

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decemcellulare* has been isolated occasionally from branch cankers on longan in northern Vietnam (L. Burgess, unpublished data) and from coffee in Dac Lac province (Dr Tran Kim Loang, pers.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



**Table 10.7** *Fusarium oxysporum* (vascular wilts)

Diseases	<p>Fusarium vascular wilt diseases are caused by formae speciales of <i>F. oxysporum</i>. 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.</p> <p>Some Fusarium vascular wilt pathogens and their associated diseases in Vietnam are:</p>														
	<table border="0"><tr><td><i>F. oxysporum</i> f. sp. <i>cubense</i></td><td>Fusarium wilt of banana (Panama disease)</td></tr><tr><td><i>F. oxysporum</i> f. sp. <i>lycopersici</i></td><td>Fusarium wilt of tomato</td></tr><tr><td><i>F. oxysporum</i> f. sp. <i>pisi</i></td><td>Fusarium wilt of peas</td></tr><tr><td><i>F. oxysporum</i> f. sp. <i>niveum</i></td><td>Fusarium wilt of watermelon</td></tr><tr><td><i>F. oxysporum</i> f. sp. <i>callistephi</i></td><td>Fusarium wilt of asters</td></tr><tr><td><i>F. oxysporum</i> f. sp. <i>zingiberi</i></td><td>Fusarium wilt of ginger</td></tr><tr><td><i>F. oxysporum</i> f. sp. <i>dianthi</i></td><td>Fusarium wilt of carnations</td></tr></table>	<i>F. oxysporum</i> f. sp. <i>cubense</i>	Fusarium wilt of banana (Panama disease)	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	Fusarium wilt of tomato	<i>F. oxysporum</i> f. sp. <i>pisi</i>	Fusarium wilt of peas	<i>F. oxysporum</i> f. sp. <i>niveum</i>	Fusarium wilt of watermelon	<i>F. oxysporum</i> f. sp. <i>callistephi</i>	Fusarium wilt of asters	<i>F. oxysporum</i> f. sp. <i>zingiberi</i>	Fusarium wilt of ginger	<i>F. oxysporum</i> f. sp. <i>dianthi</i>	Fusarium wilt of carnations
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<i>F. oxysporum</i> f. sp. <i>dianthi</i>	Fusarium wilt of carnations														
	<p>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.</p>														
Symptoms	<p>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.</p>														



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	<p><b>Banana</b></p> <p>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.</p> <p><b>Tomato</b></p> <p>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.</p> <p><b>Cucurbits</b></p> <p>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.</p>
Host range	<p>A forma specialis usually causes vascular wilt in only a single host species. For example, <i>F. oxysporum</i> f. sp. <i>niveum</i> causes wilt of watermelon.</p>
Weather	<p>Fusarium vascular wilt diseases are usually more severe in warm, wet conditions.</p>
Overseasoning	<p>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.</p>
Infection	<p>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.</p> <p>Fusarium wilt diseases are commonly associated with root knot nematodes. The <i>Fusarium</i> infects through wound sites made by the nematode.</p>

Control	<p>Fusarium wilt diseases are difficult to control as the chlamyospores persist for long periods in soil.</p> <p>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 chlamyospores in soil.</p> <p>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.</p> <p>There are no effective fungicide treatments.</p>
Isolation	<p>Fusarium wilt pathogens can be readily isolated from infected stem tissue (Section 6.3.1), using a <i>Fusarium</i>-selective medium (PPA) or WA. Isolation should be from stems with early wilt symptoms.</p>

