6 Entry and Colonization of the Host

'Whenever the little seeds of rust come to rest upon the same stalk, finding some open mouths of the exhaling vessels, there they enchase their minute radical fibers, and there they infiltrate in such a manner, that they graft into the tender and delicate arteries peculiar to the plant.' [G. Targioni-Tozzetti, 1712–1783]

To gain access to host nutrients and establish a parasitic relationship, the microorganism must first pass through the external protective layers of the host. Plant pathogens enter their hosts in a variety of ways. Some penetrate directly through the intact surface covering of the plant. Others pass through natural openings, or through regions where the external defences are especially thin. The most important such route is through the stomata, but other zones of weakness include glands, hydathodes, lenticels, nectaries and root tips. Many other pathogens enter the host via wounds resulting from physical or chemical damage, or from the activities of animal pests. Wounds can also be self-inflicted, for instance by the abscission of leaves or during the emergence of lateral roots.

A distinction may therefore be drawn between pathogens which enter directly through the protective barriers of the host, and those which bypass these defences (Fig. 6.1). Several major groups of pathogens, including the viruses, phytoplasmas and many fungi causing post-harvest diseases of fruit, are almost entirely dependent upon wounds to gain entry to the host.

The entry route is important in determining the nature of the initial host–pathogen interface formed. For instance, bacterial cells washed through stomata by rain can multiply initially in intercellular spaces, while pathogens penetrating directly through the host epidermis must cross cell walls and often grow within host cells. These differences will affect the types of nutrients available to the pathogen, and also the molecular events involved in recognition of the pathogen by the host.

The infection court

Let us assume that a pathogen has been successfully dispersed or has grown into contact with a potential host plant. Subsequent development on the surface of the host, penetration into the host and the very early stages of establishment within host tissues comprise the process of **infection**. This stage in the life cycle of the pathogen ends when the organism becomes dependent on the host for nutrients, at which point it begins to **colonize** tissues around the initial site of infection.

The initial site of contact between the pathogen and the surface of the host is described as the **infection court**. In any discussion of host penetration it is useful to distinguish at the outset between the aerial and subterranean surfaces of the plant. In one respect the problems confronting airborne and soil-borne pathogens are similar, in that both must breach the outer defensive layers of the host, but there are major differences between the two environments. Soil exerts a buffering effect against extremes of temperature, water availability, and other environmental fluctuations. A propagule landing on an aerial plant surface is exposed to wide daily fluctuations in temperature and hazards such as desiccation.

The leaf and root surfaces of plants are termed the **phylloplane** and the **rhizoplane**, respectively. The allied terms **phyllosphere** and **rhizosphere** describe the habitats adjacent to these surfaces. In recent years a great deal has been learned regarding the influence of physical, chemical and biological factors on pathogen behaviour in these two infection courts. Factors influencing the germination of fungal spores are of special significance and include humidity, dura-

