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.

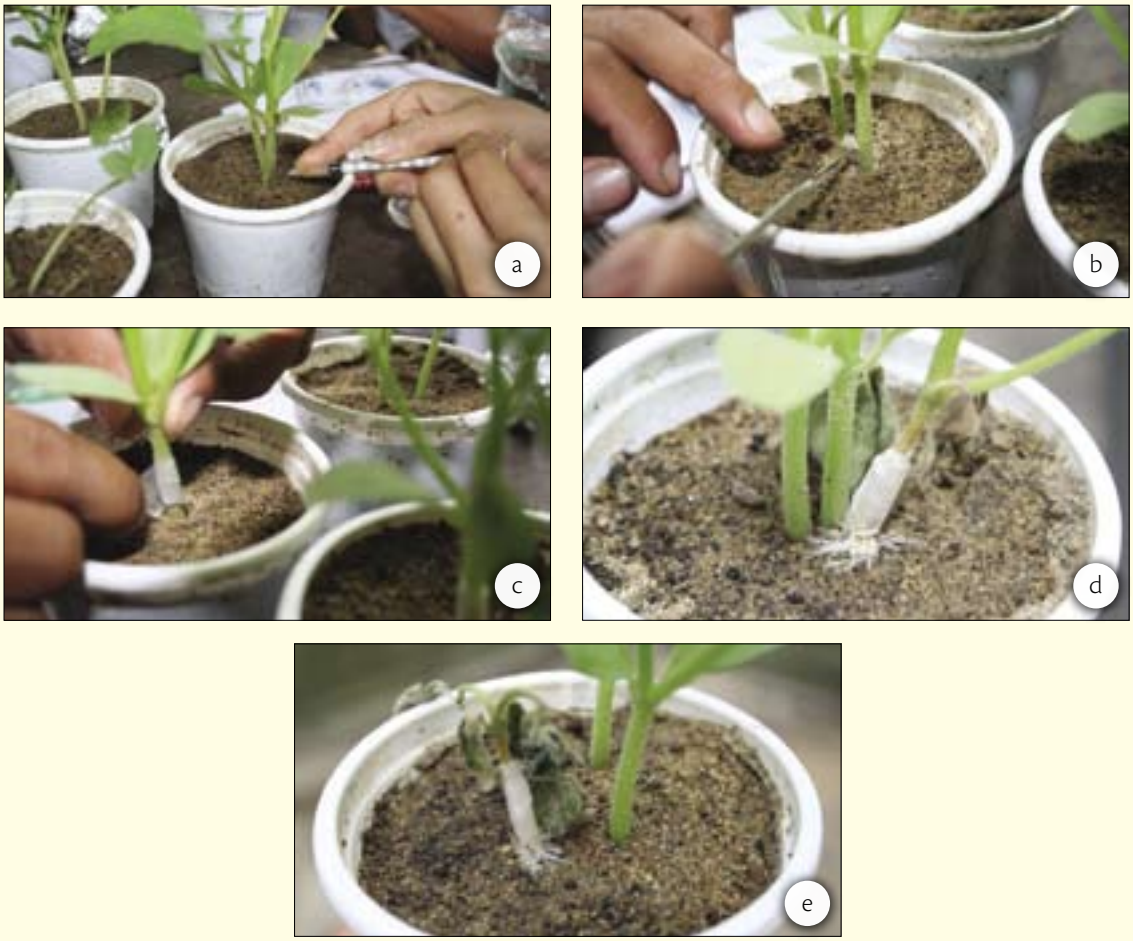

Figure 8.1 Stem inoculation technique for pathogenicity testing: (a) piercing the lower stem, (b) transferring the pure culture to the wound site, (c) wrapping the wound site in plastic, (d) mycelium developing on soil surface from diseased stem, (e) an inoculated plant (left) and an uninoculated control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