### 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
- chlamyospores (formed after 2–3 weeks)



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

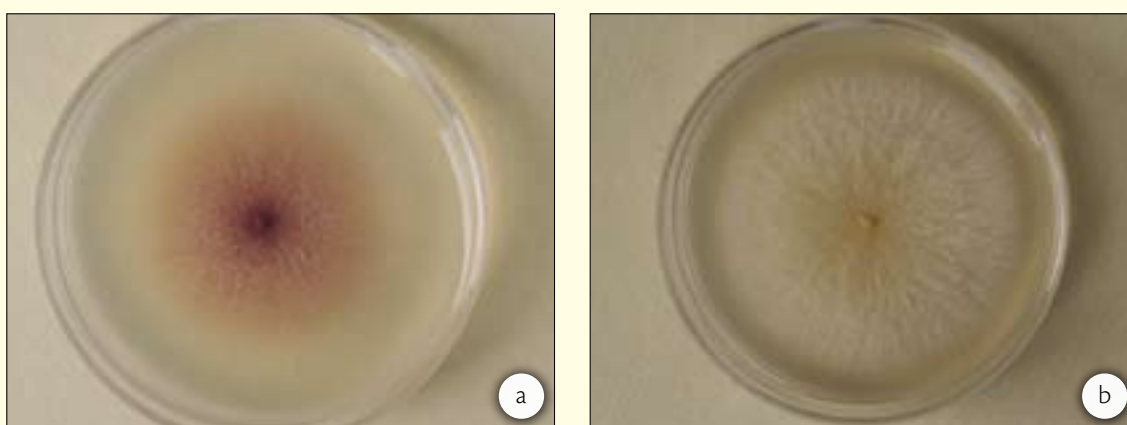
On PDA *F. oxysporum* produces:

- 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

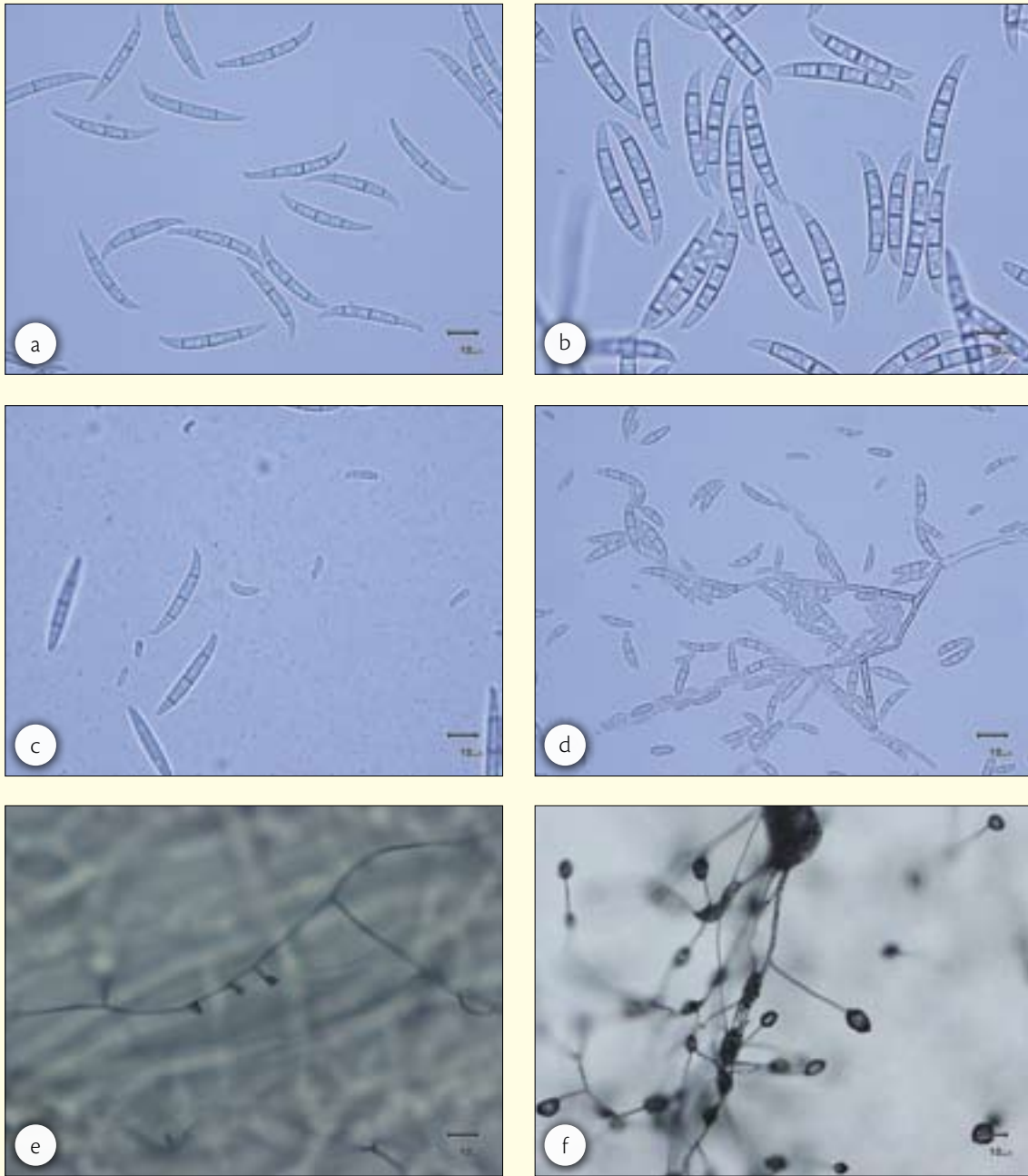
Isolates of *Fusarium oxysporum* and *F. solani* can be difficult to distinguish by inexperienced researchers (Table 10.9 and Figures 10.14–10.16). *F. oxysporum* mainly causes vascular wilt diseases, while *F. solani* mainly causes collar and root rots.