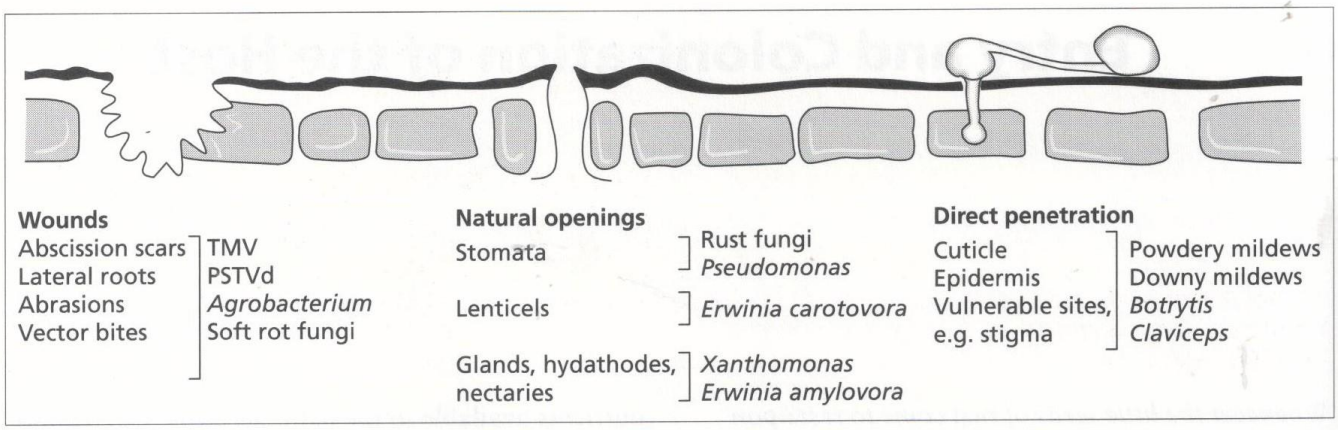


Fig. 6.1 Some entry routes for plant pathogens. TMV, tobacco mosaic virus; PSTVd, potato spindle tuber viroid.

tion of leaf surface wetness, temperature, light, pH, nutrient availability and the quality and quantity of host exudates. Exudates from leaves and roots contain numerous chemicals such as sugars, amino acids, mineral salts, phenols and alkaloids; any of these may stimulate or inhibit germination and/or growth of pathogens. Root exudates are particularly significant in determining the behaviour of soil fungi which produce motile zoospores. These are chemotactically attracted to the elongating zone of host roots where they encyst prior to entry. The initial phases of development on the host surface would seem to represent an especially vulnerable stage in the life cycle of fungal pathogens, as witnessed by the efficacy of protectant fungicides in the control of many diseases.

The principal components of the aerial surfaces of herbaceous plants are summarized in Fig. 6.2. In practice, there are considerable physical and chemical differences between the outer layers of various plant species, and even between different parts of the same plant. Thus the cuticle may vary in chemical composition and thickness on leaves, flowers and fruits. Variations are also found in different regions of the same organ, for example between the upper (adaxial) and lower (abaxial) surfaces of leaves. Other factors influencing the structure and composition of external layers include the conditions under which the plant has grown, and the developmental stage that the plant has reached. Seedling tissues are particularly prone to infection by opportunist pathogens, such as the 'damping-off' fungus *Pythium*, whereas mature plants are seldom attacked. A critical factor here is the relative ease with which the pathogen can penetrate the cuticle and epidermal layers in the young plant.

The outer cell walls of primary roots are usually impregnated with lipid materials, including suberin and cutin. These form a definite membrane comparable with the leaf cuticle, but the use of the term *'cuticle' to describe this root covering is not accepted* by some scientists. There is also some doubt as to whether such protective layers are present in the physiologically active apical region of the root. The root-hair zone is especially vulnerable to pathogens, as it is in intimate contact with a large volume of soil. The necessity for efficient water and nutrient uptake by the root hairs means that mechanical barriers, which would perhaps deter pathogens, are absent. Root-cap cells secrete a mucilaginous gel which encloses the growing root and is a distinctive feature of the rhizosphere.

There are even greater differences between the surfaces of herbaceous tissues and the stems and roots of woody perennials. Periderm, commonly termed bark, is formed following secondary thickening and the accompanying increase in girth of the organs. It comprises three layers, with the outermost being composed of dead cork cells which have suberized walls. Suberin is a complex material containing mixtures of hydroxy acids and it is very resistant to microbial attack. This substance, together with lignin and cellulose in cork cell walls and resins in their lumina, ensures that bark is virtually impregnable to invasion by microorganisms. Similar protective layers also form over abscission wounds and damaged tissues which are exposed when large branches are broken off in storms.

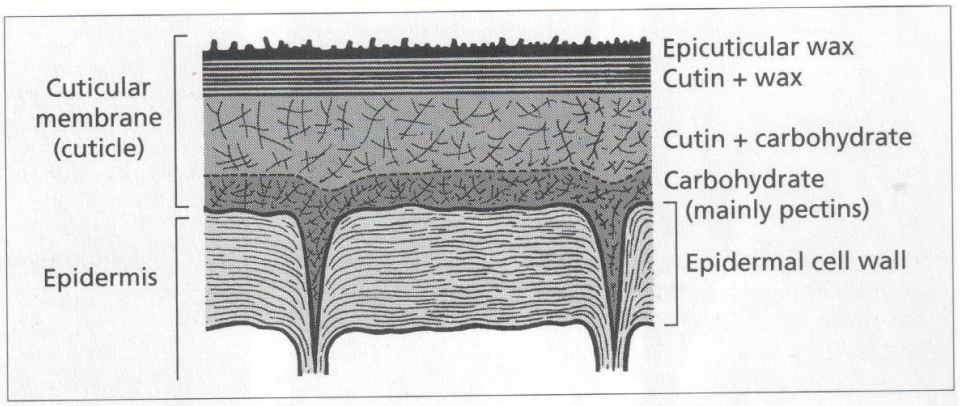


Fig. 6.2 The external layers bounding herbaceous plant organs. (After Jeffree *et al.* 1976.)

