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Diagnostic manual for plant diseases in Vietnam

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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.

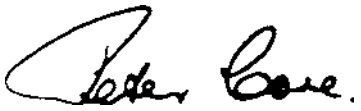
The accurate diagnosis of the cause of a disease is essential to the success of control measures. However, many diseases produce similar symptoms, making diagnosis in the field difficult or impossible. Hence, diagnostic laboratories are an essential component of a plant protection network. Staff assigned 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
Australian Centre for International Agricultural Research

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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:

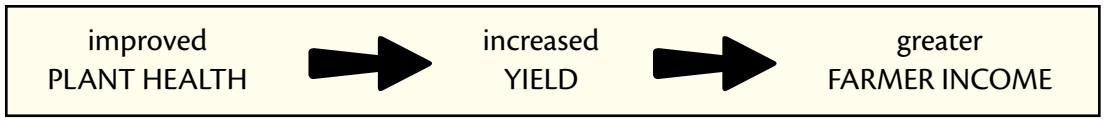
- general plant health and factors that can affect it
- field and laboratory procedures for diagnosing the causes of a disease
- symptoms of plant disease
- procedures and equipment for working in the field
- procedures and equipment for working in the laboratory
- a brief introduction to fungal taxonomy
- 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.

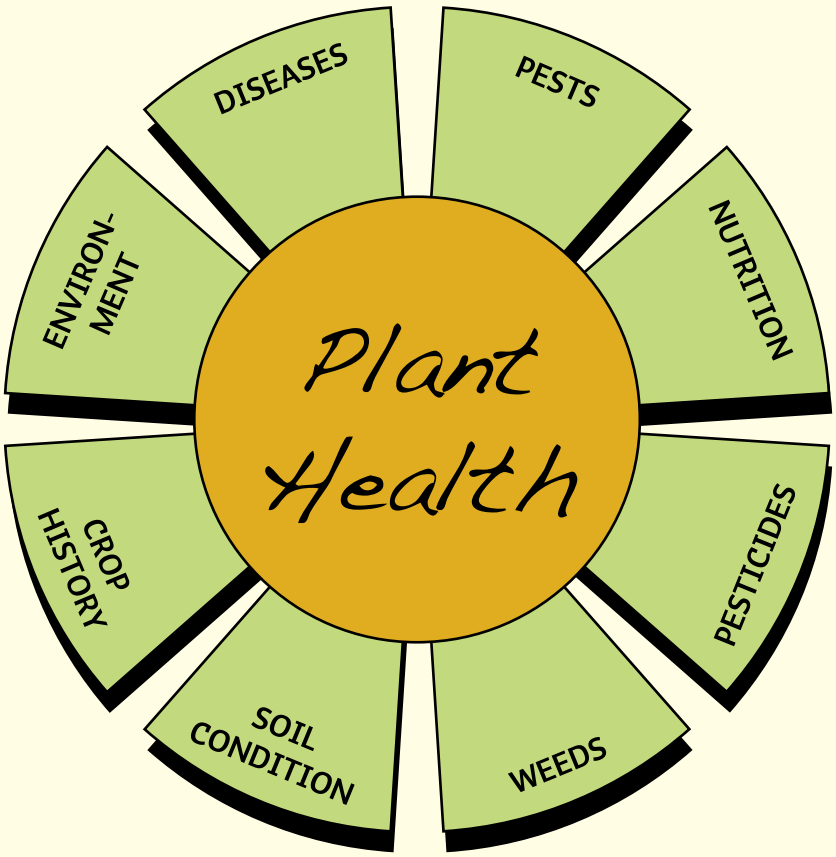


Figure 2.1 Key factors in maintaining plant health

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.