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.

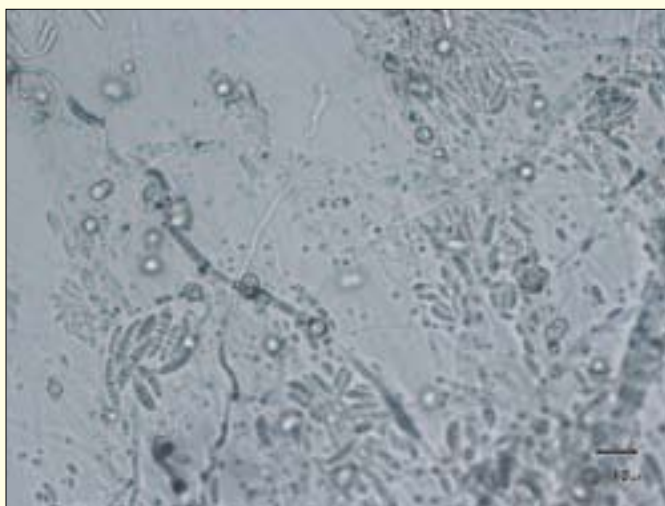


**Figure 10.15** Differentiating between *Fusarium oxysporum* (left) and *F. solani* (right): (a) and (b) macroconidia, (c) and (d) microconidia and some macroconidia, (e) and (f) microconidia in false heads on phialides (note the short phialide in *F. oxysporum* and the long phialide typical of *F.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



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

	<i>F. oxysporum</i>	<i>F. solani</i>
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 <i>F. oxysporum</i>
	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