Adhesion

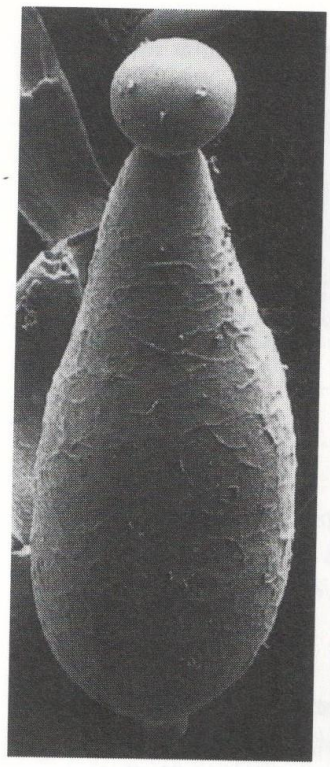
For airborne, wind- or splash-dispersed spores the first problem is effective adhesion to the host surface. Epicuticular wax is hydrophobic and repels water droplets and any microbial propagules they contain; firm attachment is essential to prevent the pathogen losing contact prior to infection. Molecules aiding microbial attachment are often described as **adhesins**. Spores of many pathogenic fungi produce an extracellular matrix which surrounds the spore and binds tightly to plant cuticles, as well as to inert hydrophobic surfaces such as Teflon, the coating of non-stick saucepans. This matrix may also protect spores from desiccation and provide a medium for immobilization of secreted enzymes. In the rice blast pathogen, *Magnaporthe grisea*, mucilage is released from the apex of conidia following hydration (Fig. 6.3a,b), serving as a form of biological 'glue'. Other pathogens which produce dry spores and which adhere to dry leaf surfaces, such as the rust and powdery mildew fungi, probably employ electrostatic mechanisms as well as adhesive materials to ensure attachment. Following contact with the host, conidia of the powdery mildew *Erysiphe graminis*, produce a small, short hypha (Fig. 6.4a). This primary germ tube helps to anchor the spore to the host surface, but may also be important in taking up water to aid pathogen growth.

Bacterial pathogens also synthesize extracellular molecules which promote adhesion. With animal-pathogenic species, small hairlike processes known as fimbriae are important in attaching cells to epithelial surfaces. Similar proteins are known to bind to plant cell walls, but their significance in plant infection is not clear. The crown gall pathogen, *Agrobacterium tumefaciens*, elaborates cellulose microfibrils which help to secure the bacterium to host cells, as well as binding further bacteria.

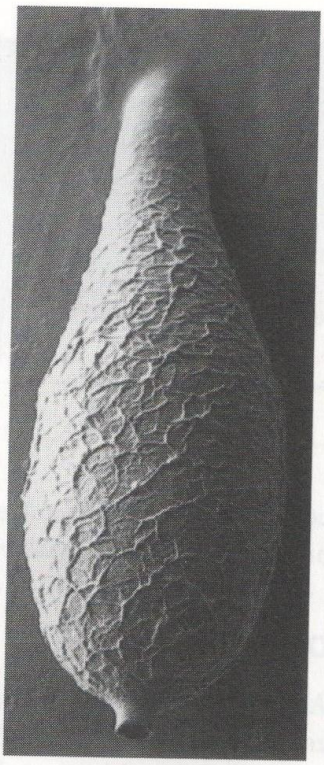
Direct penetration

As shown in Fig. 6.2, direct penetration of herbaceous tissues requires entry through layers of wax, cutin, pectin and a network of cellulose fibrils impregnated with other wall polymers, before the pathogen makes contact with host protoplasm. This would seem to be a formidable obstacle. Nevertheless, many fungal pathogens are able to enter their hosts in this way. Biotrophic fungi, such as the rusts and the downy and powdery mildews, often gain access by growing down into the epidermis, but direct penetration is by no means restricted to this type of pathogen. Even necrotrophic fungi, such as *Botrytis*, can in suitable circumstances enter hosts directly by penetration through the cuticle.