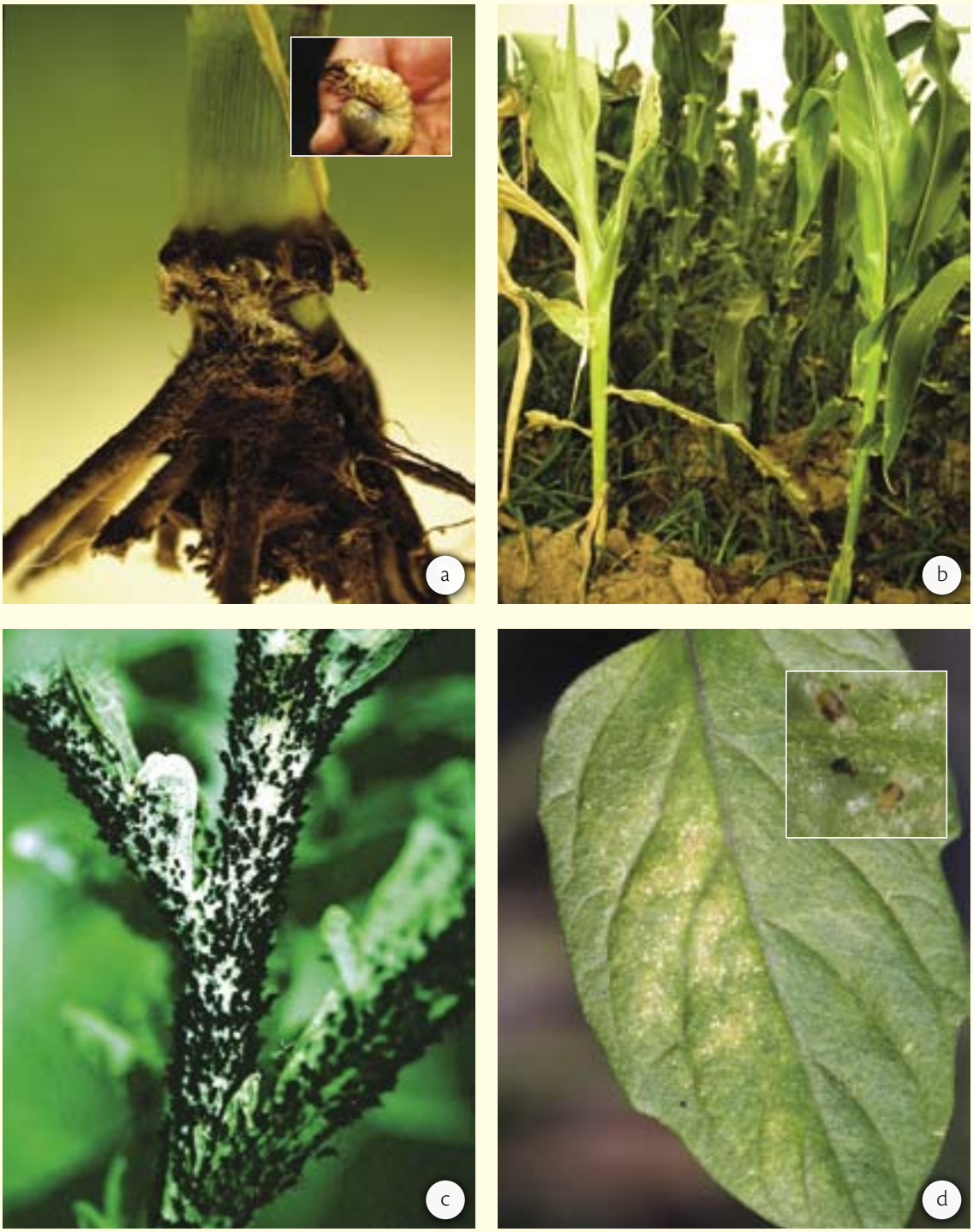


Figure 2.2 Invertebrate pest damage: (a) white grub (inset) damage to maize roots, (b) wilting maize plant affected by white grub, (c) aphid infestation, (d) typical bronzing of leaf caused by mites feeding on the underside of the leaf (inset)