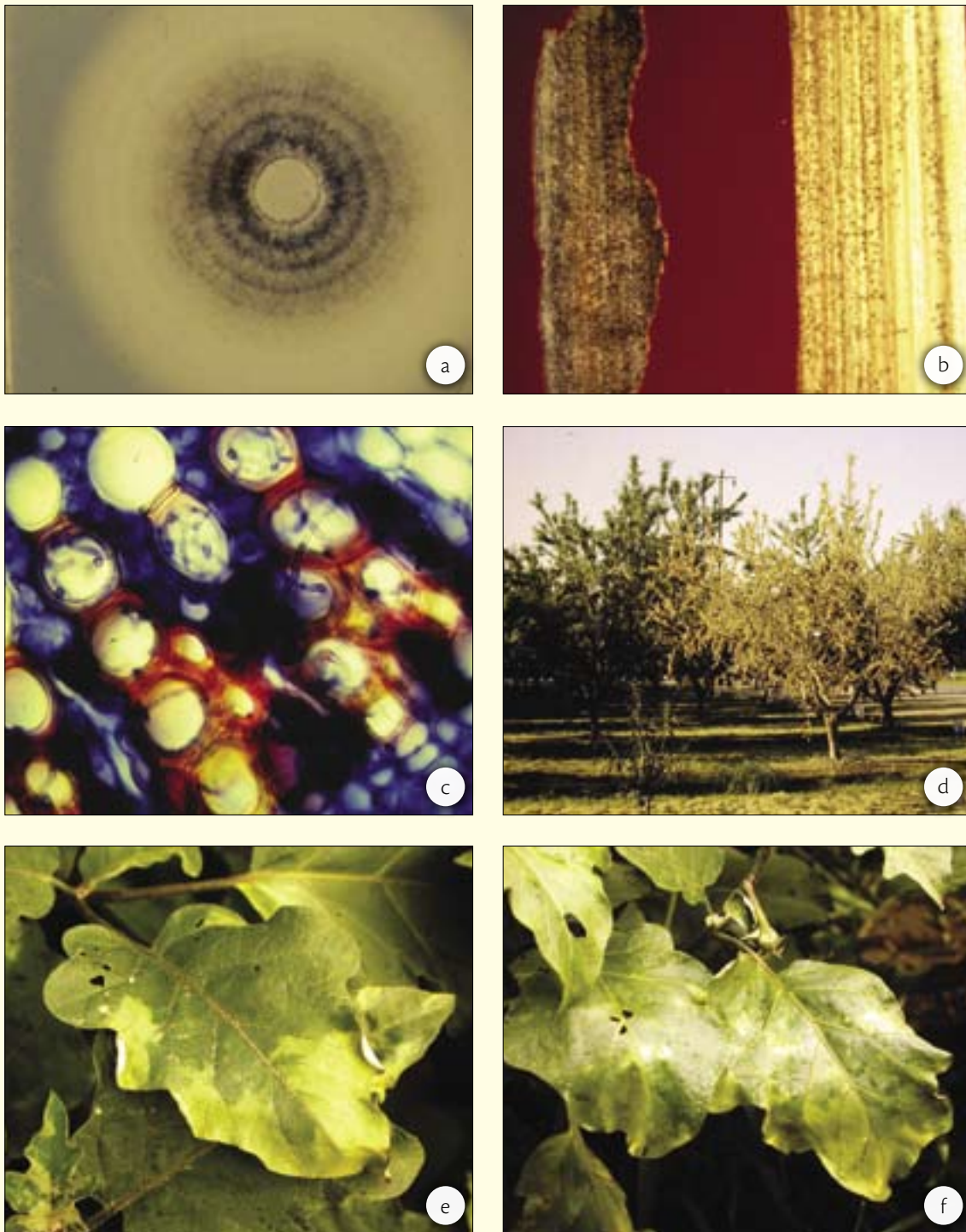


**Figure 10.16** Chlamydospores of *Fusarium solani* in culture on carnation leaf agar (CLA) (*F. oxysporum* chlamydospores look the same)

## 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.



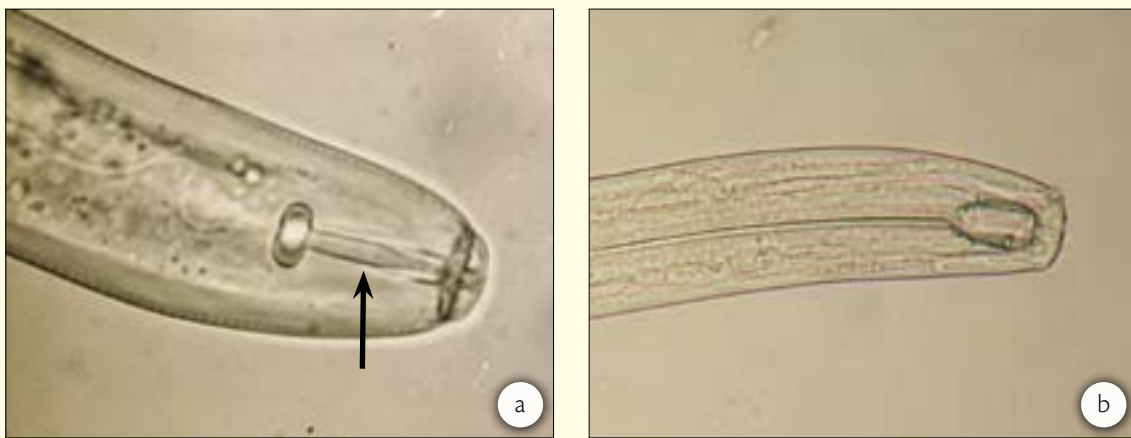
**Figure 10.17** *Verticillium dahliae*: (a) culture on potato dextrose agar (cultures grow slowly), (b) microsclerotia on old cotton stem, (c) hyphae in infected xylem vessels, (d) wilted pistachio tree affected by *V. dahliae*, (e) and (f) wilted leaves of eggplant infected by *V. dahliae*