Direct penetration of the host by fungi is frequently associated with the development of hyphal modifications known collectively as infection structures. Some examples are shown in Figs 6.3–6.5. Once the spore has germinated there follows a period of growth in which the germ tube extends over the leaf surface. This growth may appear to be random but some evidence points to the possibility that the germ tube is 'searching' for a favourable site for penetration (Fig. 6.4b). The length of germ tube developed varies but eventually extension growth ceases and the tip of the hypha swells to form an **appressorium**. This spherical or ovoid structure increases the area of contact and attachment between the fungus and the host surface (Fig. 6.3d). Penetration then takes place by the downward growth of a narrow hyphal thread or infection peg formed from the lower surface of the appressorium. There has been much debate as to the



(a)



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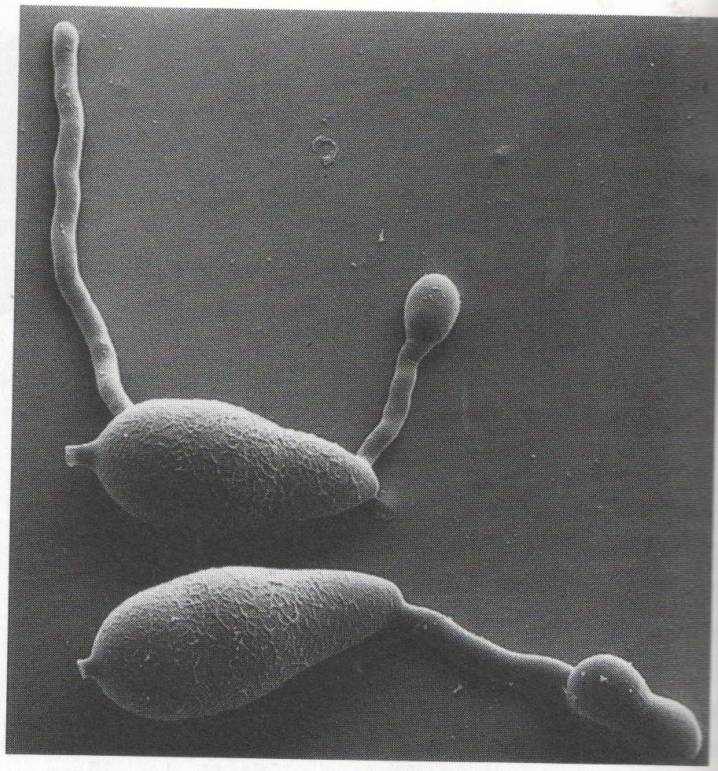