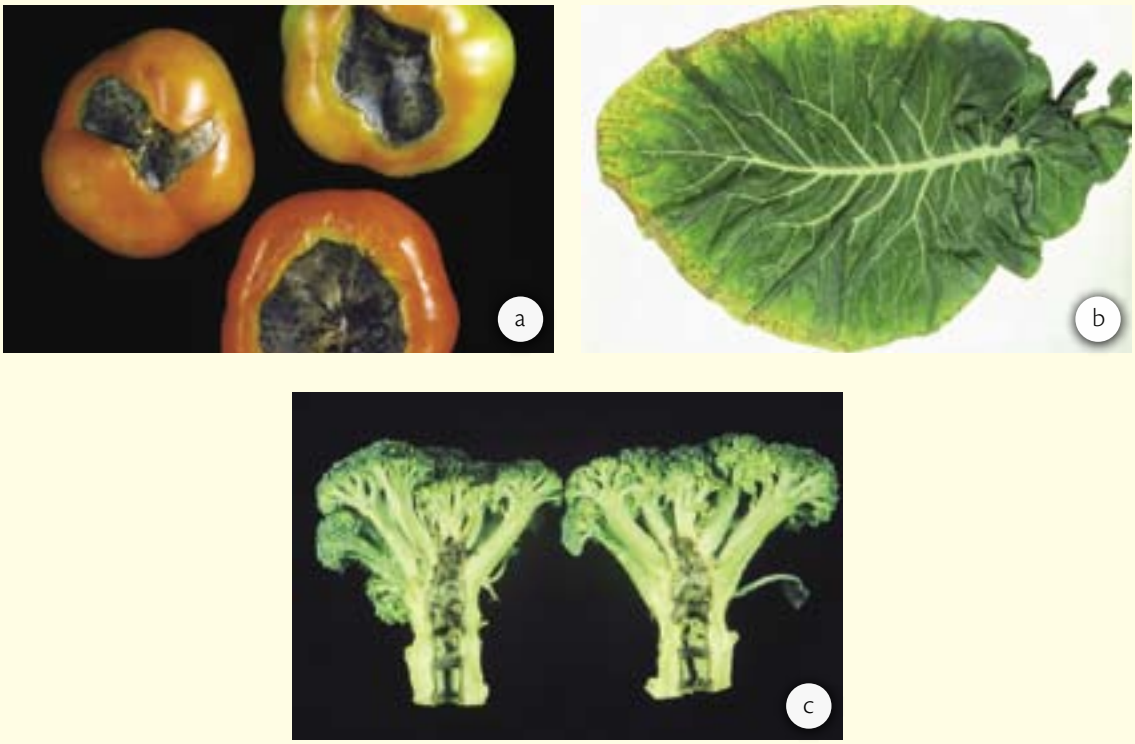


Figure 2.3 Nutrient deficiencies causing disease-like symptoms: (a) blossom end rot due to calcium deficiency of tomato, (b) potassium deficiency of crucifer, (c) boron deficiency of broccoli

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.

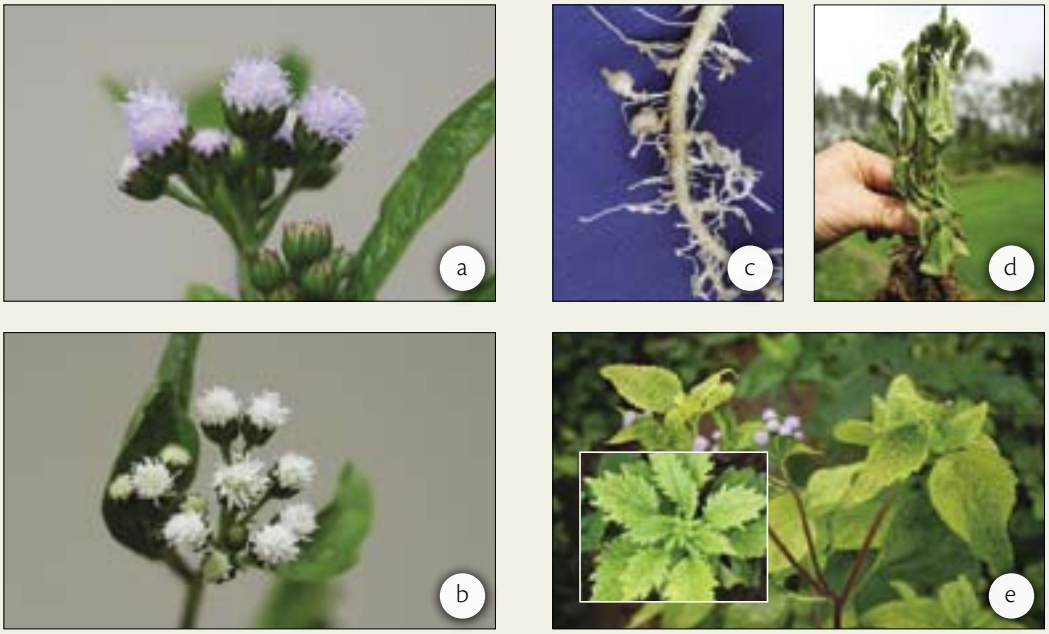


Figure 2.5 *Ageratum conyzoides*: (a) blue flowered variety, (b) white flowered variety, (c) *Ageratum conyzoides* root affected by *Meloidogyne* spp. (nematodes) causing root knot symptoms, (d) wilting *Ageratum conyzoides* caused by *Ralstonia solanacearum* (a bacterium), (e) aster yellows-like symptoms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