual. Other texts should be referred to if a viral plant pathogen is suspected.

Although viral pathogens often produce distinct symptoms, their identification usually involves the use of molecular or other diagnostic techniques. Indicator hosts may assist with identification.

Plant virus particles are very small and cannot be seen with a compound light microscope. An electron microscope is needed to see plant virus particles. A virus particle is called a virion. The shape of plant viruses can, for example, be long thin rods, spherical or bacilliform. All plant viruses are composed of infectious nucleic acid, usually RNA; however, some contain DNA. Most plant viruses have a protein shell.

Plant viruses cannot be isolated and grown on agar media, as they can only replicate in a living plant host cell.

Plant viruses can only infect the host plant cell through small wounds made by insect or other vectors, or by mechanical damage (abrasion). The virus replicates in the plant cell disrupting its normal behaviour. The disruption of the plant cell affects the host plant and may cause the development of obvious symptoms. The virus particles move from cell to cell, spreading to other plant parts (Figure 10.29).

Plants can be infected by more than one plant virus. Some infected hosts of a plant virus may be symptomless.

Symptoms of virus diseases include stunting, yellowing, mosaic or mottling of the leaves, yellow or necrotic lesions on the leaf, ringspots, dwarfing of leaves, leaf roll, stunting and, in some diseases, death of the infected plant. Some symptoms of plant viruses are similar to signs of nutrient disorder or symptoms caused by other types of pathogens.

Plant viruses can be disseminated by insect vectors, infected tubers, rhizomes, bulbs, rootstocks or scions used in grafting. Some viruses are disseminated in infected seed. Some plant viruses can be transmitted mechanically from plant to plant in plant sap on grafting knives and pruning shears (and for some viruses, on hands). Tobacco mosaic virus is easily transmitted on cutting tools and hands, and can even be found in tobacco in cigarettes, which leads to contamination of hands.

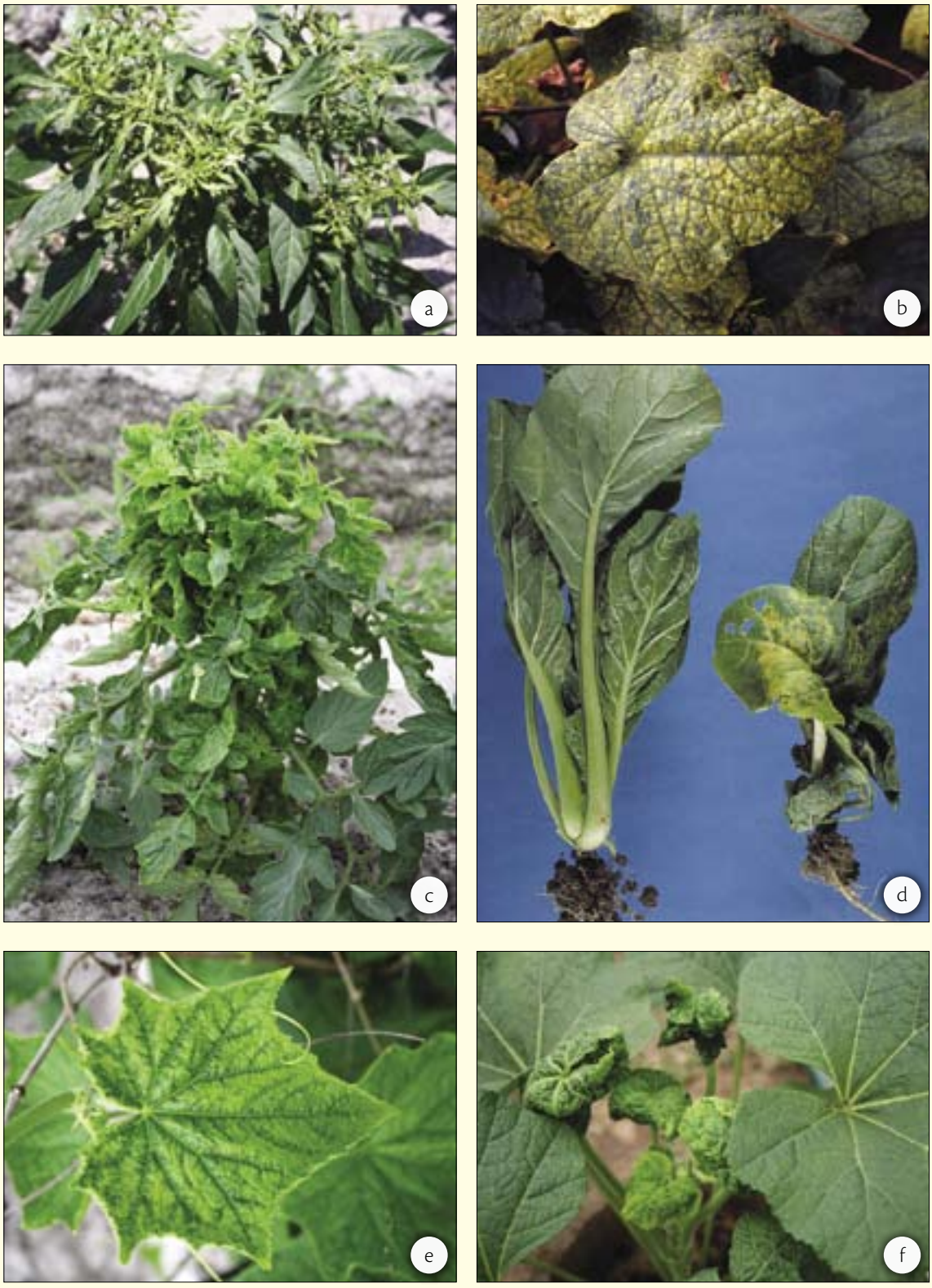


Figure 10.29 Virus diseases: (a) tomato spotted wilt virus on chilli, (b) beet pseudo-yellows in cucumbers, (c) yellow leaf curl virus in tomato, (d) turnip mosiac virus on leafy brassica (right), healthy plant (left), (e) virus on cucumber, (f) crumple caused by a virus in hollyhock (*Althaea rosea*)

The identification of plant viruses requires a specialist laboratory. It is recommended that Provincial diagnostic laboratories in Vietnam seek assistance from the Plant Protection Research Institute to diagnose virus diseases. There are some diagnostic kits for some plant viruses for use in the field, but these kits are relatively expensive.

In the absence of the crop host plant, viruses overseason mainly in weed hosts. However, some persist in seeds and can be found in asexually propagated planting material.

Control of a plant virus disease depends on the nature of the virus, host range, the methods of transmission and overseasoning. Control measures include:

- removal of weed hosts of the virus and the vector
- control of the virus vector within the crop
- use of virus-free planting material
- use of indexing schemes to provide virus-free planting material
- good crop hygiene
  - minimal contact with infected plants
  - sterilisation of pruning equipment between use.

10.10 References

Erwin D.C. and Ribeiro O.K. 1996. Phytophthora diseases worldwide. American Phytopathological Society Press: St. Paul, Minnesota.

Drenth A. and Sendall B. 2001. Practical guide to detection and identification of *Phytophthora*. CRC for Tropical Plant Protection: Brisbane, Australia.

11 Common diseases of some economically important crops

In this section the common diseases of a range of vegetable crops and one field crop are recorded to illustrate the diversity of diseases in Vietnam. The diseases listed in each table also provide a checklist to help with observations in the field. The pathogens responsible for many of these diseases can only be diagnosed accurately in the laboratory.

Accurate diagnosis is essential before recommendations can be made on an integrated disease management strategy. For example, fungal root rots can be caused by many pathogens such as species of *Pythium, Phytophthora, Rhizoctonia* and *Phoma*. The appropriate disease management strategy differs between these genera.

A diagram of each crop plant is included to assist the reader in learning where to look for symptoms of each disease.

A thorough understanding of these diseases will assist the reader in their diagnosis of diseases in many other crops.

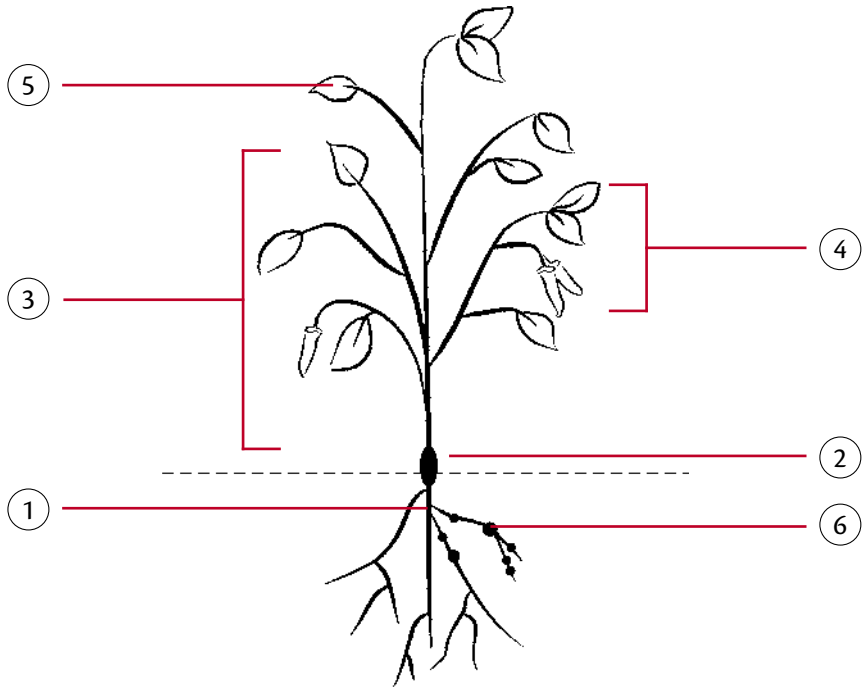
11.1 Common diseases of chilli

Table 11.1 provides a list of the common diseases of chilli in Vietnam (numbers refer to diagram). All diseases may be present in a single crop, and one plant can be affected by one or more of these diseases (Figure 11.1).

Phytophthora root rot, basal stem rot, bacterial wilt, root knot nematode and stem boring insects all cause similar wilting symptoms.

Table 11.1 Common diseases of chilli

Disease	Pathogen	Key diagnostic sign
1 Phytophthora root rot	*Phytophthora capsici*	Root rot and wilt
2 Basal stem rot	*Sclerotium rolfsii*	Small brown round sclerotia and white mycelium on stem base
3 Bacterial wilt	*Ralstonia solanacearum*	Bacterial ooze in stem, stem browning
4 Anthracnose	*Colletotrichum* sp.	Black sunken lesion
5 Viral disease	Plant virus	Dwarfing of younger leaves
6 Root knot nematode	*Meloidogyne* sp.	Galls on roots



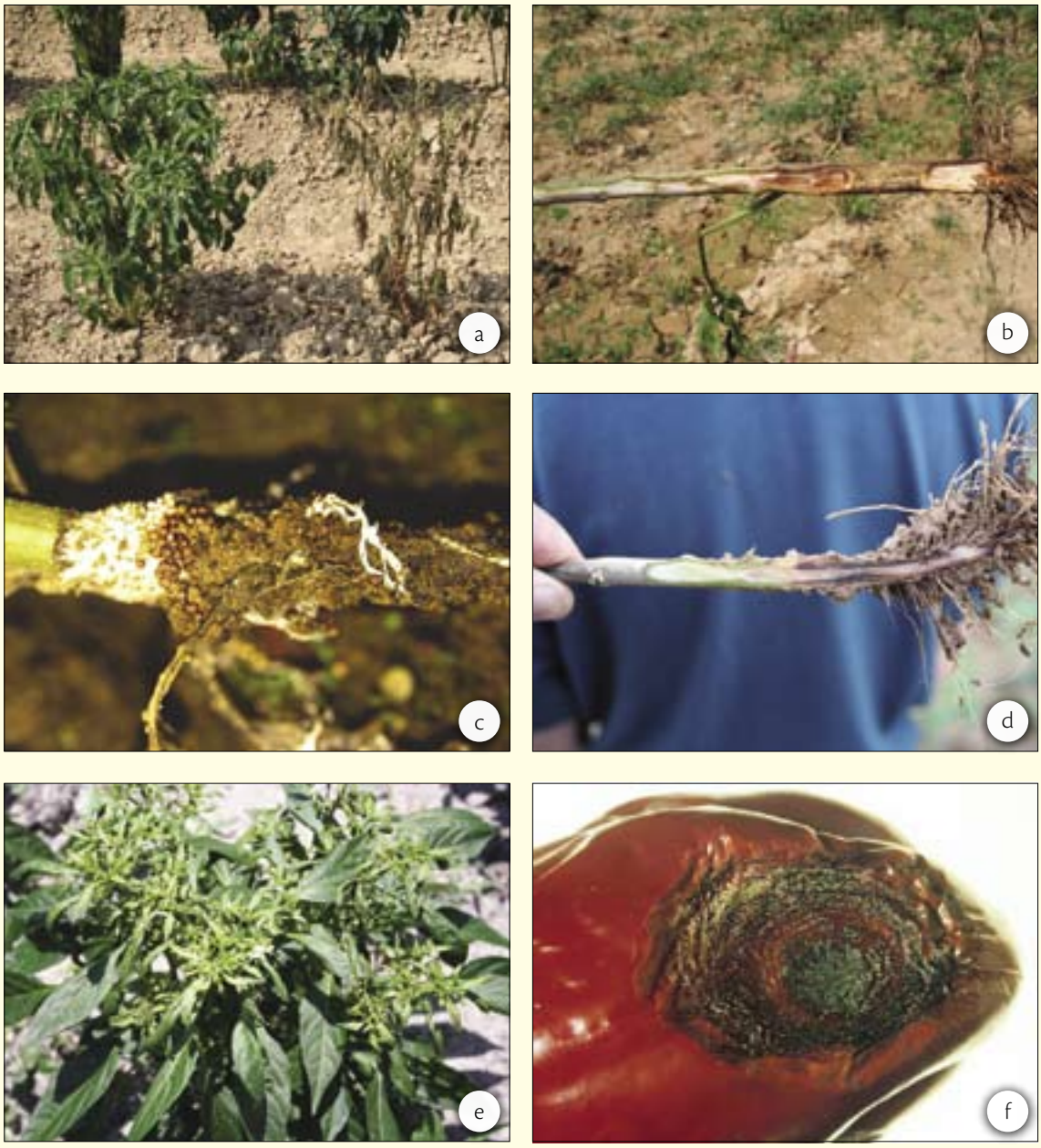


Figure 11.1 Diseases of chilli: (a) healthy chilli plant (left) and wilted (right), which can be caused by several diseases, (b) stem browning, a typical symptom of bacterial wilt caused by *Ralstonia solanacearum*, (c) basal rot caused by *Sclerotium rolfsii*, (d) Phytophthora root rot caused by *Phytophthora capsici*, (e) chilli affected by tomato spotted wilt virus, (f) chilli fruit affected by anthracnose, caused by *Colletotrichum* sp.