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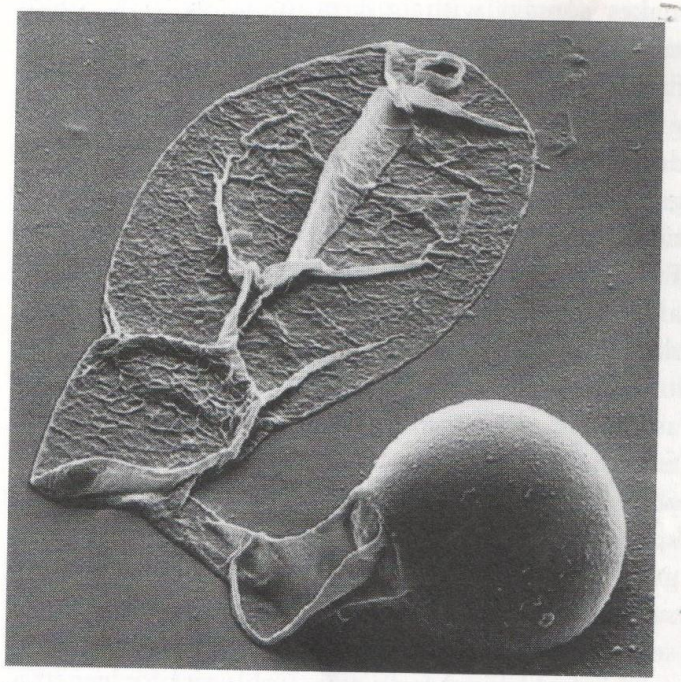
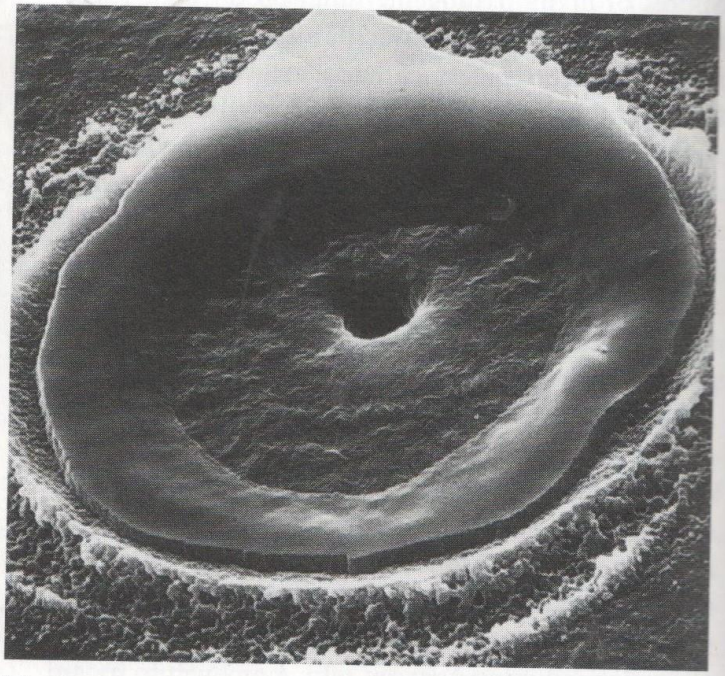


(d)



(e)

Fig. 6.3 Early infection stages of the rice blast fungus, *Magnaporthe grisea*. (a) Conidium with apical droplet of spore tip mucilage (×2900). (b) Conidium attached to substrate by adhesion of spore tip mucilage (×2900). (c) Germination of conidia and early stages of appressorium development, seen as swelling of germ tube apex (centre and lower right) (×1100). (d) Mature, globose, turgid appressorium attached to collapsed germ tube and conidium. A septum separates the appressorium from the germ tube (×3000). (e) Remnants of appressorium attached to a polyethylene surface. The upper part of the cell has been removed by sonication. What remains is the appressorial pore, with the dent made by mechanical force of the penetration peg clearly visible, and part of the smooth surrounding wall, composed of melanin. Note the halo of extracellular matrix material around the attachment site (×15 500). (a, d & e, From Braun & Howard 1994; b & c, from Howard 1994.)