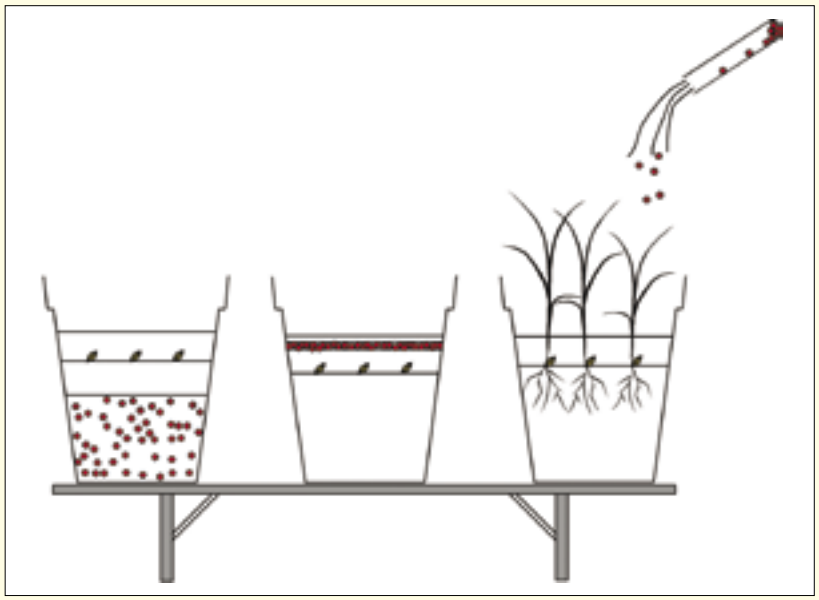

Figure 8.2 Different methods for inoculating soil to produce disease in the glasshouse

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.

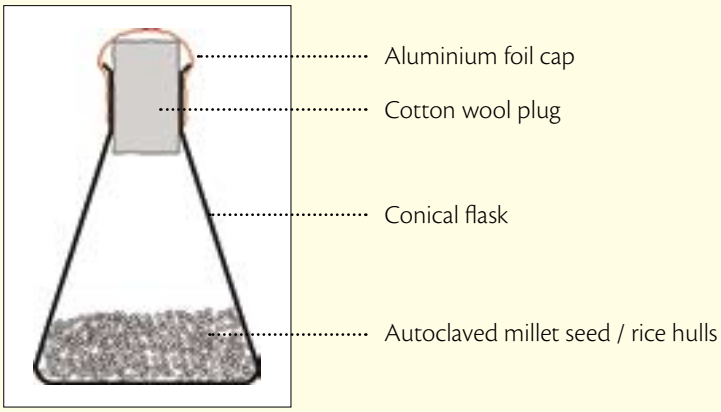

Figure 8.3 An inoculum flask

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).