11.2 Common diseases of tomato

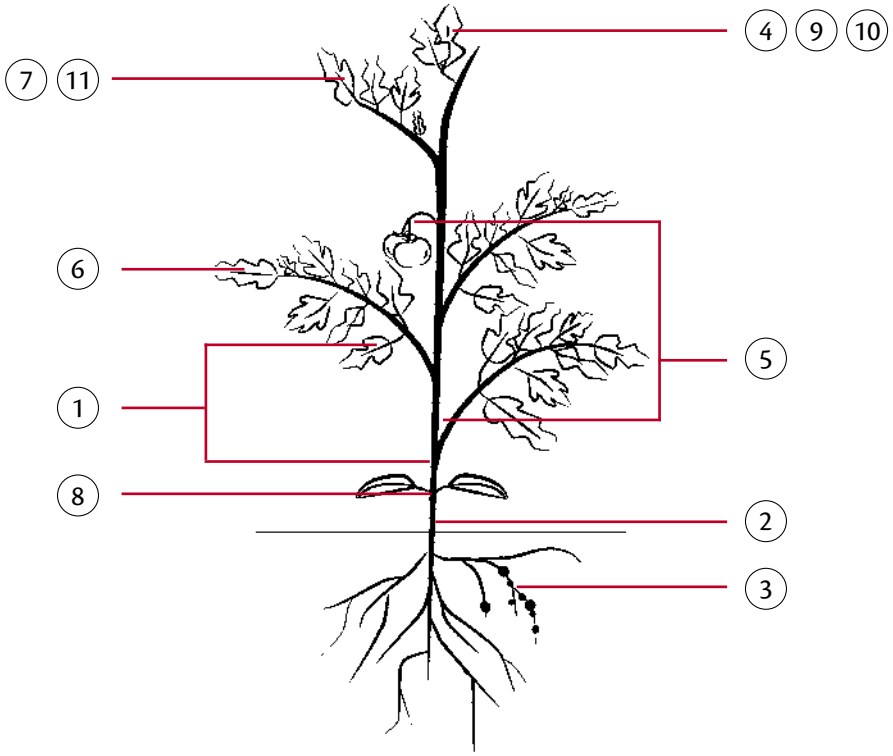
Tomato is susceptible to a very wide range of diseases (Table 11.2). There is a need for more disease surveys of tomatoes in Vietnam to identify all the serious diseases present. In particular, diagnostic studies are needed on the viruses and bacterial pathogens on tomato.

Tomato crops in Vietnam are commonly affected by several diseases. Individual plants can be affected by more than one disease, which can make diagnosis difficult (Figure 11.2).

Table 11.2 Common diseases of tomato

Disease		Pathogen	Key diagnostic sign
1	Bacterial wilt	*Ralstonia solanacearum*	Wilt, bacterial ooze in stem, stem browning
2	Basal stem rot	*Sclerotium rolfsii*	Small brown round sclerotia and white mycelium on stem base
3	Root knot nematode	*Meloidogyne* sp.	Wilt, galls on roots
4	Late blight	*Phytophthora infestans*	Grey fungal growth on underside of leaf
5	Bacterial canker[a]	*Clavibacter michiganensis*	Leaf yellowing, wilting, stem browning, fruit spotting
6	Bacterial speck[a]	*Pseudomonas syringae*	Necrotic spots on leaves
7	Tomato spotted wilt virus[a]	Virus	Small areas of browning (bronzing) on young leaves, dark spots or rings on old leaves
8	Fusarium wilt[a]	*Fusarium oxysporum* f. sp. *lycopersici*	Wilt, vascular stem browning
9	Target spot/early blight	*Alternaria solani*	Concentric circular black lesions on leaves
10	Leaf mould	*Cladosporium fulvum (Fulvia fulva)*	Grey/purple fungal growth on underside of leaf
11	Yellow top virus	Virus	Small yellow curled leaves

a The presence of these pathogens in Vietnam needs to be confirmed.



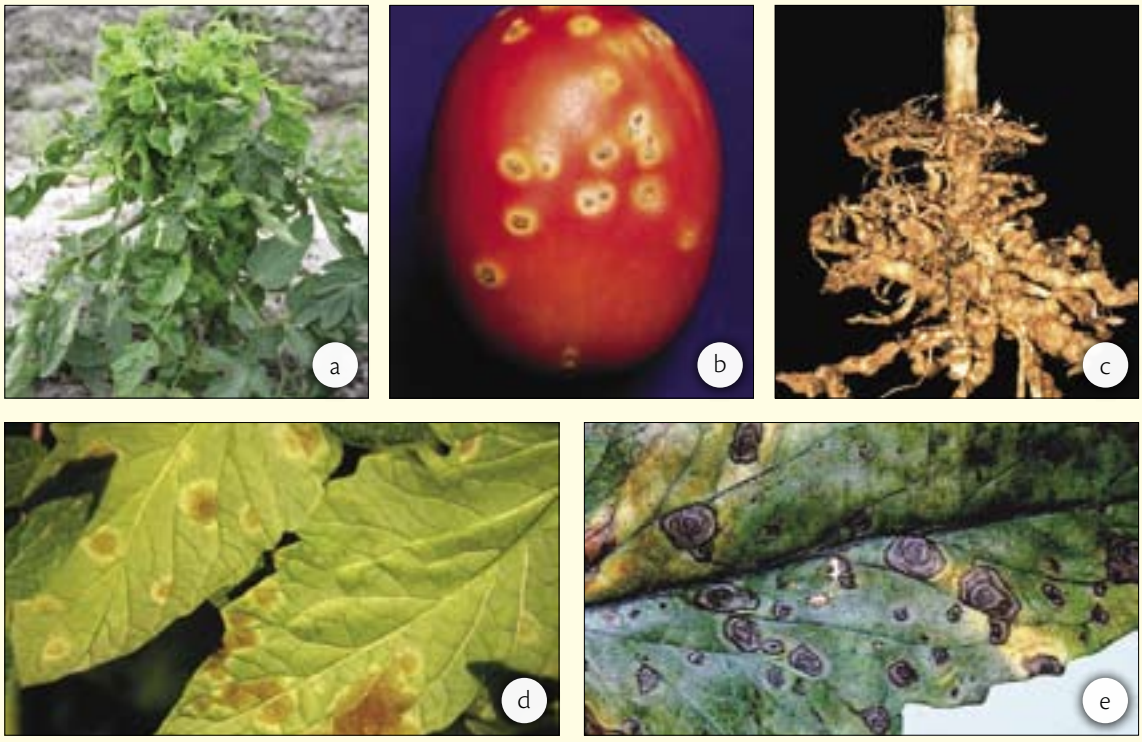


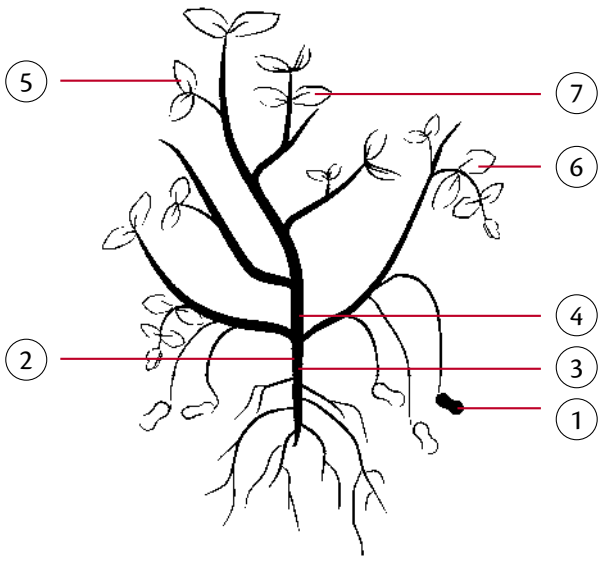
Figure 11.2 Tomato diseases: (a) tomato showing symptoms of yellow leaf curl virus in new growth, (b) tomato fruit showing bacterial speck lesions caused by *Pseudomonas syringae*, (c) root knot nematode caused by *Meloidogyne* sp., (d) velvet leaf spot caused by *Cladosporium fulvum*, (e) target spot caused by *Alternaria solani*

11.3 Common diseases of peanut

Peanuts are susceptible to root, pod, stem and leaf diseases (Table 11.3 and Figure 11.3). The root and pod rot diseases need more diagnostic research to determine the key pathogens involved.

Table 11.3 Common diseases of peanut

Disease	Pathogen	Key diagnostic sign
1 Root and pod rot	*Pythium/Rhizoctonia*	Seedling death/root rot Yellowing and wilting Stunting Browning of lateral roots mid-season Tap-root rot late in season and pod rot
2 Basal stem rot	*Sclerotium rolfsii*	Small brown round sclerotia and white mycelium on stem base
3 Crown rot	*Aspergillus niger*	Stunting and wilting Black mycelium and spores on stem base and cotyledons
4 Stem rot	*Sclerotinia sclerotiorum*	Wilting, wet rot of stems and leaves, large black sclerotia
5 Rust	*Puccinia arachidis*	Reddish rust pustules on leaves
6 Cercospora leaf spot	*Cercospora arachidicola*	Dark chocolate brown lesions
7 Mosaic virus	Virus	Mosaic, laboratory diagnosis required



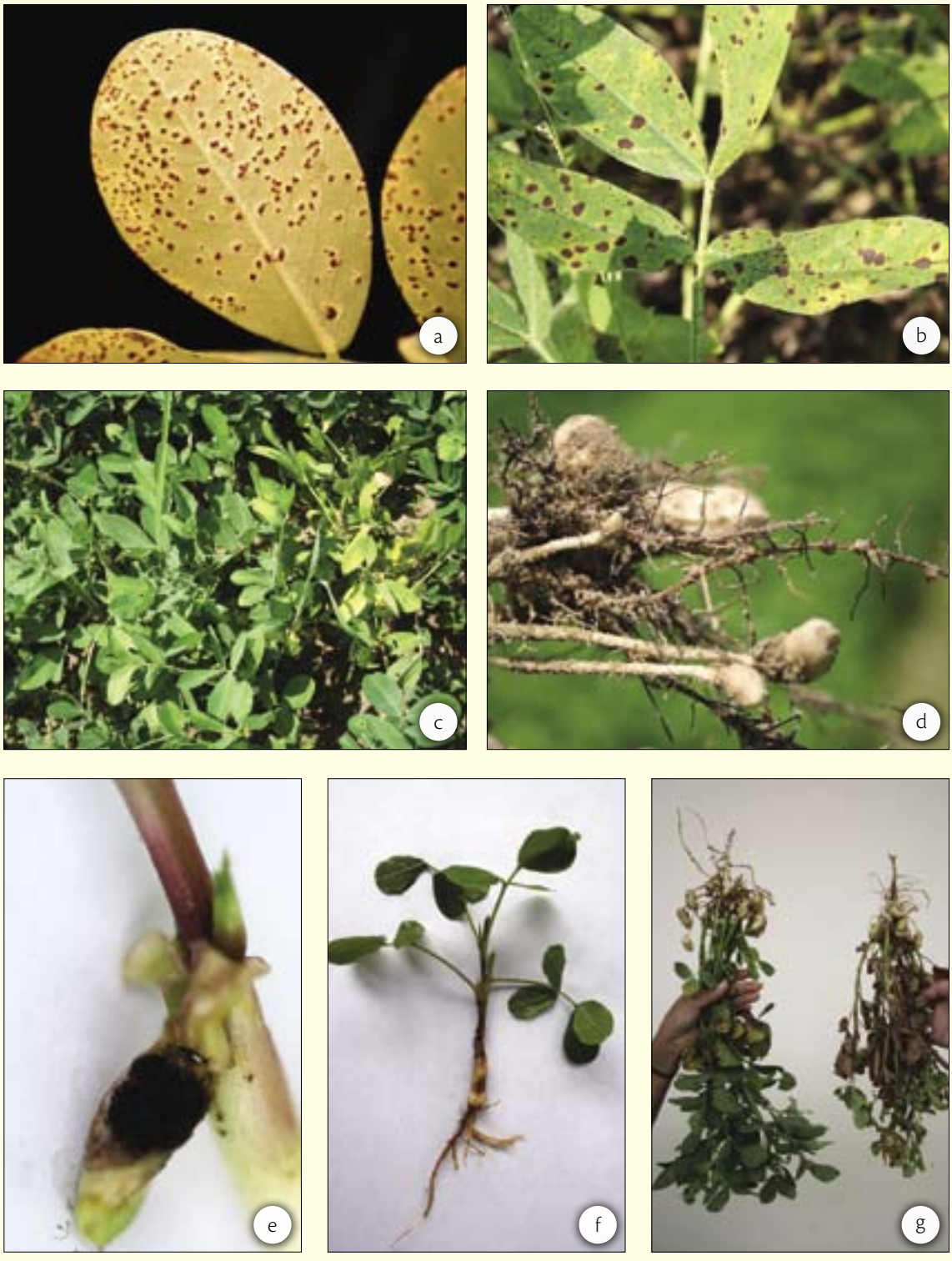


Figure 11.3 Peanut diseases: (a) peanut rust caused by *Puccinia arachidis*, (b) Cercospora leaf spot (*Cercospora arachidicola*) and rust, (c) peanuts affected by root rot showing yellowing and stunting symptoms, (d) feeder root rot and pod rot caused by *Pythium* sp., (e) necrotic peanut cotyledon showing abundant sporulation of the pathogen *Aspergillus niger*, (f) Pythium root rot on peanut seedling, (g) healthy peanut plant (left) and stunted root rot affected plant (right)