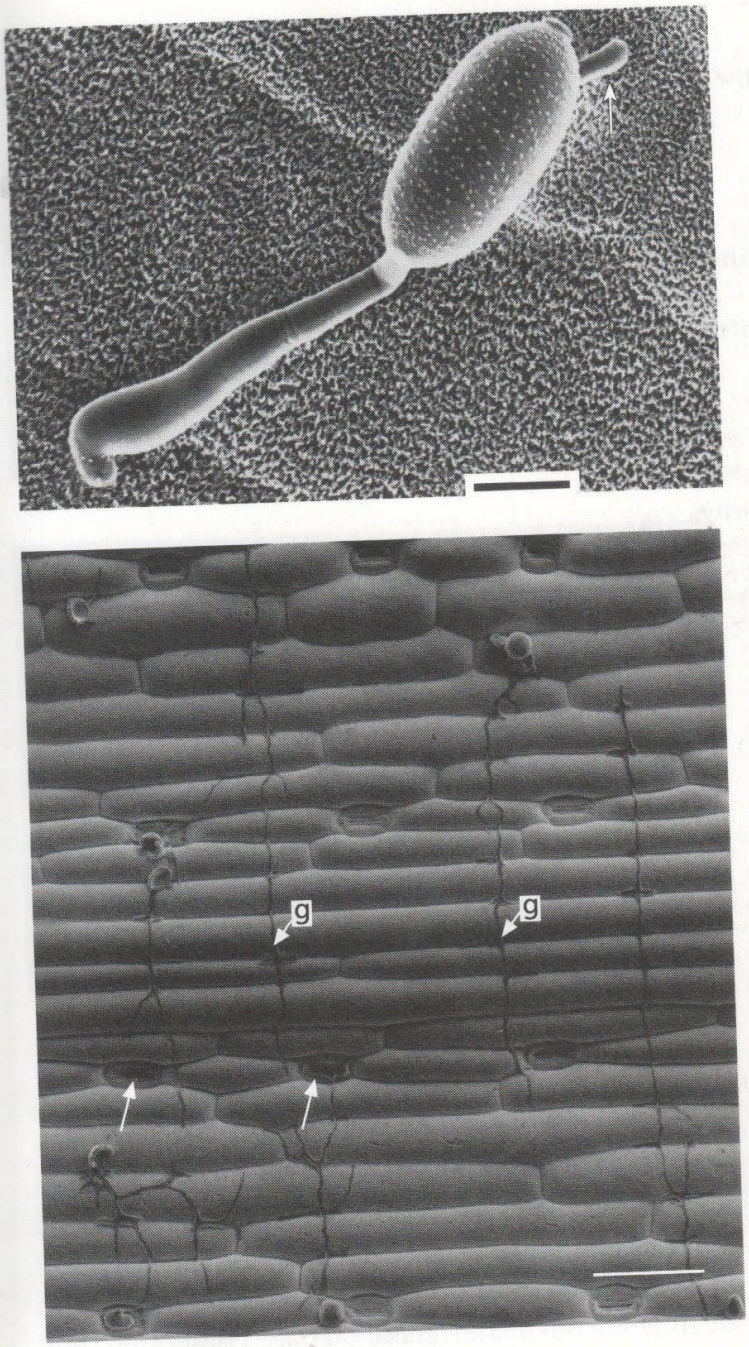


Fig. 6.4 Early development of fungal pathogens on the surface of barley leaves viewed by scanning electron microscopy. (a) Germinating spore of the powdery mildew *Erysiphe graminis* showing small, primary germ tube (arrow) and larger appressorial germ tube with hooklike tip. Scale bar = 10 µm. (Courtesy of Tim Carver.) (b) Germ tubes (g) of brown rust (*Puccinia hordei*) showing growth perpendicular to the orientation of epidermal cells, short branches formed at cell junctions, and appressoria (arrows) formed over stomata, which occur in rows. Scale bar = 100 µm. (From Read *et al.* 1992.)

actual mechanics of penetration. Early workers showed that many fungi will successfully penetrate artificial materials such as gold leaf, suggesting that the process is entirely mechanical. However, scanning and transmission electron microscope studies suggest that some pathogens degrade the cuticle and cell-wall polymers during penetration. Differential staining techniques have revealed localized dissolution of the cuticle and cell walls around infection pegs, implicating the action of hydrolytic enzymes in penetration by fungi.

The mechanisms employed by pathogenic fungi to penetrate plant surfaces have recently been analysed using elegant molecular and genetic techniques. Several fungi are known to produce cutinase, an enzyme able to degrade cutin; cutinase from the pea pathogen *Fusarium solani* f.sp. *pisi* was purified and used to raise antibodies specific for the enzyme. Such antibodies can be utilized either to detect production of the enzyme, for instance by immunolabelling in electron micrographs, or to inhibit enzyme activity. A combination of these approaches suggested that cutinase is a vital factor in breaching the host surface (Table 6.1). The gene coding for cutinase in *F. solani* has now been cloned and sequenced, and its regulation studied in detail. In germinating spores, cutinase synthesis is induced by breakdown products of cutin. Low levels of constitutive activity release cutin fragments which induce rapid expression of the cutinase gene. This system of regulation ensures that the enzyme is only synthesized in any quantity when pathogen spores contact a plant surface. Further evidence that cutinase is required for direct penetration has been obtained by introducing the *Fusarium* cutinase gene by transformation into another fungus, *Mycosphaerella*, which normally requires a wound to infect the host. Possession of the cutinase gene enabled the transformants to penetrate intact host surfaces.