**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-leaved (dicotyledonous) plants including tomato, potato, cotton, cucurbits, strawberry and some temperate fruit crops such as almonds and walnuts.
Overseasoning	<i>Verticillium albo-atrum</i> survives as hyphae in host residues. <i>V. dahliae</i> 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	<p><i>V. albo-atrum</i> and <i>V. dahliae</i> 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.</p> <ol style="list-style-type: none"> <li>1. Surface sterilise stem sections for 1 minute in 70% ethyl alcohol.</li> <li>2. Dry on paper tissue.</li> <li>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).</li> <li>4. Subculture, purify by single spore technique and grow on PDA and green rice stem piece agar.</li> </ol> <p><i>V. dahliae</i> will produce black microsclerotia in culture, especially on pieces of straw or sterile host root fragments.</p> <p><b>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.</b></p>

## 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.



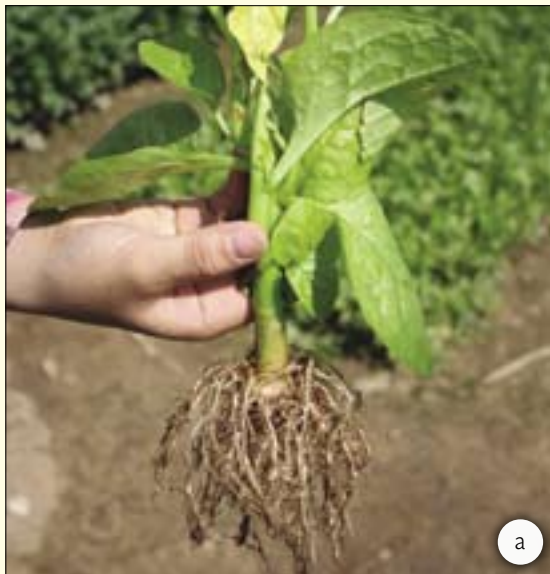
**Figure 10.18** Nematodes: (a) plant parasitic with piercing stylet (mouth spear), (b) non-plant parasitic with no stylet

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).

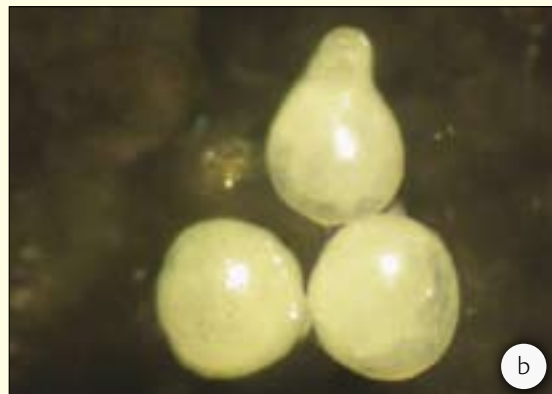
Cyst nematodes infect roots, causing root proliferation (a cluster of small roots). The female cyst is obvious, being attached to the outside of the root at the point of root proliferation.

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.