11.4 Common fungal diseases of onions

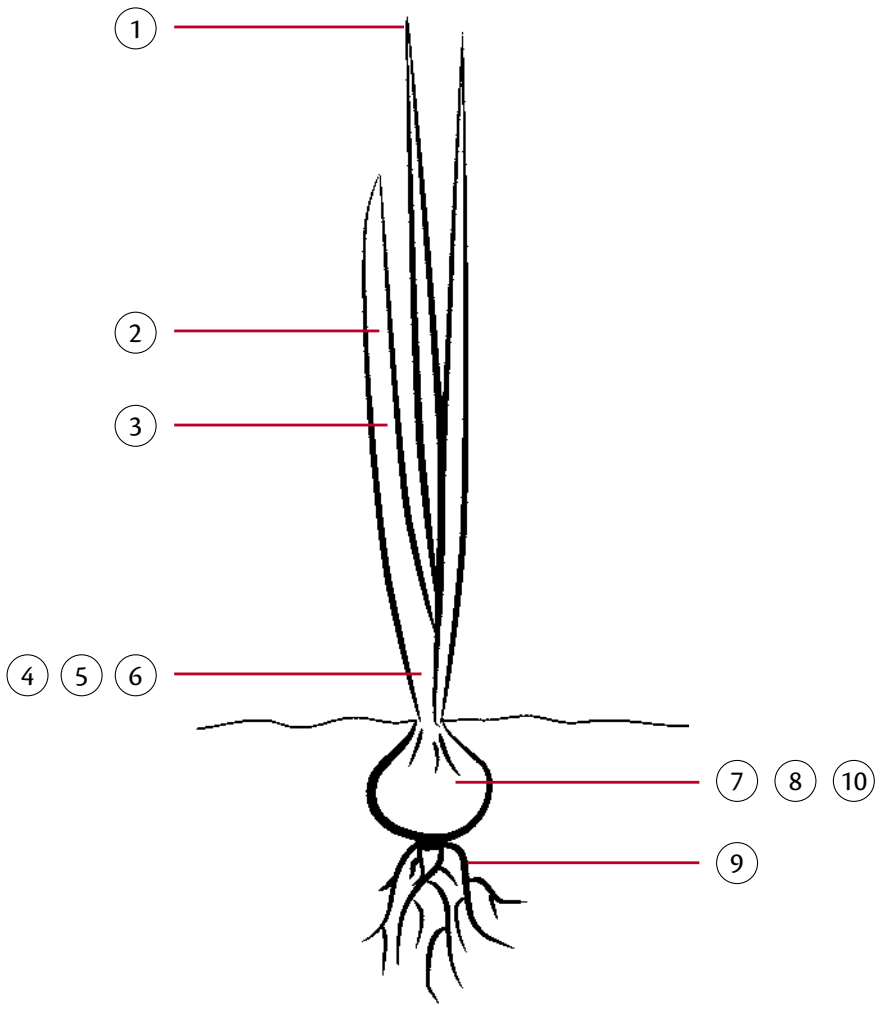
Onions are affected by a wide range of fungal diseases of the leaves, bulb and roots (Table 11.4). Most of the fungal pathogens can be isolated on culture media relatively easily. Note that downy mildew is an obligate fungal pathogen and cannot be grown on artificial culture media.

The diseases listed in Table 11.4 have distinctive symptoms and can usually be distinguished readily in the field, and then confirmed in the laboratory. The fungi which cause bulb rots can continue to cause problems during storage.

Table 11.4 Common fungal diseases of onions

Disease	Pathogen	Key diagnostic sign
1 Tip blight	*Colletotrichum* sp.	Brown-white tip, acervuli present
2 Downy mildew	*Peronospora* sp.	Grey fungal growth
3 Stemphylium leaf spot	*Stemphylium* sp.	Target-like leaf spot
4 Neck rot	*Botrytis byssoidea*	Grey-brown fungal growth and spore masses on bulb
5 White rot	*Sclerotium rolfsii*	White mycelium and brown sclerotia on stem base
6 Leaf base (wet) rot	*Sclerotinia sclerotiorum*	White mycelium, large black sclerotia
7 Fusarium rot	*Fusarium* spp.	White to pale violet mycelium, no sclerotia
8 Black mould (bulb rot)	*Aspergillus niger*	Black powdery spore masses (also a storage rot)
9 Pink root rot	*Phoma terrestris* (*Pyrenochaeta terrestris*)	Pink roots and pink outer scales
10 Bulb rot	*Rhizopus stolonifer* (*R. nigricans*)	Extensive cottony fungal growth with obvious black sporangia

Onions are also affected by bacterial leaf blights, bacterial bulb rots, a number of plant viruses, and several nematode diseases of the roots (Figure 11.4). Nematode diseases mainly cause stunting and rarely lead to plant death, so these are commonly overlooked.



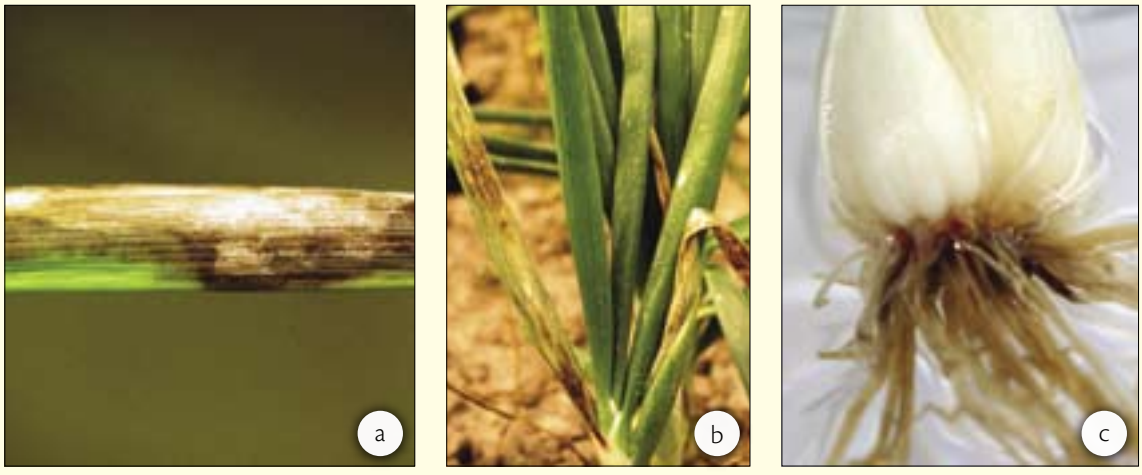


Figure 11.4 Diseases of onion: (a) Stemphylium leaf spot, (b) downy mildew caused by *Peronospora* sp., (c) symptoms of pink root rot caused by *Phoma terrestris*

11.5 Common fungal diseases of maize

Maize is strongly recommended for rotation with vegetable crops for the control of many pathogens which survive in soil. Maize is resistant to bacterial wilt (*Ralstonia solanacearum*), *Sclerotinia sclerotiorum*, most common *Phytophthora* species, and root knot nematode. However, it is susceptible to common species of *Pythium* and moderately susceptible to *Sclerotium rolfsii* and *Rhizoctonia* spp. (Table 11.5 and Figure 11.5) Maize is also susceptible to stalk and cob rots caused by several *Fusarium* species but these do not normally affect vegetable crops. A more exhaustive list of maize diseases can be found on the internet (http://www.cimmyt.org/english/docs/field_guides/maize/diseases.htm).

Table 11.5 Common fungal diseases of maize

Disease		Pathogen	Key diagnostic signs
1	Common (boil) smut	*Ustilago maydis*	Large white galls replace kernels, black spore masses; can also infect the tassel and stalk.
2	Fusarium stalk, cob and root rots	*Fusarium graminearum*	Stalks rot internally usually with 'shredded' appearance of pith. Pink to red pigments and hyphal growth may be present in rotted stalks and cobs.
		Fusarium verticillioides *Fusarium sublutinans* *Fusarium proliferatum*	Stalks rot internally usually with 'shredded' appearance of pith. Pith usually pigmented violet to purple. White mycelium develops on diseased cobs under hulls.
3	Common rust	*Puccinia sorghi*	Elongated necrotic pustules forming on leaves.
4	Rhizoctonia leaf, stalk and root rots	*Rhizoctonia* spp.	Causes large irregular pale-brown lesions on leaves and stalk. Brown irregular-shaped sclerotia usually present on diseased areas.
5	Southern leaf blight	*Bipolaris maydis* (*Cochliobolus heterostrophus*)	Necrotic lesions form on leaves.
6	Turcicum leaf blight	*Exserohilum turcicum*	Small oval water-soaked lesions on leaves changing to larger necrotic lesions.
7	Pythium stalk and root rot	*Pythium* spp.	Wet rot of stalk tissues and brown lesions on roots.
8	Downy mildews	*Peronosclerospora* spp. *Sclerospora* sp. *Sclerophthora* spp.	Grey fungal growth (sporangiophores) on underside of leaf.

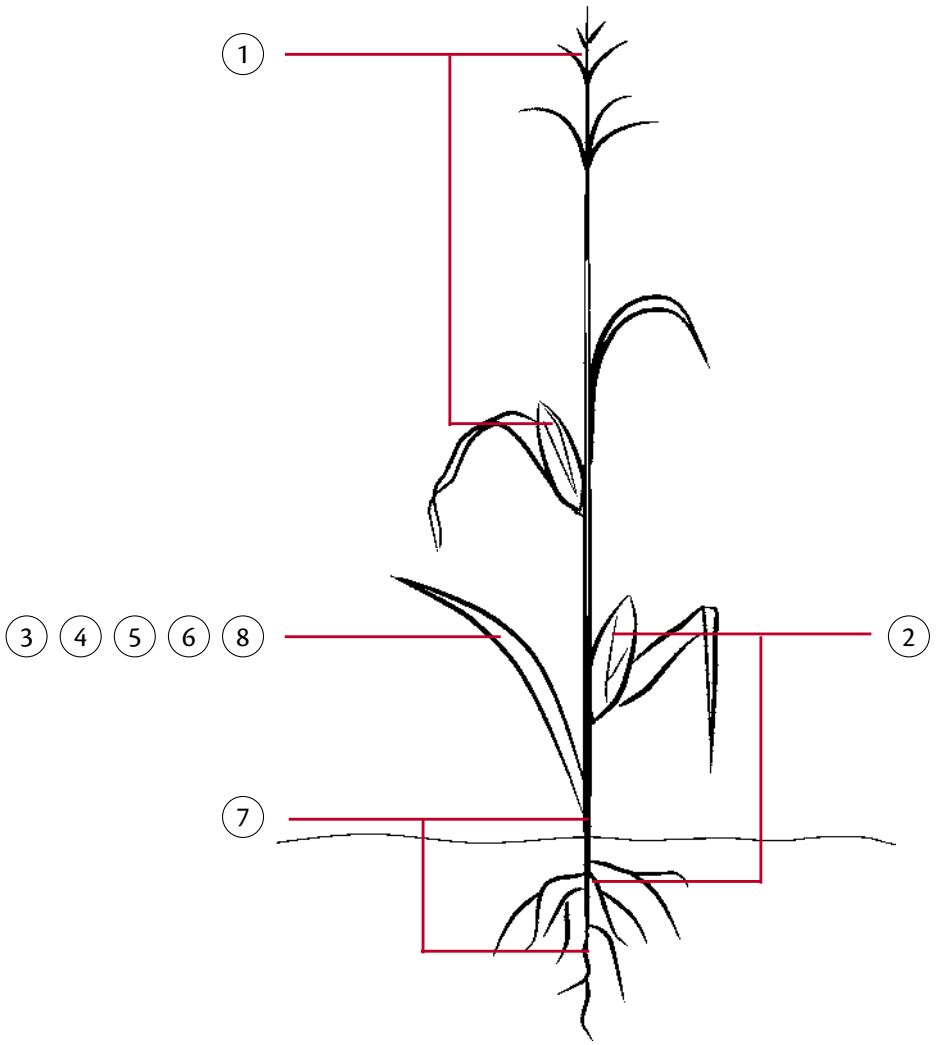


Figure 11.5 Diseases of maize: (a) common (boil) smut on maize cob caused by *Ustilago maydis*, (b) banded sheath blight caused by *Rhizoctonia solani*, (c) white mycelial growth on infected cob caused by *Fusarium verticillioides*

12 Fungi, humans and animals: health issues

Some fungi cause diseases of humans and other animals—these diseases are called mycoses. For example, *Aspergilllus flavus* can infect the human lung, causing chronic respiratory disease. Therefore, it is important to take great care with cultures of *A. flavus* (see Section 12.2.1). *Fusarium oxysporum* and *F. solani* have been associated with diseases of the eye and of the fingernails and toenails.

Some fungi which infect plants also have the ability to produce toxic secondary metabolites called mycotoxins. Mycotoxins can contaminate human food or animal feed and cause mycotoxicoses. For example *A. flavus* produces aflatoxins, one of the most important group of mycotoxins. Aflatoxins are found in a range of products such as peanuts and corn.

Mycotoxins are produced by fungal hyphae and diffuse into the substrate (e.g. grain, hay or fruit, see Figure 12.1).

Mycotoxins can be produced and contaminate the substrate before harvest or during grain storage after harvest (post-harvest). It is important to store grain under dry conditions to minimise post-harvest growth of fungi and mycotoxin contamination.

Mycotoxin production varies between species. *Fusarium graminearum*, for example, produces zearalenone in corn grain but not in wheat grain. *Aspergillus flavus* prefers hot, humid conditions for growth and production of aflatoxins in corn and peanuts (Figure 12.2). Even within a species, mycotoxin production can differ significantly. Within the species *F. graminearum,* isolates can either be deoxynivalenol or nivalenol producers. These differences are very important, as their toxicities and effects on animal species are significantly different.

Some mycotoxogenic fungi produce toxins in fungal structures such as ergots (sclerotia) and spores. Such structures may contaminate grains or hay and so affect humans or animals that eat the contaminated food. Ergots of *Claviceps purpurea*, for example, are quite toxic.