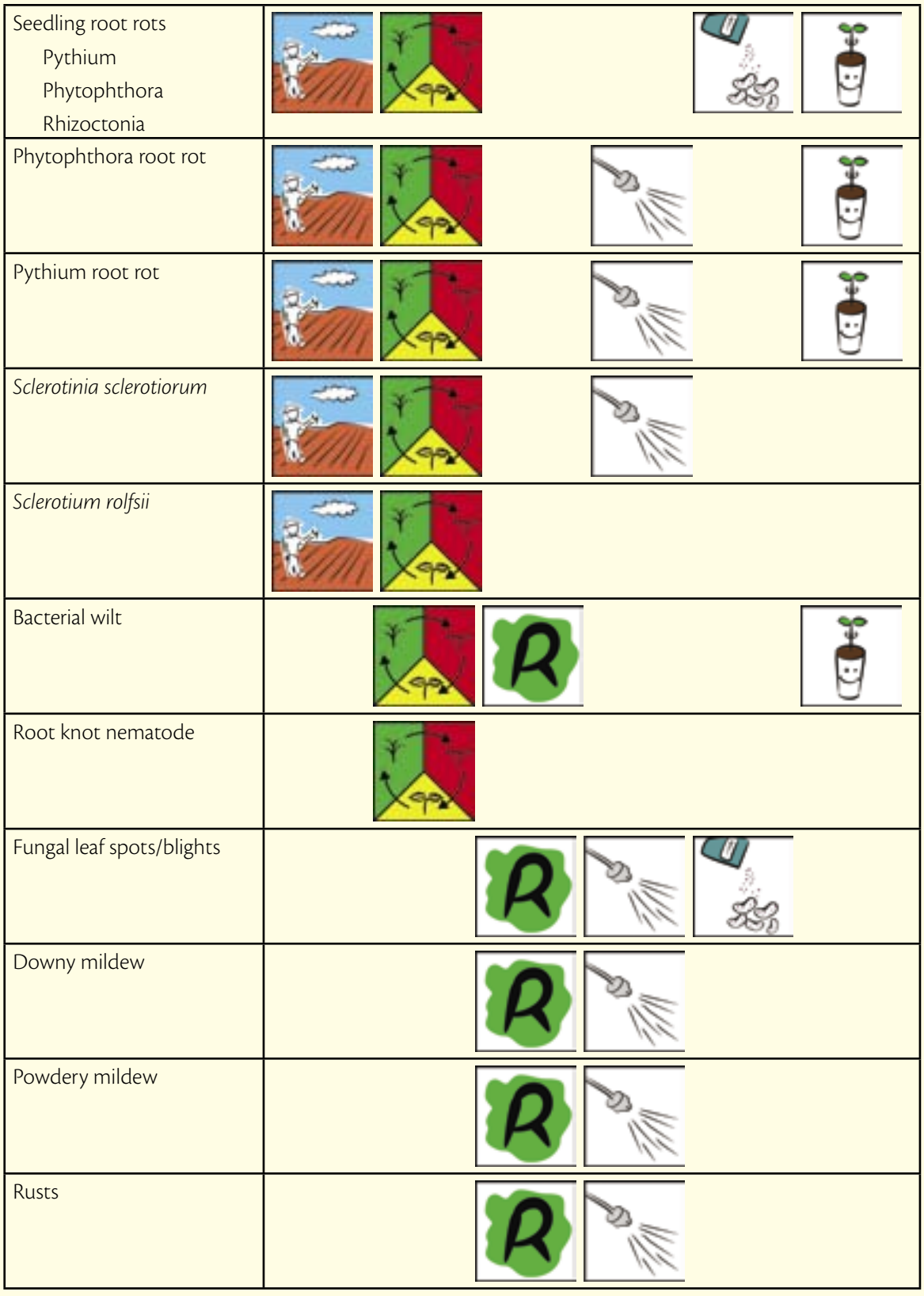

Figure 9.1 Diagrammatic summary of appropriate control measures for common groups of diseases

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.

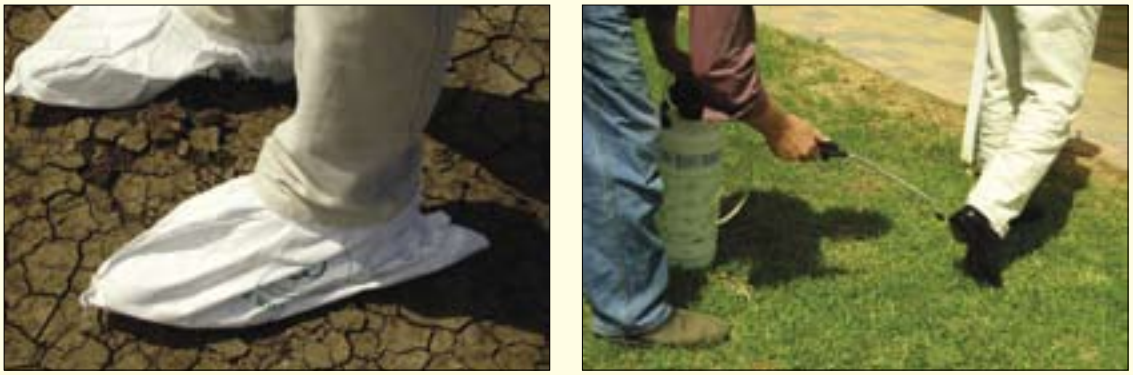

Figure 9.3 Measures for preventing transfer of contaminated soil on footwear: disposable synthetic overshoes (left) and disinfecting shoes after inspecting a crop affected by a pathogen which survives in soil (right)

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.