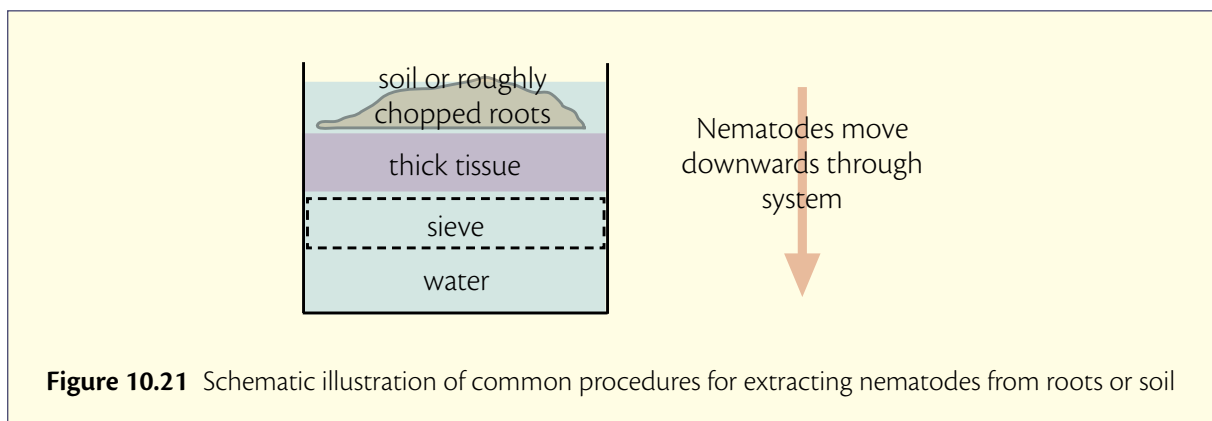
### 10.7.1 Nematode extraction from soil and small roots.

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.

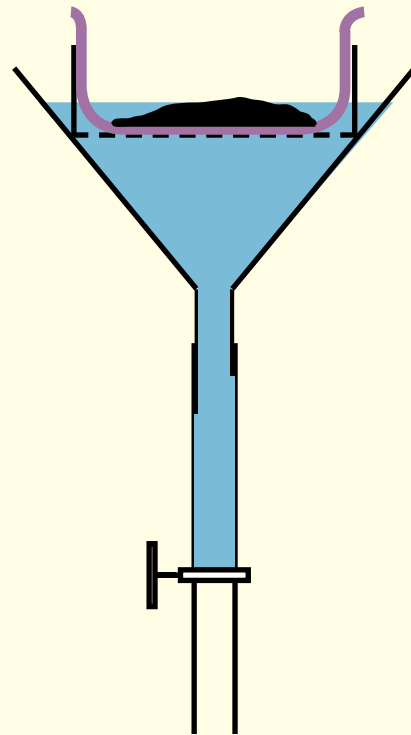
1. Take a small subsample from a composite, well-mixed soil sample from the root zones of 10 plants.
2. Pour the subsample gently into the water in a glass or plastic funnel containing paper tissue, which is supported by a plastic sieve with small (1 mm) mesh. Do not tear the tissue.



**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.

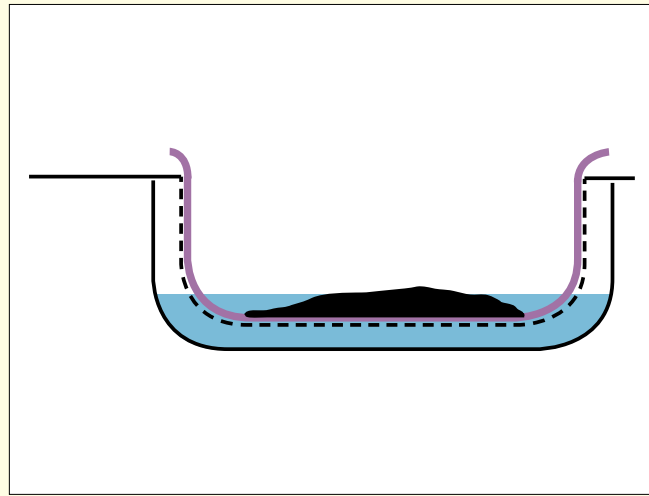
4. After 24 hours, pour the water into a glass beaker or jar and allow the nematodes to settle to the bottom of the jar.
5. Pipette the water from the bottom of the jar into a small Petri plate for examination under the dissecting microscope.