Many mycotoxins are not affected by heating, and can therefore survive cooking in processed foods such as grain (cereal) and nut products. Some mycotoxins in farm animal feed can also pass into meat, milk and eggs. Humans ingest mycotoxins in food from contaminated grains, nuts, or other processed foods.

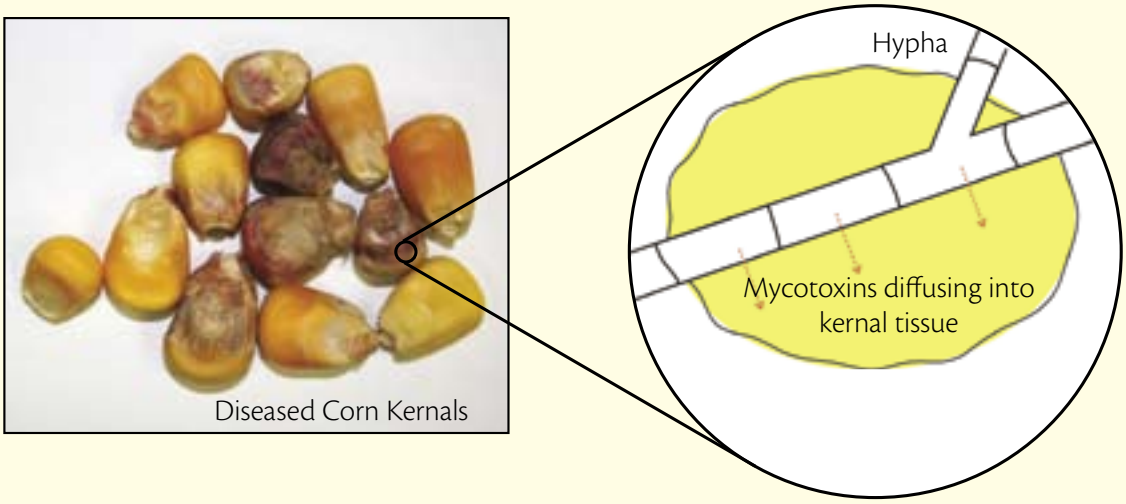


Figure 12.1 Corn kernels infected with *Fusarium graminearum* and a diagrammatic illustration of the diffusion of mycotoxins from fungal hyphae into kernel tissue

Figure 12.2 *Aspergillus flavus* sporulating on infected peanut seeds on isolation medium

12.1 Key mycotoxigenic fungi in Vietnam

Table 12.1 provides a list of key mycotoxigenic fungi in Vietnam, along with the toxins they produce and the crops and animals they affect.

Table 12.1 Key mycotoxigenic fungi in Vietnam

Species	Toxin	Crop	Animal
Aspergillus flavus	Aflatoxins	Peanuts, corn	Many species
Fusarium verticillioides	Fumonisins	Corn	Horse, pigs
Fusarium graminearum	Deoxynivalenol	Wheat, barley, corn	Pigs, poultry
	Nivalenol	Wheat, barley, corn	Pigs, poultry
	Zearalenone	Corn	Pigs
Penicillium	Cyclopiazonic acid	Cereals	See literature
	Patulin	Fruit	See literature
	Ochratoxin A	Fruit	See literature

Mycotoxin production by a fungus is influenced by a number of factors including:

- substrate
- temperature
- moisture levels in the substrate
- strain of the fungus.

12.2 Mycotoxigenic *Aspergillus* species

12.2.1 *Aspergillus flavus*

Sources

Aspergillus flavus is common in peanuts and maize grain in tropical regions. It can also be found in other stored commodities including spices.

Plant pathogenicity

Aspergillus flavus colonises peanut plants, but does not appear to be pathogenic to the growing plant. *A. flavus* is associated with cob rot in maize under hot, humid conditions.

Mycotoxins

Aspergillus flavus can produce aflatoxins and cyclopiazonic acid. Some isolates are highly toxigenic. Aflatoxins are potential carcinogens and can cause liver cancer.

Precautions

This species grows at 37 °C and can be pathogenic to humans, causing lung infections. The conidia may contain aflatoxins. Care should be taken in handling cultures of this species (Figure 12.3). Avoid inhaling spores (conidia).

Figure 12.3 *Aspergillus flavus*, three colonies on Czapek yeast autolysate agar (left), conidia produced abundantly on heads on conidiophore (centre), conidia (right)

Description

Aspergillus flavus produces yellow-green colonies which grow well, especially at 30–37 °C. Some isolates produce dark brown to black sclerotia. *Aspergillus* heads are yellow-green and mop-like when viewed under the stereomicroscope. The heads are usually biseriate, but some only have phialides.

Do not open plates containing *Aspergillus flavus*. This fungus is pathogenic to humans, causing serious lung infections.

12.2.2 *Aspergillus niger*

Sources

Aspergillus niger is one of the most common *Aspergillus* species. It is common in peanuts, and can be isolated from almost any durable commodity (e.g. grains, legumes, pulses, spices) as well as dried fruit (Figure 12.4).

Plant pathogenicity

Aspergillus niger causes a wide range of plant diseases, including crown rot of peanuts, damping off and seedling rots, vine canker, bunch rot in grapes, black rot of onions and garlic, as well as a range of post harvest rots in fruits and vegetables.

Mycotoxins

A small number of *A. niger* strains can produce ochratoxin A. The similiar species *A. carbonarius* is an important producer of ochratoxin A and is probably the primary source of ochratoxin in grape products and in coffee.

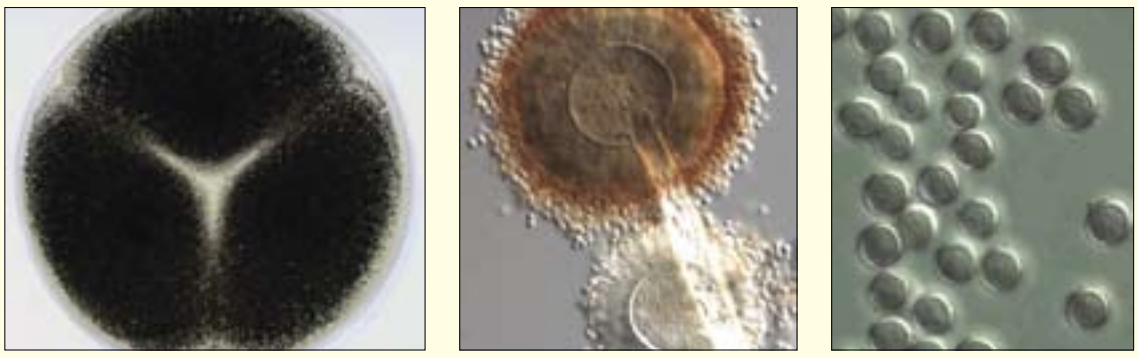


Figure 12.4 *Aspergillus niger*, three colonies on Czapek yeast autolysate agar (left), conidia produced abundantly on heads on long conidiophore (centre), conidia (right)

Precautions

Aspergillus niger and other black Aspergilli grow well at 37 °C and are potentially pathogenic to humans. They are quite commonly isolated from human ear infections. Care should be taken in handling cultures of this species. Avoid inhaling spores (conidia).

Description

Colonies of *A. niger* are chocolate brown to black and grow well, especially at 30–37 °C. Several different species are included in the *A. niger* complex (aggregate). The heads of this species are generally dark brown to black, produced on long stipes and are mop-like when viewed under the stereomicroscope. Most species produce biseriate heads which have large metulae.

12.2.3 *Aspergillus ochraceus*

Sources

Aspergillus ochraceus is essentially a storage fungus. It has been reported from a wide variety of stored commodities, particularly in tropical areas. *A. ochraceus* and other related species are thought to be responsible for ochratoxin A contamination in coffee, cocoa, and stored oilseeds and nuts.

Plant pathogenicity

Not pathogenic under normal weather conditions.

Mycotoxins

Ochratoxin A was first discovered in cultures of *A. ochraceus*. This mycotoxin is produced by a number of species in the *A. ochraceus* group.

Precautions

Aspergillus ochraceus has rarely been reported to be pathogenic to humans. However, as with all fungi, care should be taken to avoid inhaling spores (conidia).

Description

Colonies of *A. ochraceus* are pale yellow brown (ochre) coloured, often with a pinkish-brown reverse. Many strains also produce pinkish-brown sclerotia. There are a number of similar species within the *A. ochraceus* group (Figure 12.5). *A. ochraceus* grows more slowly than *A. flavus* and *A. niger*, especially at 37 °C. Some species within this group do not grow at 37 °C.

The authors wish to thank Dr Ailsa Hocking for her contribution of species descriptions and images for this section.

Figure 12.5 *Aspergillus ochraceus*, three colonies on Czapek yeast autolysate agar (left), conidia produced abundantly on heads on conidiophore (centre), conidia (right)

12.3 Mycotoxigenic *Fusarium* species

Fusarium verticillioides and *F. graminearum* are the two most common toxigenic *Fusarium* species on maize in Vietnam. These species can occur in the same areas. Other *Fusarium* species occur in maize, but usually are less common than the two species discussed here.

12.3.1 *Fusarium verticillioides*

Sources

Mainly associated with maize but is occasionally isolated from other plants.

Plant pathogenicity

Causes root, stalk and cob rot in maize. Most common in warm to hot, dry conditions, when plants are drought stressed. Cob rot is also more severe in cobs damaged by insects. This fungus can cause symptomless infection of maize stalks under good growing conditions.

Mycotoxins

Fusarium verticillioides produces the fumonisin group of mycotoxins in corn. Fumonisin B1 is the most common and most toxic of the group. Fumonisin B1 causes pulmonary oedema in pigs and liquefaction of the brain of horses. Fumonisin B1 has also been associated with oesophageal cancer in humans. There are restrictions on the trade in corn contaminated by fumonisin B1.

Description

Produces white mycelium on PDA and violet pigment in the agar (Figure 12.6). On water agar containing sterile carnation leaf or green rice stem pieces, *F. verticillioides* produces long, thin and relatively straight macroconidia in sporodochia in the leaf/stem pieces, and long chains of oval microconidia from monophialides. It does not produce chlamydospores.

Figure 12.6 Fusarium cob rot caused by *Fusarium verticillioides* (left), and pure cultures on potato dextrose agar (right)

12.3.2 *Fusarium graminearum*

Sources

In Vietnam, *Fusarium graminearum* most commonly occurs on maize. In Sapa region, it has also been observed on some grasses.

Plant pathogenicity

Causes stalk, root and cob rot in maize in warm temperate growing conditions. It also causes head blights of wheat and pearl millet.

Mycotoxins

Produces trichothecenes, especially deoxynivalenol and nivalenol. These can be found in animal and human food made from contaminated maize grain. Deoxynivalenol (sometimes shortened to DON) is also known as 'vomitoxin', as it causes feed refusal or vomiting in pigs depending on the concentration in the feed. *F. graminearum* also produces zearalenone, an estrogenic mycotoxin. This mycotoxin causes infertility especially in pigs, but may also affect cattle and other animals.

Description

Produces rose to burgundy ('red') mycelium on PDA and burgundy pigment in the agar (Figure 12.7). There can be some pale yellow mycelium. It produces slightly curved macroconidia of medium length in small sporodochia in CLA or green rice stem pieces in water agar. Does not produce microconidia or chlamydospores. Produces abundant black fertile perithecia homothallically on CLA or other water agar medium containing a suitable plant material, at 20–23°C under lights. Perithecia do not usually form above 25°C in culture. Perithecia are also produced on old maize stalks and old hulls under cool humid conditions.

Figure 12.7 Fusarium cob rot caused by *F. graminearum* (left), and pure cultures on potato dextrose agar (right)

13 The diagnostic laboratory and greenhouse

13.1 The diagnostic laboratory

The following recommendations are based on existing diagnostic laboratories established in Quang Nam Plant Protection Sub-department (PPSD), Hue PPSD, Nghe An PPSD and the School of Agriculture and Forestry at Hue University through ACIAR funding (Diseases of Crops in the Central Provinces of Vietnam: Diagnosis, Extension and Control, CP/2002/115). These laboratories were established to assist mainly with the laboratory diagnosis of fungal diseases. However, the facilities are also suitable for the isolation of common bacterial plant pathogens. Before working in any laboratory potential safety issues and health risks must be considered. Appendix 2, health and safety, outlines common risks encountered in a diagnostic plant pathology laboratory, however please consult the laboratory supervisor before entering an unfamiliar laboratory.

13.1.1 Location of the laboratory

The diagnostic laboratory should be in a building with walls protected from rain. In tropical regions fungi commonly grow on the inside of walls exposed to rain. Such fungal growth can produce spores which contaminate cultures. Ideally, the laboratory should be located on the second level of the building. This reduces problems with rats and other pests such as ants. It is recommended that the laboratory consist of two large rooms, a preparation room and a clean room.

We also recommend that a room or covered area be used for initially examining field samples and removing soil from root samples by washing. In this area small plant samples should be selected for later isolation of fungal or bacterial pathogens in the clean room. This area can also be used to extract plant parasitic nematodes from soil.

Figure 13.1 Typical arrangement of equipment in a diagnostic laboratory (laboratory in Nghe An PPSD): (a) and (b) two views of clean room, (c) and (d) two views of preparation room.

13.1.2 Preparation room

The preparation room is used for preparing media, including sterilising items in the autoclave, sterilising Petri dishes in an oven, washing glassware and storing glassware, chemicals and other basic items. This room should have an exhaust fan to remove hot air produced by the autoclave and the oven.

13.1.3 Clean room

The clean room is used for isolating fungi and bacteria from cleaned subsamples of diseased plant tissue into pure cultures. It is also used for growing cultures under clean conditions. The microscopes are located in this room for examining cultures and fungal structures.

Do not examine large plants in the clean room. Isolate from small plant samples that have been washed free of dust or soil outside the laboratory.

This room should be air-conditioned, if possible, to protect equipment and cultures. It should also be kept free from dust and insects. However, do not have an airtight clean room or humidity will be too high and fungus (mould) will develop on walls and equipment. A dehumidifier is useful in this room. No soil is allowed in the clean room as soil is a source of fungus-eating mites that can contaminate cultures.

13.2 Laboratory layout

When designing a laboratory there are many aspects to consider. It is important that work can be carried out in a logical order and that particular parts of the diagnostic protocol are separated from one another. The following is a layout of a diagnostic laboratory (Figure 13.2), mainly concerned with the diagnosis of fungal plant pathogens.

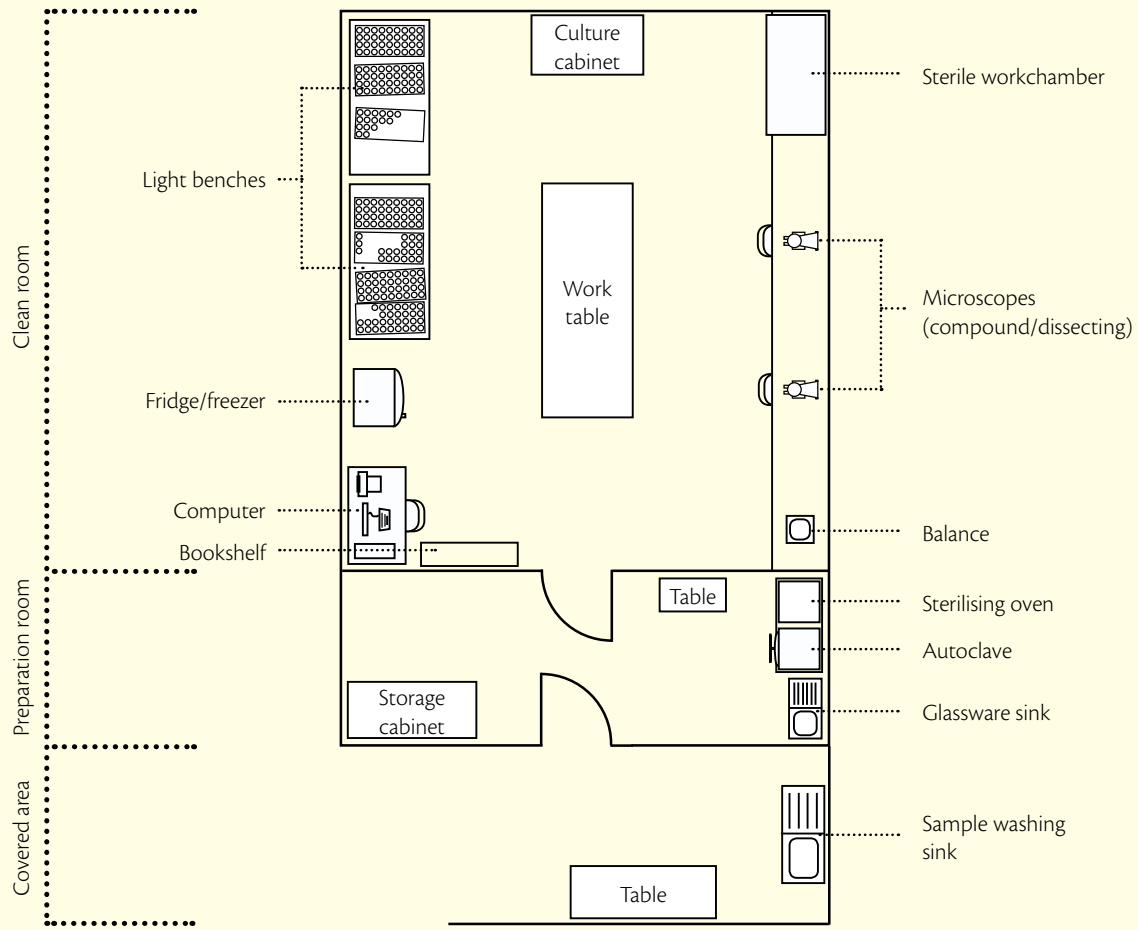


Figure 13.2 Floor plan of diagnostic laboratory, indicating suggested layout of equipment and benches

13.3 Laboratory equipment

13.3.1 Equipment for the clean room

Essential items of equipment for this room are listed below and shown in Figure 13.2:

- A compound microscope fitted with ×10, ×20, ×40 and ×100 (oil immersion) objective lenses. A basic student-grade microscope is sufficient for most diagnostic work. If funds are available the microscope can be fitted with a ×20 metallurgical lens with a long working distance. This lens is ideal for examining fungal structures *in situ* in cultures, as it has a long depth of field (see Section 6.2.2).
- A dissecting microscope for examining diseased plant samples for fungal structures. This is especially important for many leaf infecting pathogens which cannot be grown in artificial media. It is also used for transferring germinated single spores or hyphal tips for purifying cultures, and for studies of plant pathogenic nematodes (see Section 6.2.1).
- A sterile work chamber for pouring media and isolating fungi from plant tissues. The tropical climate of Vietnam means that there are many fungal spores in the air. These spores contaminate media while pouring it, plating tissue or performing culture transfers, unless a sterile work chamber is used.
- A bench with overhead fluorescent lights for stimulating sporulation and pigment production of many fungal species, either in culture or on leaves in moist chambers. Ideally there should be one bench for clean cultures and one bench for plates with diseased tissues. A culture cupboard is useful for incubating cultures in the dark. This is necessary for cultures on media containing antibiotics that are affected by light (e.g. *Phytophthora* selective medium).
- A refrigerator for storing media in bottles, Petri dishes with media (in plastic bags or foil to stop the media drying out), as well as antibiotics, cultures and small tissue samples.
- An electronic balance with an accuracy of 0.001 g is recommended for weighing small amounts of antibiotics or chemicals.
- Large work benches, one for microscopes and the electronic balance, and one for general isolation and cultural work.
- Comfortable chairs for sitting at work benches.
- Accession books, for recording details of each diagnosis and for recording a list of stored cultures.

- A bookshelf containing a wide range of printed information on diseases:
  - textbooks
  - manuals
  - compendia of disease
  - research papers.
- At least one computer with internet access and printer for:
  - database work
  - searching for information
  - access to picture libraries
  - communication via email.
- Small instruments for isolation and cultural work, including:
  - fine forceps
  - inoculating needles
  - surgical scalpel handles
  - transfer loops (for bacterial work)
  - surgical scalpel blades
  - marker pens
  - small knives
  - ethyl alcohol
  - transfer needles (flat tip)
  - tissues
  - cutting boards
  - microscope slides and coverslips
  - filter paper.

Check the walls and equipment regularly for fungal growth.

The floor of the clean room should be mopped regularly to remove any dust particles from the area. Fans should also be turned off and windows closed whilst culturing, to reduce the movement of air in the laboratory. Critical work should be carried out in a laminar flow cabinet which has been wiped down with 70% alcohol.

Surface disinfect as necessary.

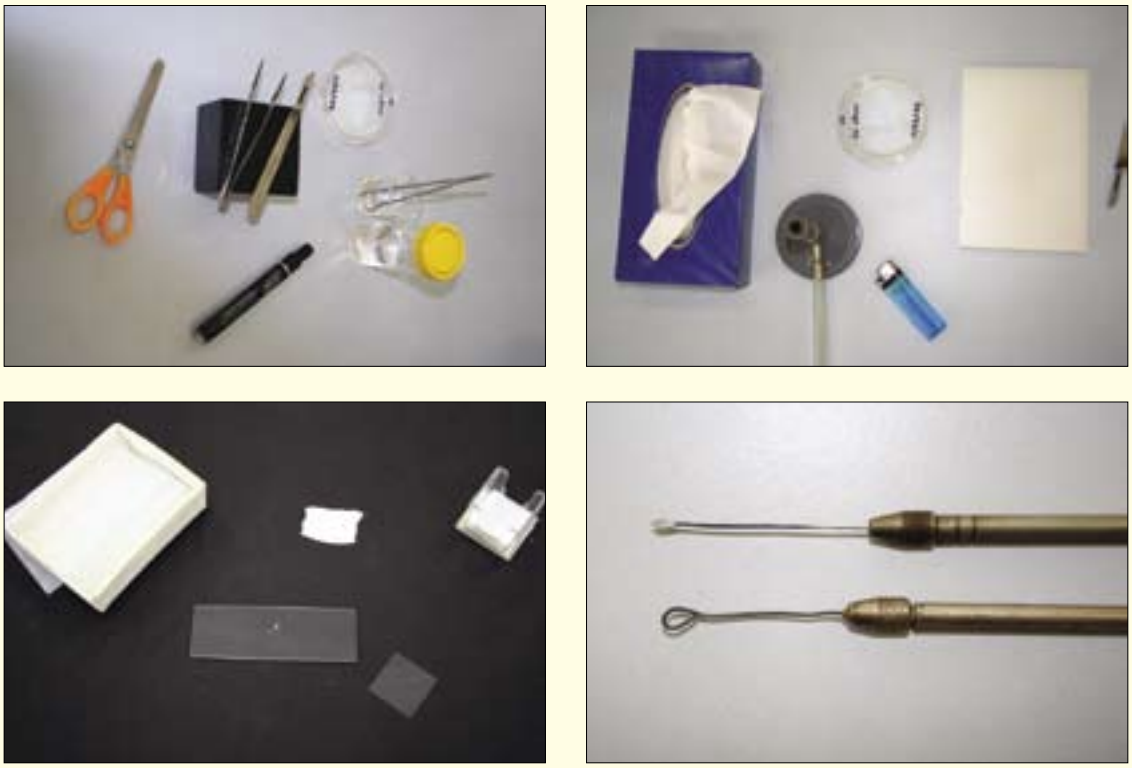


Figure 13.3 Essential instruments for isolation, subculturing, purification and identification of fungal and bacterial plant pathogens

Sterilise the bench and wash hands before working with any pure cultures to reduce the chance of contamination.

13.3.2 Equipment for the preparation room

Essential items of equipment for the preparation room are listed below and shown in Figure 13.1:

- An oven for sterilising glass Petri dishes, which should be wrapped in newspaper or in paper bags.
- A small autoclave suitable for sterilising volumes of 1–2 litres of media or water in flasks or Schott bottles. The autoclave is also used to sterilise media or water in glass test tubes or McCartney bottles, pipettes and other glassware wrapped in paper or aluminium foil.

- A pressure cooker to sterilise small amounts of media and water. This can be purchased at most large markets.
- A balance (0.1 g accuracy) for weighing chemicals, potatoes, carrots and so on for media preparation.
- An electric hot plate for boiling potatoes and carrots for media.
- A bench for media preparation.
- A sink for washing Petri dishes and other glassware.
- A storage cabinet.

13.4 Greenhouse for plant disease studies

A greenhouse is an important part of a diagnostic laboratory as it is required for pathogenicity testing, evaluating fungicides and other disease control methods. The design should allow good plant growth and prevent cross contamination in pathogenicity tests and other experiments (Figure 13.4).

A basic greenhouse should include:

- a transparent roof
- a sloping concrete floor with good drainage
- good ventilation for hot weather (wind driven exhaust fans are very effective) (Figure 13.5)
- a rat-proof design
- a good water supply
- benches (Figure 13.5)
- a preparation area within or near the greenhouse.

The transparent roof should allow at least 75% transmission of sunlight. A corrugated polycarbonate material is ideal for the roof as it is ultraviolet resistant, very durable and easy to attach to a steel or timber roof frame.

Plastic sheeting can also be used for roofing material, but will only last for 1–2 years. Glass roofs are not suitable for regions affected by typhoons or hail. Ideally the roofing should be attached so as to provide eaves that are boxed in (for typhoon protection). Shade cloth can be used in mid-summer to decrease the temperature in the greenhouse (Figure 13.5).

A sloping concrete floor drains well and can be kept clean by hosing with water.

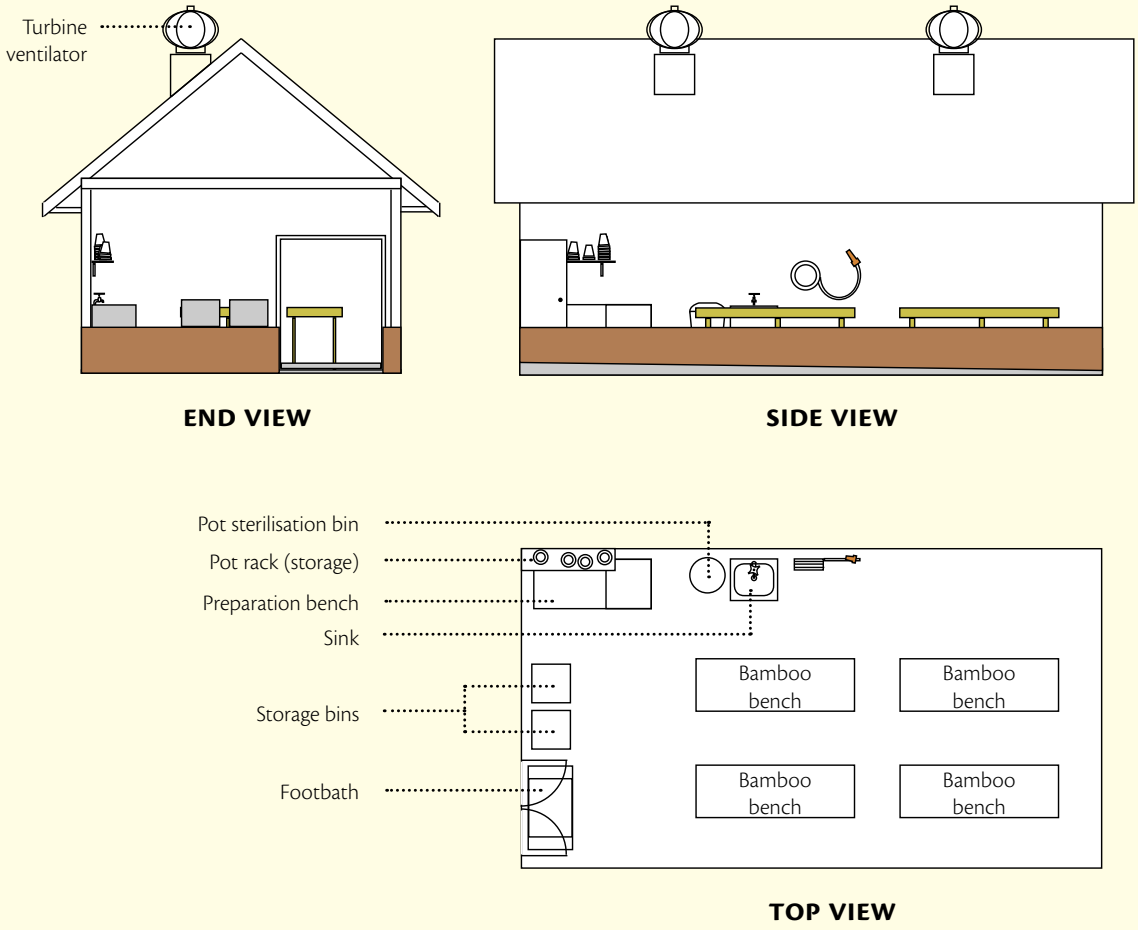


Figure 13.4 Diagrammatic illustration of a suggested design for a greenhouse suitable for pathogenicity testing and other experimental work with plant pathogens

Figure 13.5 Plant pathology greenhouse at Quang Nam PPSD: (a) general view of greenhouse showing insect-proof screens, (b) shade cloth sun screen and flat polycarbonate roofing with wind driven ventilator units

The sides of the greenhouse can be brick walls (height approximately 1 m). Wire netting (such as B40 wire netting) or galvanised wire mesh (with holes approximately 1 cm diameter) can be fixed between pillars, the low brick wall and the roof supports. The wire netting or mesh allows good ventilation and helps to stop rats and birds entering the greenhouse. Insect-proof mesh is expensive, but is important because it prevents insect pests from entering the greenhouse.