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  $\times 100$  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

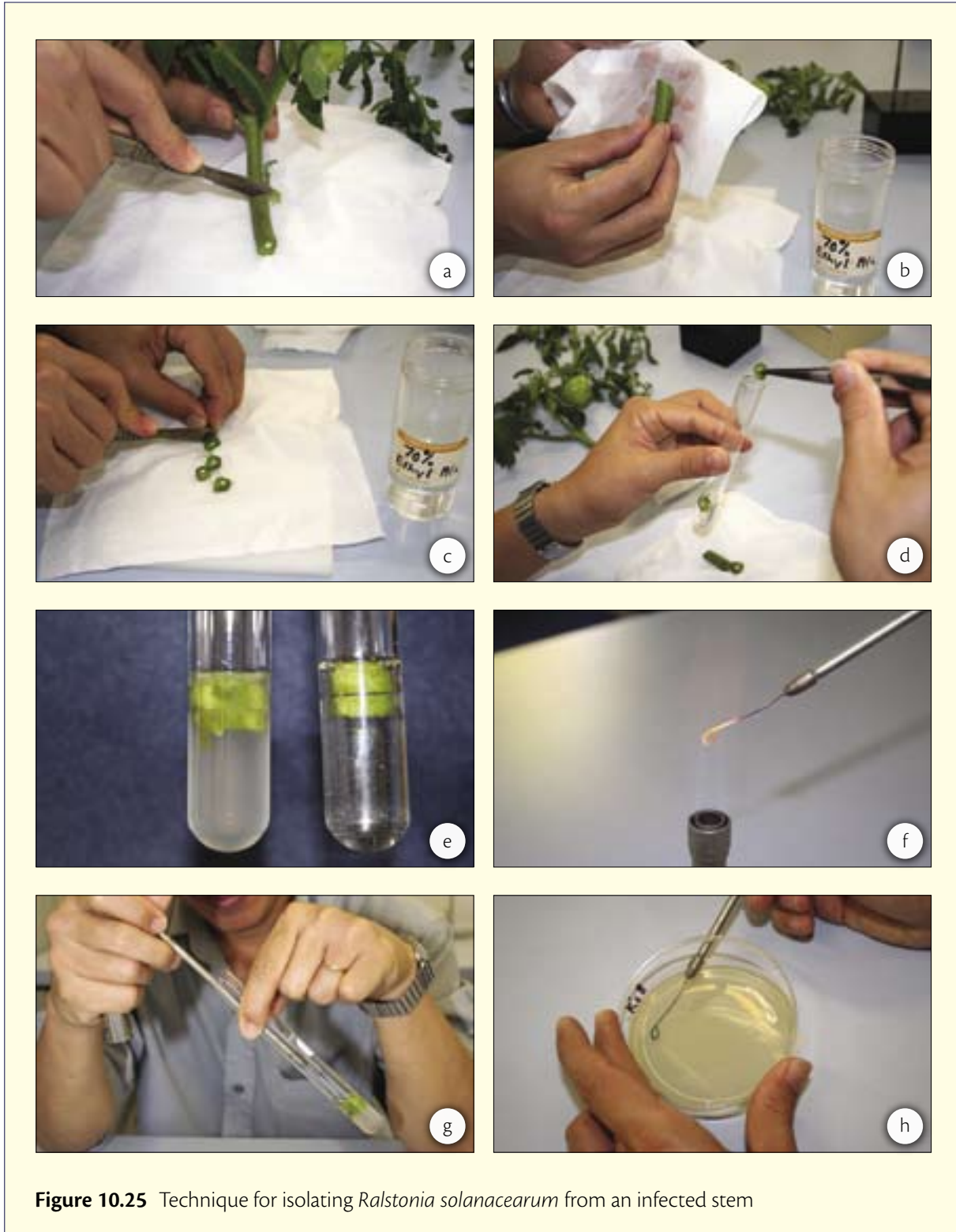
Bacterial wilt and bacterial leaf spots and blights are common in Vietnam. Many of these pathogens can be isolated and purified in a basic laboratory. Pure cultures can then be tested for pathogenicity using Koch's postulates (see Box 8.1). If cultures are pathogenic they can be sent to a bacteriology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