A good water supply is needed to keep the greenhouse floor clean and to provide pathogen-free water for the plants. The hose should be hung on the wall so the nozzle is never in contact with the floor of the greenhouse.

Electricity for lighting and for instruments is useful.

Rust-proof steel benches for pots of plants should be approximately 1 m high and 2–3 m long. This height minimises the risk of contamination from the floor. The benches should be portable, so that they can be removed easily if the greenhouse is needed for tall plants, vines on trellises or young fruit trees in large pots. Bamboo benches can be used but must be treated with copper fungicide to inhibit mould growth.

A 10 kg pan balance should be located in the greenhouse for use in weighing pots to monitor the water content of the potting mixture.

13.4.1 Preparation area

The preparation area can be located in the greenhouse or in a nearby building. It should contain storage well above the floor level for all pots and equipment. It should also contain a facility for storing pathogen-free potting soil (mixtures) or sand, coconut fibre, composted saw dust, or other materials used for growing plants for pathogenicity tests. A bench is needed for preparing pots of plants, inoculating soil and other activities. The bench should have a top suitable for easy surface sterilisation, such as stainless steel or marble.

13.4.2 Potting mixture

Pathogen-free potting mixture is essential for pathogenicity tests and many experiments. It is also essential for producing pathogen-free seedlings and cuttings for transplanting for field experiments.

There are many types of potting mixtures. The main features of a good potting mixture are that it has a good water holding capacity and it drains easily. Several types of potting mixture are used in Vietnam. Common materials include composted sawdust, coconut fibre, sand, peat and pelleted chicken manure. Some of these components may be contaminated with plant pathogens; sand may be contaminated with pathogens such as *Pythium* and *Phytophthora*. Coconut fibre and sawdust are usually free of contamination.

Field soil usually contains many plant pathogens. These pathogens should be killed by fumigation or heat treatment (pasteurisation with steam/air mixture at 60 °C for 30 minutes) before the soil can be used for pathogenicity tests. Untreated field soil should not be brought into a greenhouse as the pathogens in the soil can contaminate the greenhouse.

Sawdust compost potting mixture can be made by mixing sawdust, sand and pelleted chicken manure (70:28:2 by volume) and composting it for 4–6 months. Initially this potting mixture should heat up to approximately 50 °C for an extended period which will kill any plant pathogens in the mixture. The potting mixture should be composted in large bins. It is essential that it is kept free from contact with field soil or diseased plants. Coconut fibre can also be a valuable component of potting mixes. Potting mixes can also be pasteurised with steam/air mixture if not composted.

Potting mixtures can be mixed in an electric concrete mixer that has been disinfected. Granular fertiliser can be added during this mixing process.

13.4.3 Greenhouse hygiene

It is essential to have strict rules for staff using the greenhouse to avoid contamination of pathogenicity tests or other studies with plant pathogens in field soil. Equipment and procedures to follow include:

- installing a footbath at the entrance (doorway)
- having rubber boots or sandals for use only in the greenhouse
- not taking field soil or diseased plants into the greenhouse
- removing experimental plants and soil immediately after an experiment is completed and burning diseased plants
- using pathogen-free water
- always keeping the end of the hose away from the floor
- hosing the floor regularly
- staff not entering the greenhouse directly after visiting the field, but showering and putting on clean clothing before using the greenhouse
- sterilising all pots with a strong disinfectant, such as 1% sodium hypochlorite in water for 24 hours, after use in experiments
- storing sterilised pots on shelves well above the floor
- treating bamboo benches with copper fungicide.

13.4.4 Plant management and nutrition

Growing plants in pots for pathogenicity tests and other studies requires careful management of plant nutrition.

It is recommended that pots have holes in the base for good drainage. Small stones can also be placed in the bottom of the pot to allow for good drainage. The aim is to prevent water-logging (saturation) of the material in which the roots grow. Weigh pots regularly to maintain uniform moisture in the potting mixture and prevent water-logging. Soil should only be wet to field capacity.

Plants should be grown in pathogen-free potting mixture. The choice of potting mixture depends on the plants involved, the availability of materials and the nature of the experiment. Nutrition should be adequate for normal plant growth. Granular fertiliser may need to be added to the potting medium before planting. Usually a liquid fertiliser such as Hoagland's solution or a commercial product is applied every 1–2 weeks to maintain normal growth (Figure 13.6). Regular applications of liquid fertiliser are particularly necessary if larger plants are grown in relatively small pots for long periods of time. Commercial liquid N–P–K concentrate and micronutrient liquid concentrate are readily available in Vietnam in small packets.

Figure 13.6 Preparation of commercial fertiliser for greenhouse use

Alternatively, Hoagland's solution can be used (see formula in Box 13.1). This is particularly helpful if the nutrient status needs to be monitored closely or particular nutrients are being left out as part of a nutritional study.

Box 13.1 Hoagland's solution

This solution contains all essential nutrients for good plant growth and development. Hoagland's solution is made up from a range of pre-made stock solutions, which are mixed with water before being used.

For each litre of water add:

- 5 mL potassium nitrate solution
- 5 mL calcium nitrate solution
- 1 mL potassium acid phosphate solution
- 2 mL magnesium sulfate
- 1 mL micronutrient stock solution
- 10 mL iron-EDDHA stock solution.

Stock solutions:

- 1 M KNO_3 potassium nitrate (approx. 101 g in 1 L)
- 1 M $Ca(NO_3)_2.4H_2O$ calcium nitrate (approx. 236 g in 1 L)
- 1 M KH_2PO_4 potassium acid phosphate (approx. 136 g in 1 L)
- 1 M $MgSO_4.7H_2O$ magnesium sulfate (approx. 246.5 g in 1 L).

Micronutrient stock:

- 0.046 M H_3BO_3 boric acid (approx. 2.86 g in 1 L)
- 0.009 M $MnCl_2.4H_20$ magnesium chloride (approx. 1.81 g in 1 L)
- 0.765mM $ZnSO_4.7H_2O$ zinc sulfate (approx. 0.22 g in 1 L)
- 0.320mM $CuSO_4.5H_2O$ copper sulfate (approx. 0.08 g in 1 L)
- 0.111mM $H_2MoO_4.H_2O$ molybdic acid (85%) (approx. 0.02 g in 1 L)

Iron-EDDHA stock

- 10mM $Fe(NO_3)_3$ iron-EDDHA (approx. 2.45 g in 1 L)

Appendix 1

Making a flat transfer needle

The flat transfer needle is one of the most important tools in the laboratory. NiChrome (Nickel and Chromium Alloy, 80:20) 1 mm diameter wire, commonly used for heating hair driers, has been found to be the most suitable material (Figure A1.1).

1. Cut a 60 mm length of wire.
2. Flatten one end of the wire to approximately three times the original width.
3. Trim the flattened wire to a point using sidecutters or heavy-duty scissors.
4. File off the rough edges of the flat area.
5. Mount the needle in a handle.
6. Completed flat transfer needle.

Figure A1.1 A step-by-step guide to making a flat transfer needle

Appendix 2
Health and safety

In the field

- Take care to follow all recommended safety precautions when applying pesticides, particularly those used for insects (insecticides). Only use registered chemicals.
- Wash hands carefully before eating meals, especially when soil has been handled.
- Drink adequate water on hot days in the field.
- Take care with machetes so that you do not cut yourself or other people.

In the laboratory

- Check the safety aspects of all chemicals before use. Such information can be found on the product packaging or on the internet. Major chemical companies supply links to the chemical Material Safety Data Sheets that correspond with their products.
- Use gloves where appropriate.
- Ethyl alcohol is highly flammable. Do not wipe benches near a flame.
- Keep a fire blanket in the laboratory to put out clothing fires.
- Wear shoes in the laboratory to protect feet from sharp instruments dropped accidentally. Closed shoes also protect feet from broken glass and chemicals.
- Do not open the autoclave until the internal air pressure reaches atmospheric pressure (reading 0 on the dial). Always use heavy duty material gloves when removing any material from the autoclave or oven.
- Take care when opening the oven. High temperatures and steam can cause serious burns.

Appendix 3

Media, sterilisation and preservation of cultures

The media section includes the recipes for a number of commonly used media. There are many more types of media which have been developed for specific fungi or experimental procedures. These are described in scientific literature, particularly journal articles.

It is important to understand the basic principles of heat sterilisation of media, glassware and other equipment. Treatment times need to be adjusted to correspond to the volume and nature of the material being sterilised. Treatment times also differ significantly between wet heat (autoclave) and dry heat (oven).

There are many types of preservation techniques to preserve living cultures of fungi. A few common methods are outlined in this section and many others have been documented in other literature.

A large number of media have been developed for culturing fungi. Many of these are general purpose media, such as water agar (WA) and potato dextrose agar (PDA), suitable for growing most fungi. Other media, such as *Phytophthora* selective medium (PSM) and peptone pentachloronitrobenzene agar (PPA) are selective and are used for the isolation of particular fungi from plants or soil.

Synthetic media, those made entirely from defined chemical compounds, are by nature uniform as their chemical composition is standard. Natural media, for example PDA or potato carrot agar (PCA), are inexpensive and encourage good growth of fungi. However, natural media (those made from natural material, usually plant extracts) are variable depending on the extract from the plant. If using natural media for distinguishing morphological characters or growth rate studies it is important that the same batch of media is used across all isolates. Some natural media such as PDA have high carbohydrate levels, giving rapid growth of fungi, with abundant aerial mycelium. Repeated subculturing on these types of media can lead to rapid degeneration of the culture and loss of virulence. Therefore, low nutrient media are preferred for maintenance of cultures.

Remember when making media to loosely screw on lids of bottles during autoclaving and tighten afterwards. This will prevent bottles from exploding under pressure and a lot of clean-up work.

We recommend that glass Petri plates be used in small diagnostic laboratories in tropical areas. Our experience indicates that there is less contamination from airborne fungal spores of media in glass plates than the contamination of plastic plates.

A3.1 Comments on some components of media

Water

Tap water is suitable for use for most media, as it contains trace elements which may be missing from distilled water. However in some areas tap water may contain substances which are toxic to fungi. One of the most significant is copper, which is inhibitory to many fungal species. In these cases distilled water is preferred.

Agar

Agar is an extract from algae, and its quality can vary depending on its source. It is available as a powder, or in a block or flake form. Many powdered agars dissolve readily during autoclaving; the recipes given below are for agar of this type.

Use a good grade of agar so that media such as water agar (WA) are transparent. It is essential that WA used for isolation studies, single sporing, hyphal tipping and identification be transparent. This allows hyphae and spores to be seen clearly under the dissecting microscope.

Use a lower quality agar only for media for which transparency is not as important, such as PDA and PCA. However it is best to avoid lower quality agar if possible.

Water agar is the most useful general purpose isolation medium. Do not use PDA for isolating fungi from plant material. Use PDA only to grow cultures to determine colony morphology and pigmentation. Use other media for encouraging reproduction and sporulation, such as sterile leaf or stem pieces in water agar, or sterile bean pods in water agar. Selective media are very useful for isolating fungi from roots or severely diseased tissue with contaminating saprophytic fungi and bacteria.

Antibiotics

Antibiotics may be added to fungal isolation media to prevent the growth of bacteria or unwanted fungi (Table A3.1). Most antibiotics (except chloramphenicol; see below) are unstable if heated and need to be added to the medium after autoclaving. These antibiotics are dissolved in a small quantity of sterile distilled water, according to the recipe. For most purposes this may be added directly to the medium but, for critical work the antibiotic solution should be filter-sterilised before use.

Table A3.1 Commonly used antibiotics

Antibiotic	Active against	Solubility
Penicillins	Gram-positive bacteria	Water soluble
Streptomycin	Gram-negative bacteria	Water soluble
Neomycin	Gram-positive bacteria	Water soluble
Chloramphenicol	Gram-positive and negative bacteria	Ethanol soluble

Chloramphenicol may be added to the medium before autoclaving. Chloramphenicol is a suspected carcinogen, and it and all other antibiotics must be handled with care.

Fungicides are frequently used in selective media. For example, *Fusarium* species are relatively tolerant to pentachloronitrobenzene (PCNB; Terrachlor® or Quintozene) and dichloronitroaniline (DCNA; Allisan®) and these fungicides are added to media selective for *Fusarium*.

Rose Bengal is added to some media used for isolating fungi from soil. It inhibits the growth of all fungi, and is added to prevent fast-growing species from overgrowing colonies of slow growing fungi. Rose Bengal becomes more toxic on exposure to light. Plates of Rose Bengal media should be stored and incubated in the dark.

When adding antibiotics to media it is important that they are completely dissolved in 10 mL of sterile water to ensure an even spread of antibiotics throughout the media. Once poured into the medium (at 55 °C) the antibiotics should be mixed into the medium by swirling carefully to avoid making excess air bubbles.