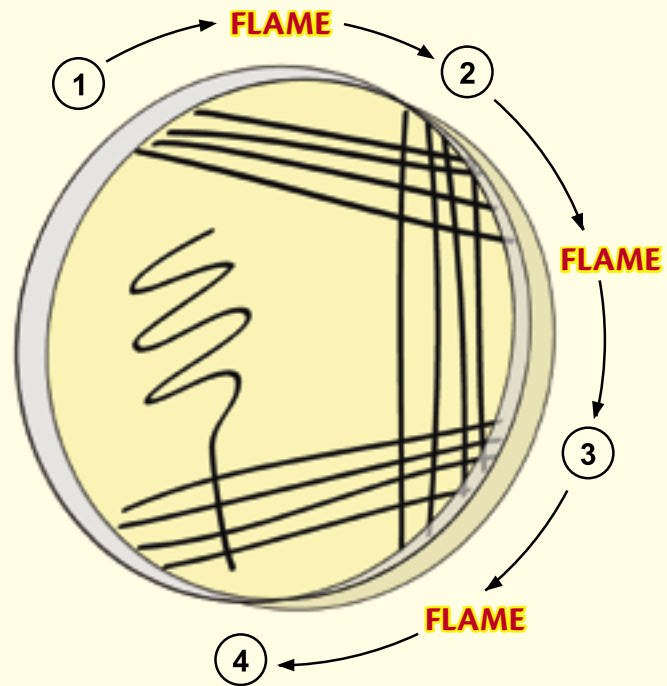
#### Isolation procedure for *Ralstonia solanacearum*, the cause of bacterial wilt

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.

- 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



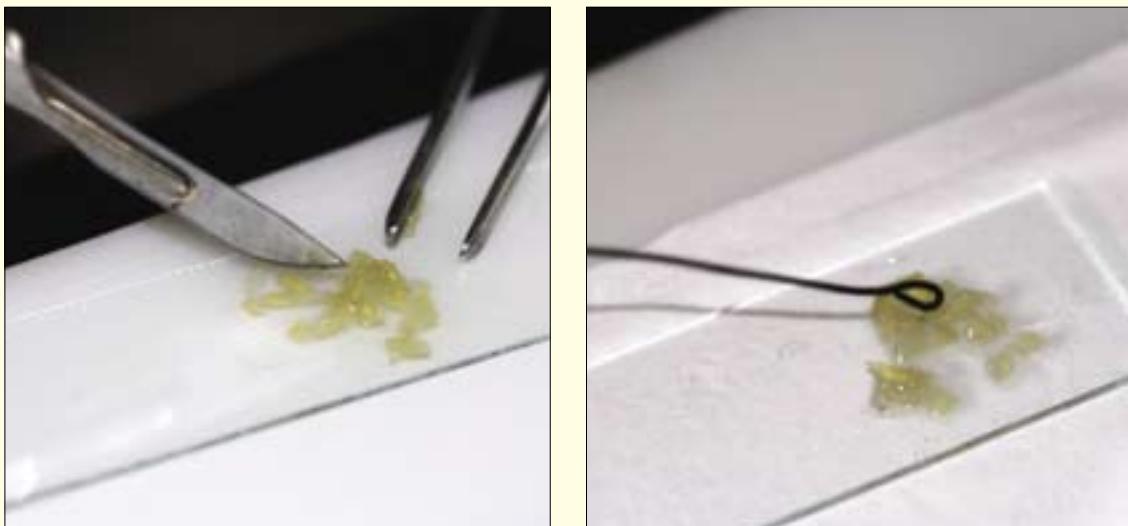
**Figure 10.27** Bacterial streak plate after 2 days growth at 25 °C

### 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 ( $\times 10$  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 mosaic 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.