Often a range of different media are used in the laboratory at the same time. It is a good idea to devise a system for marking the edges of Petri dishes with dashes made with different coloured permanent markers so that different media can be easily differentiated. The system can be then posted on the wall of the laboratory to avoid any confusion.

A3.2 General purpose media for fungi

Water agar (WA)

WA (2%) consists of 20 g agar in 1 L of water and is recommended as the substrate for the germination of conidia used to initiate single spore cultures. Hyphal growth is sparse on this medium so it is suitable for cultures from which single hyphal tips are to be taken for the initiation of new colonies. Sparse growth on WA also facilitates the isolation of fungi from plant material, particularly roots.

For single sporing and hyphal tipping it is suggested that plates be poured when the medium is still quite hot so that thin plates can be produced—this restricts fungal growth and makes it easier to cut out the spores or hyphal tips.

WA (0.05%), 0.5 g agar in 1 L of water, is used in the preparation of soil dilution series. The small quantity of agar slightly retards sedimentation rates of fungal propagules. The agar is dissolved in water before being dispensed into McCartney bottles. Bottles are capped loosely during sterilisation and caps are tightened when sterilisation is complete.

Carnation leaf-piece agar (CLA) or other natural plant substrate agar

CLA is a natural substrate medium (Fisher et al. 1982) prepared by placing sterile carnation leaf pieces (approximately 1 piece per 2 mL agar) in a Petri plate and then adding sterile 2% WA.

The carnation leaf pieces are prepared from fresh carnation leaves free from fungicide or insecticide residue. Immediately after collection the leaves are cut into 5–8 mm pieces and dried in a forced-air oven at approximately 70 °C for 3–4 hours until brittle. Leaf pieces can also be dried in a microwave oven. The dried leaf pieces are packaged in aluminium or polycarbonate containers and sterilised by gamma irradiation (25 kilograys). Sterilised leaf pieces can be stored at 2–5 °C for up to 12 months before use.

Many species sporulate on CLA in 6–10 days. On this medium, conidial shapes are more uniform than when using carbohydrate rich media such as PDA. Macroconidia of *Fusarium* are formed mainly in sporodochia, which usually develop on the leaf pieces. Macroconidia formed in sporodochia are preferred in identification, as they are more consistent in shape and length than macroconidia formed from solitary monophialides on hyphae on the agar. Microconidia are more common on hyphae growing on the agar, often away from the leaf pieces. The mode of formation of microconidia, the presence of chains of microconidia, and the presence of chlamydospores can be determined by direct examination with a compound microscope when small plates of CLA (5 cm diameter) are used for routine identification of *Fusarium* cultures. CLA is also suitable for producing large numbers of conidia for experimental work.

A variety of plant parts such as green rice stem pieces and bean pod pieces can be substituted for carnation leaf pieces. If necessary sterilise these plant pieces by autoclaving. You should experiment to find the most suitable plant pieces for your laboratory.

Potato dextrose agar (PDA)

PDA is a carbohydrate rich medium which contains 20 g dextrose, 20 g agar and the broth from 250 g white potatoes made up to 1 L with tap water. The potatoes are unpeeled, but are washed and diced before boiling until just soft. The boiled potatoes are filtered through cheesecloth, leaving some sediment in the broth.

Conidia formed on PDA are usually variable in shape and size, and so are less reliable for use in identification. However, colony morphology, pigmentation and growth rates of many fungal species on PDA are reasonably consistent, as long as the medium is prepared carefully and the cultures are initiated from standard inocula and incubated under standard conditions. These colony characteristics are useful secondary criteria for identification. Although PDA is used for the isolation of some fungal pathogens, many saprophytic fungi and bacteria also grow rapidly on PDA and may inhibit the recovery of the pathogen. We do not recommend using PDA for isolation studies. Do not use PDA for isolation from roots.

It is recommended that one quarter strength PDA be used for isolation purposes, emended with antibiotics when isolating from stem or leaf tissue.

Spezieller Nährstoffarmer agar (SNA)

SNA is a weak nutrient agar which can be used for the identification and maintenance of *Fusarium* and *Cylindrocarpon* isolates (Nirenberg 1976). In addition to limiting cultural degeneration, this medium promotes uniform sporulation of microconidia in particular. SNA is prepared by autoclaving, in 1 L distilled water:

Agar	20 g
KH_2PO_4	1 g
KNO_3	1 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
Glucose	0.2 g
Sucrose	0.2 g

Two pieces of sterile filter paper (1 cm square), placed on the agar surface when set, assist in stimulating sporulation.

Because SNA is transparent, cultures can be viewed by direct examination under the microscope or small blocks can be mounted on a slide with a drop of water and cover slip for observation. A liquid broth made from this medium, but with no addition of agar, is often used for preparing mycelium for DNA extraction.

Potato carrot agar (PCA)

Carrot puree	20 g
Potato puree	20 g (made from peeled potatoes)
Agar	20 g

Peel the potato and dice the potato and carrot into small pieces. Place in a beaker containing approximately 200 mL of distilled water and gently boil for 30 minutes on low heat. The vegetables can then be forced through a fine sieve or blended to create a puree. Add agar and then distilled water to make up to 1 litre. Mix and autoclave. When pouring the media occasionally swirl to keep the carrot/potato mix in suspension.

The carrot puree is rich in sterols, which are essential for oogonial production in Oomycete species. PCA is an important medium for stimulating the production of oogonia in *Pythium* and *Phytophthora*.

A3.3 Selective media for specific fungi

Phytophthora selective medium (PSM)

This recipe includes penicillin and was originally recommended for use in Vietnam to the authors by Mr Nguyen Vinh Truong.

Agar	8 g
Carrot puree	20 mL (recipe below)
Potato puree	80 mL (recipe below)

Make up to 1 L with distilled water, autoclave and when cooled to 55 °C, add:

Hymexazol	3.7 mL of stock solution in water
Pimaricin	400 µL
Penicillin	200 mg

Wrap the plates in plastic wrap and store them in the fridge out of the light. Discard after a month. To make a medium selective for both *Phytophthora* and *Pythium,* do not add Hymexazol.

Carrot puree

Wash and dice 400 g carrots and autoclave for 10 minutes in 400 mL distilled water. Puree the mix, then add an additional 500 mL water. This can be measured out and frozen in plastic containers until needed.

Potato puree

Dice 200 g potato and boil in 500 mL tap water until tender. Puree and make up to a total of 800 mL with additional water. Store as above.

Hymexazol stock solution

Add 0.3 g pure hymexazol to 20 mL sterile water.

Pimaricin

Pimaricin can be added directly to the molten agar. Shake well before dispensing. Store wrapped in foil in the fridge.

Peptone PCNB agar (PPA / Nash–Snyder medium)

PPA is comprised of a basal medium to which antibiotics and fungicides are added, and it enables the selective isolation of *Fusarium* species from soil dilutions (Nash and Snyder 1962) or plant material. It is highly inhibitory to most other fungi and bacteria, but allows slow growth of *Fusarium*, which form small colonies of 5–10 mm diameter after 5–7 days.

Basal medium in 1 L water:

Agar	20 g
Peptone	15 g
KH_2PO_4	1 g
$MgSO_4.7H_20$	0.5 g
Terrachlor®	1 g (contains PCNB 75% w/w)

Autoclave basal medium and cool to 55 °C before adding, in 10 mL sterile water:

Streptomycin sulfate	1 g
Neomycin sulfate	0.12 g

The prepared plates should be allowed to 'dry' in a cool dark place before use so that the water used for the soil suspension is rapidly absorbed. Most species of *Fusarium* do not form distinctive colonies on PPA; sporulation is poor and conidial morphology abnormal. Colonies must be subcultured and purified for identification. *Fusarium* cultures should not be maintained on PPA, because the metabolism of peptone leads to the accumulation of toxic ammonia.

Quarter-strength PDA with antibiotics

This medium is designed primarily for routine isolation of *Fusarium* species from plant tissue, such as stems infected with *F. oxysporum* wilt pathogens. It can also be used with a range of other pathogens, but test it before use in an important experiment. It is a useful medium for diagnostic studies.

Do not use this medium for isolating *Fusarium* or other fungi from soil.

In 1 L of water, mix:

Potato extract	Broth from 62.5 g of cooked potato
Agar	20 g
Dextrose	5 g
PCNB (Terrachlor®)	0.1 g

Autoclave basal medium and cool to 55 °C before adding, in 10 mL sterile water:

Streptomycin sulfate	0.16 g
Neomycin sulfate	0.06 g

Dichloran chloramphenicol peptone agar (DCPA)

DCPA was developed for the selective isolation of *Fusarium* species and dematiaceous hyphomycetes from cereal grains (Andrews and Pitt 1986). The basal medium, made up with 1 L distilled water, contains:

Agar	20 g
Peptone	15 g
K_2HPO_4	1 g
$MgSO_4.7H_2O$	0.5 g
Chloramphenicol	0.2 g (broad spectrum antibiotic—can be autoclaved)

After autoclaving add, in 10 mL ethanol:

Dichloran	0.002 g

DCPA should not be used as a maintenance medium because the metabolism of peptone leads to accumulation of ammonia to toxic levels. Mucoraceous fungi are suppressed by dichloran, and the absence of a carbohydrate source is selective against *Aspergillus* and *Penicillium* species.

Rice leaf (or grass leaf) medium for *Pythium*

This medium is useful for stimulating and observing the formation of sporangia and oogonia by many *Pythium* species. The sporangia and oogonia form in mycelium growing on the surface of the water near the leaf pieces. This medium can be prepared by floating pieces of sterile rice or grass leaves in Petri plates of water:

1. Cut rice leaves into pieces about 3 cm long.
2. Autoclave and place 4–5 pieces into large Petri plates containing 15 mL sterile water.
3. Inoculate with a plug from an agar culture.

The fungus will colonise the grass pieces, and mycelium will grow over the surface of the water. To mount the fungus for microscopic observation:

1. Place a coverslip under the surface of the water.
2. Carefully tease off some of the mycelium and draw the material onto the coverslip.
3. Remove the coverslip from the culture, invert, and place onto a drop of water on a microscope slide.

A3.4 Media for use with bacteria

King's B medium (KBM)

Agar	15 g
Proteose peptone No. 3	20 g
Glycerol, C.P.	10 mL
K_2HPO_4	1.5 g
$MgSO_4$	1.5 g
Distilled water	1 litre

Combine all ingredients except $MgSO_4$. Adjust pH to 7.2 ± 0.2. Add $MgSO_4$ slowly and mix. Autoclave and pour into 90 mm Petri dishes.

Sucrose peptone agar (SPA)

Sucrose	20 g
Peptone	5 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.25 g
Agar	20 g
Distilled water	1 L

Combine all ingredients. Adjust pH to 7.2 ± 0.2. Autoclave and pour into 90 mm Petri dishes.

Tetrazolium medium

This medium (Kelman 1954) can be used to differentiate between mutant and wild type colonies of *Ralstonia solanacearum*. Mutants commonly form round, butyrous, deep red colonies with a narrow bluish border. Wild type colonies are irregularly round, fluidal white colonies with a pink centre.

Peptone	10 g
Casein hydrolysate	1 g
Glucose	5 g
Agar	17 g
Triphenyl tetrazolium chloride	0.05 g
Distilled water	1 L

Combine all ingredients. Autoclave and pour into 90 mm Petri dishes.

A3.5 Sterilisation

Sterilisation is the process of killing all living organisms in a culture medium or on the surface of glassware used for sterile work, such as glass Petri dishes.

Heat sterilisation

The temperature and time required for killing are inversely related. Table A3.2 shows the minimum times required for effective sterilisation at the temperatures given for both moist and dry heat:

Table A3.2 Required times for sterilisation using moist and dry heat over a range of temperatures

Temperature	Moist heat	Dry heat
100 °C	20 hours	
110 °C	2.5 hours	
121 °C	15 minutes	8.0 hours
130 °C	2.5 minutes	
140 °C		2.5 hours

These times do not guarantee sterility. They are times calculated from experience and are based on normal levels of contamination with heat resistant organisms.

The species, strain and spore forming ability of a microbe greatly affects its susceptibility to heat. In moist heat the vegetative forms of most bacteria, yeasts and fungi and most animal viruses, are killed in 10 minutes by temperatures between 50 °C and 60 °C. However bacterial spores require 15 minutes at temperatures ranging from 100 °C to 121 °C. In dry heat bacterial spores require 1 hour at 160 °C.

The nature of the material in which the organisms are heated is also an important factor. A high content of organic substances generally tends to protect spores and vegetative organisms against the lethal action of heat. Proteins, gelatin, sugars, starch, nucleic acids, fats and oils all act in this way. The effect of fats and oils is greatest in moist heat as it prevents access of moisture to the microbes. The pH is also very important. The heat resistance of bacterial spores is greatest at neutral pH and decreases with increasing acidity or alkalinity.

Dry heat sterilisation

Dry heat kills microbes by oxidation. The dry heat process is the best method for the sterilisation of dry glassware such as test tubes, glass Petri dishes, flasks, pipettes, all glass syringes and instruments such as forceps, scalpels and scissors.

Glassware should be packed so as to allow proper penetration of the hot air throughout the load. This is aided by the fan. The holding period required for sterilisation is 160 °C for 1 hour. However most ovens, particularly if packed, will take 2 to 3 hours to reach temperature. Thus 4 hours at 160 °C would be the minimum for a big load. Four hours at 170 °C allows a safety margin.

Ovens must not be opened during their cycle, as one opening for a few seconds may drop the temperature by up to 70 °C, which takes the oven perhaps an hour to recover. This leads to the non-sterilisation of that load.

Moist heat sterilisation

Moist heat kills microorganisms, probably by coagulating and denaturing their enzymes and structural proteins, a process in which water participates. All culture media therefore are sterilised by moist heat.

Autoclaving at temperatures greater than 100 °C is the most reliable method and widely used for the sterilisation of culture media. Most autoclaves and pressure cookers operate at 121 °C, at which the minimum holding period for sterilisation is 15 minutes. It is essential that all air is expelled from the autoclave, otherwise it will not reach the correct temperature. Many large autoclaves do this automatically.

If using a pressure cooker or a manual autoclave, allow steam to hiss from the outlet for 2–3 minutes before closing the valve or placing on the cap. Baskets and not tins should be used for autoclaving and pipettes should not be autoclaved in canisters as localised air pockets will make for inefficient sterilisation. Temperature and NOT pressure is the true criterion for sterilisation procedures.

The autoclave should be adjusted so that the chamber pressure does not fall too rapidly as this results in media boiling over and wetting plugs. Media should be left in the autoclave for about 5 minutes after it has returned to atmospheric pressure, as sometimes solutions remain superheated and, when disturbed, spray boiling medium or agar over the operator, resulting in nasty burns. If left in the autoclave for longer periods, excessive loss of volume will occur as a vacuum builds up in the autoclave.

An effort should be made to avoid sterilising large and small volumes of media in one load as time must be allowed for large volumes to reach the required holding temperature, and this will result in small volumes receiving too much heat. Table A3.3 provides a rough guide to the extra time that must be added to reach holding temperature:

Table A3.3 Suggested times for sterilisation of different volumes of liquid

Volume of liquid	Extra time (minutes)	Total time at 121 °C (minutes)
100 mL bottle	10	25
250 mL bottle	12	27
500 mL bottle	18	33
1000 mL bottle	22	37
2000 mL bottle	27	42

Sterilisation of instruments

Forceps, inoculating needles and other instruments must be sterilised before contact with a culture to avoid cross-contamination. Inoculating needles are best sterilised by heating to red heat in a flame.

The needle must be allowed to cool to room temperature again before being used. Hot needles are the most common cause of failure of subculturing, hyphal tipping and single sporing.

Forceps and scalpels are sterilised by dipping in alcohol. Before use, the alcohol is burnt off by passing the forceps through a flame to ignite it. Do not hold the instrument in the flame, since this will heat it up too much. Be very careful not to place hot or flaming instruments in or near alcohol, since this is a fire hazard.

Sterilisation of work surfaces

Trays, benches and other surfaces may be sterilised with a liquid disinfectant. Alcohol is the most commonly used. Alcohol works best as a sterilant if it contains some water, and a solution of 70% ethyl alcohol is suitable. Methylated spirits is also suitable.

A3.6 Preservation of cultures

Preservation of living cultures

Living cultures are stored for use as reference cultures, or for later use in pathogenicity tests or other experiments. Cultures are stored in national culture collections as part of reference materials that support a national database of plant pathogens.

Storage in sterile water—*Pythium* and *Phytophthora*

This is a low-cost, simple method that is particularly suitable for *Pythium* and *Phytophthora*. A sterile work chamber should be used for this procedure. Agar blocks 1 cm square are cut from the margin of a young, actively growing fungal colony. These are placed in sterile water in a McCartney bottle and the cap is screwed down. The bottles are stored under cool conditions. Do not store in a refrigerator as some species are killed at low temperatures. Cultures can be stored between 6 months to 2 years, depending on the species. Cultures are revived by removing a block of agar from the bottle and placing mycelium side down on fresh medium. It is essential to ensure that the water and agar blocks are not contaminated by bacteria—the presence of bacteria will lead to rapid death of the fungus.

Storage of sclerotia

Sclerotia can be stored for long periods under cool dry conditions in a small screw cap glass bottle or ampoule. This is a suitable technique for storage of species such as *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia* spp. (sclerotial forming isolates).

In tropical regions it is best to store sclerotia on sterile paper tissue over blue silica gel in a McCartney bottle (or similar screw cap bottle) to ensure very low humidity for storage.

Storage as colonised pieces of plant stem or leaves

Cultures are grown on sterile WA containing pieces of sterile plant tissue or seeds. The colonised pieces are air dried and then stored in a small glass tube. Alternatively they can be stored on a sealed container on sterile paper above blue silica gel to ensure very dry storage conditions.

For more in-depth information regarding the preservation of cultures see Shivas and Beasley (2005), Management of Plant Pathogen Collections.

Lyophilisation by freeze drying

Lyophilisation, or freeze-drying, is the method of choice for long-term preservation of many fungi and is used routinely in most major culture collections. Its major drawback is the requirement and expense of specialised equipment. It is best suited to fungi which grow and sporulate well in culture on sterile plant tissue such as green rice stem-pieces or carnation leaf-pieces. There are also many fungi which cannot be freeze-dried successfully, such as Oomycetes, rusts and mildews.

Cultures are lyophilised by drying colonised stem or leaf pieces in small glass ampoules under high vacuum (10^{-1} to 10^{-2} Torr). The ampoules are prepared by inserting a small cotton wool plug and then autoclaving in a loosely covered beaker. Five stem or leaf pieces are taken from a culture (which is two weeks old and initiated from a single conidium), and aseptically transferred to the ampoule. The ampoule is replugged, labelled (with an internal label) then heated and drawn out to an hourglass shape using a gas torch. The ampoules are attached to the freeze dryer for 12–24 hours, then sealed under high vacuum and stored at room temperature or at 5 °C. Many species of *Fusarium* and other fungal genera have been successfully lyophilised using this technique and have retained viability for many years.

Cultures can be revived by aseptically plating the dried stem or leaf pieces onto a suitable medium. The ampoule is first surface sterilised before it is shattered to release the leaf pieces.

Other preservation techniques for living cultures

Cultures can also be stored as spore suspensions in glycerol in a –80 °C freezer for long-term storage. Many species have also been stored successfully in liquid nitrogen. However, these are very expensive techniques.

Preservation of fungal cultures for herbarium records

Holotype specimens grown on PDA must be lodged in an internationally recognised herbarium when a formal description of a new species is published.

Cultures are initiated from single germinated conidia and grown under standard conditions of temperature and light for 2 to 3 weeks. Cultures are then killed by exposing the plates to formalin in a closed container for 3 days. Preservation of the culture is achieved using agar and glycerine. Three grams of agar are dissolved in 147 mL water, which is then dispensed as 6 mL aliquots into test tubes before autoclaving. The lid of the culture dish is inverted, 1.5–1.75 mL glycerine is added and then the 6 mL aliquot of hot agar is poured over the glycerine. The culture is aseptically lifted from the Petri dish and floated on the mixture in the lid. Cultures

are then allowed to dry in a drawer for 3–5 days covered with a sheet of paper. When dried, the culture is rubbery and can be removed from the Petri dish for storage. This procedure was originally developed for use with *Fusarium* species at the Fusarium Research Centre, Pennsylvania State University. It is suitable for many fungi.

Preservation of fungi under mineral oil

Many fungi can be stored in culture under sterile mineral (paraffin) oil for 4–5 years at 15–20 °C. Cultures should be grown on PDA amended with 0.1% concentrated yeast extract (e.g. Vegemite®). The mineral oil should be prepared as follows:

1. Dispense 11 mL paraffin oil into 25 mL McCartney bottles without rubber seals.
2. Replace lids loosely and autoclave at 121 °C for 20 minutes.
3. Allow to cool completely in the autoclave.
4. Remove any water from the oil by heating at 120 °C in an oven for 8 hours and leaving in the oven overnight to slowly cool to room temperature. Discard any bottles that contain cloudy oil or repeat the oven-heating process.

Cultures should be grown on slopes of PDA with yeast extract in 25 mL McCartney bottles (without rubber seals) until all of the agar is covered with mycelium. To preserve the cultures, aseptically add 11 mL of sterile mineral oil to each culture in a sterile work chamber. Label carefully with the accession number and date.

Cultures can be re-grown as follows:

1. Aseptically remove a small piece of agar from the preserved culture.
2. Blot the piece on sterile filter paper or blotting paper to remove the oil.
3. Plate the piece of agar on appropriate medium.

Note: It is recommended that three cultures of each fungal isolate are preserved at any one time, and that mineral oil cultures are renewed every 4–5 years.

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Acronyms and abbreviations

ACIAR	Australian Centre for International Agricultural Research
ATSE	Academy of Technological Sciences and Engineering
CFU	colony-forming units
CLA	carnation leaf agar
DCNA	dichloronitroaniline
DCPA	dichloran chloramphenicol peptone agar
DNA	deoxyribonucleic acid
DON	deoxynivalenol
EDDHA	ethylmediamine-di-o-hydroxyphenylacetic acid
IDM	integrated disease management
KBM	King's B medium
PCA	potato carrot agar
PCNB	pentachloronitrobenzene
PDA	potato dextrose agar
PPA	peptone PCNB agar (Nash–Snyder medium)
PPSD	Plant Protection Sub-department
PSM	*Phytophthora* selective medium
RNA	ribonucleic acid
SNA	Spezieller Nährstoffarmer agar
SPA	sucrose peptone agar
UV	ultraviolet
WA	water agar

Glossary

Acervulus (pl. acervuli)
An asexual saucer-shaped fruiting body that produces conidia.

Antheridium (pl. antheridia)
The 'male' sexual organ found in some fungal species.

Antibiotic
A chemical compound, synthesised naturally or artificially, that inhibits or kills targeted organisms.

Ascospore
A sexually produced spore formed in an ascus, produced by ascomycetes.

Ascomycetes
A class of true fungi that produce ascospores sexually within asci.

Ascus (pl. asci)
A sac-like body in which ascospores are formed.

Asymptomatic
Possessing no symptoms.

Basidiomycetes
A class of fungi sexually producing basidiospores on a basidium.

Basidium (pl. basidia)
A clavate body on which basidiospores are produced.

Blight
A disease characterised by rapid death of plant tissue.

Chlamydospore
A thick-walled, asexually produced survival spore.

Colonisation
The process of colony establishment on a substrate.

Conidiophore
A specialised hypha on which conidia are produced.

Conidium (pl. conidia)
An asexually produced fungal spore.

Cultivar
A variety of a plant produced by selective breeding.

Damping-off
Rot at the soil level, causing young seedlings to collapse and die suddenly. Usually associated with excess moisture in the soil.

Deuteromycetes

A large and heterogeneous grouping consisting of true fungi of which no sexual reproductive stage is known.

Diagnostic

A characteristic that can be used to differentiate one organism from another.

Disease cycle

The cyclic sequence of events involved in the life of a pathogen, including infection, development, reproduction and survival stages.

Eukaryote

An organism in which the genetic material (DNA) is contained in a nucleus.

Fungicide

A chemical compound that is toxic to fungi.

Forma specialis (f. sp.) (pl. formae speciales)

A specific biotype of a pathogen that can only infect a specific genus or species of plants.

Gamete

A reproductive cell containing half the required genetic material for reproduction.

Heterothallic fungi

Fungi that require two individuals in order to sexually reproduce, each possessing either a 'male' or a 'female' gamete.

Homothallic fungus

An individual fungus capable of producing both 'male' and 'female' gametes for sexual reproduction.

Hypha (pl. hyphae)

A singular somatic filament produced by fungi.

Infection

The establishment of a parasitic organism within a host.

Infested

An individual plant or a specific area affected by a large number of parasitic organisms.

Inoculate

The process of artificially infecting a host organism with a pathogen.

Isolation

The process of obtaining a pathogen from a host for further study.

Koch's postulates

A set of conditions, formulated by Robert Koch, that are designed to test if a microorganism is the causative agent of a disease.

Macerate

To break up into small pieces with the aid of water.

Mosaic

Irregular angular patterning commonly found on leaves of plants infected by a viral pathogen.

Mottling

An irregular patterning of light and dark regions.

Mycelium

A mass of fungal hyphae.

Mycotoxins

Secondary metabolites produced by fungi on infected plant material that can cause illness in livestock and humans when ingested.

Necrotic

Dead and discoloured organic material produced in and around diseased regions of plants.

Nematode

An unsegmented round worm. Some nematodes are parasites of plants.

Non-specific symptoms

Symptoms that do not provide a diagnostic.

Oogonium (pl. oogonia)

The 'female' sexual organ found in some fungal-like species.

Oomycete

A classification of fungal-like organisms, some of which asexually produce motile spores for infection.

Oospore

A sexually produced spore from the phylum Oomycota.

Overseason

The ability of a pathogen to survive between infection stages on a host crop.

Pathogen

An organism possessing the ability to cause disease.

Pathogenicity

The ability to cause disease.

Perithecium (pl. perithecia)

A sexual fruiting body producing ascospores.

Phialides

A specialised cell on which conidia are produced.

Prokaryote

A micro-organism whose genetic material is not contained in a membrane-bound nucleus.

Propagule

A part of an organism that can be detached from a parent and give rise to a new organism.

Pycnidium (pl. pycnidia)

An asexual fruiting body producing conidia.

Rhizome

A horizontally growing underground stem capable of producing both shoots and roots.

Saprophyte

An organism that uses dead organic matter as a source of food.

Sclerotium (pl. sclerotia)

A compact mass of hyphae covered with a dark rind, capable of surviving for extended periods of time.

Septate

Hyphae possessing cross walls.

Sporodochia

An asexual spore-producing structure containing a mass of conidiophores on a hyphal mass.

Spore

The reproductive propagule of fungi. Spores may be either sexually or asexually produced.

Sporangium (pl. sporangia)

A sac-like structure containing asexually produced spores. In some cases, the sporangium itself can act as an infective propagule.

Vector

An organism that transmits a pathogen.

Virulence

The degree of pathogenicity of a disease-causing organism.

Zoospore

An asexually produced spore, possessing flagella. The flagella enable movement in free water.

Bookshelf

The bookshelf is the most important resource in a diagnostic laboratory. We suggest one of the most valuable books to have on the bookshelf is *Plant Pathology* (Agrios 2005). This book contains valuable information on each type of pathogen — fungi, bacteria, viruses, mollicutes and nematodes. A wide range of diseases are illustrated in this text and many excellent diagrams are provided.

The American Phytopathological Society publishes compendia of plant diseases of individual crops or groups of crops. These are valuable resources, and are highly recommended for any diagnostic plant pathology laboratory. Many international agencies such as ACIAR also produce manuals on a wide range of subjects, many produced at low or no cost.

A great deal of information is also available from official websites of government departments of agriculture and from universities. Make use of these sources of information and file them for future reference, either on the basis of the crop of interest or by taxonomic grouping.

The bookshelf should be continually updated with references on current diseases and methods in disease diagnosis.

The following is a list of literature recommended by the authors to be added to your bookshelf in the diagnostic laboratory.

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