More recently this apparently conclusive story has been questioned following experiments using a technique known as gene disruption. In this procedure the functional gene for cutinase was replaced by a defective copy unable to produce the enzyme. Fungal transformants containing the disrupted gene, and in which cutinase synthesis was abolished were still pathogenic to pea seedlings, and apparently able to penetrate the intact host surface. It is difficult to reconcile these conflicting results, as molecular genetic evidence suggests that there is only a single copy of the cutinase gene in the pathogen, and no detectable enzyme activity was present in the pathogenic transformants containing a defective gene.

The possibility that fungal enzymes such as cuti-

Evidence that cutinase is required for host penetration
Immunolocalization shows that the enzyme is present at the site of penetration
Antibodies against cutinase prevent infection
Chemical inhibitors of cutinase prevent infection
Mutants lacking cutinase activity are non-pathogenic
Wounding of the plant surface restores pathogenicity of cutinase^{-ve} mutants
Insertion of the cutinase gene into a wound pathogen confers ability to penetrate intact surface
Evidence that cutinase is not required
Gene replacement, to disrupt a single cutinase gene, abolishes cutinase mRNA and enzyme activity, but does not alter pathogenicity

Table 6.1 The cutinase debate.

nase might be required for direct host entry does not preclude a role for mechanical forces in penetration. Confirmation of the importance of such forces has come, perhaps unexpectedly, from studies on the mode of action of certain fungicides. The compound tricyclazole (Table 11.2) effectively controls several fungi, including *Magnaporthe grisea*. This fungus penetrates rice plants directly from dark, pigmented appressoria; appressoria formed in the presence of the fungicide are non-pigmented, and no penetration takes place from them. The biochemical target of tricyclazole turns out to be melanin synthesis, so that production of the pigment is inhibited in treated appressoria. Normally melanin is deposited in the appressorial wall (Fig. 6.3e), making it rigid and impermeable to solutes. As the appressorium matures, hydrostatic pressure builds up inside until sufficient force is generated to push the infection peg down through the cuticle. Functional appressoria formed on inert plastic surfaces actually leave a microscopic dent at the point where the peg projects (Fig. 6.3e). In non-melanized appressoria the wall remains relatively thin, flexible and permeable, and the infection peg appears unable to breach the surface. Albino mutants of *M. grisea* which are unable to synthesize melanin are similarly incapable of achieving penetration from non-pigmented appressoria. Thus in this, and other similar pathogens with pigmented appressoria, mechanical force would appear to be the primary means for penetrating the host.

Root-infecting fungi also form infection structures which are generally more complex than those produced by fungi attacking aerial tissues. Some isolates of *Gaeumannomyces* produce appressoria in the form of short side branches from runner hyphae, beneath which narrow penetration hyphae enter the root cortex. *Rhizoctonia solani*, a versatile pathogen attacking a wide variety of hosts, forms both lobed appressoria and more complex aggregations of repeatedly branched hyphae, called infection cushions. The former tend to be produced on aerial tissues and the latter on roots and other subterranean organs. Multiple infection hyphae are produced from the lower surface of infection cushions, and enter the host. The eyespot fungus, *Pseudocercosporella* (*Tapesia*) *herpotrichoides*, infects the stem base of wheat by colonizing the coleoptile and then penetrating through successive leaf sheaths. Multicellular plates of mycelium, termed infection plaques, are produced on the surface of each leaf sheaf (Fig. 6.5), and these act as compound appressoria enabling the fungus to penetrate epidermal cells at numerous sites. Fungi attacking perennial hosts, such as trees, in which the surface is protected by a layer of bark or periderm, often infect from compound structures. When rhizomorphs of *Armillaria mellea* encounter a suitable host the concerted action of the numerous hyphae comprising these strands (see p. 32) is often sufficient to penetrate the intact surface layers.

It was noted above that the root hair zone is particularly vulnerable to invasion by pathogens. *Plasmodiophora brassicae* enters root hairs at an early stage in its life cycle (it subsequently returns to the soil, re-enters the root epidermis and proliferates in cortical cells causing the club root symptoms). The mode of entry of *Plasmodiophora* into root hairs

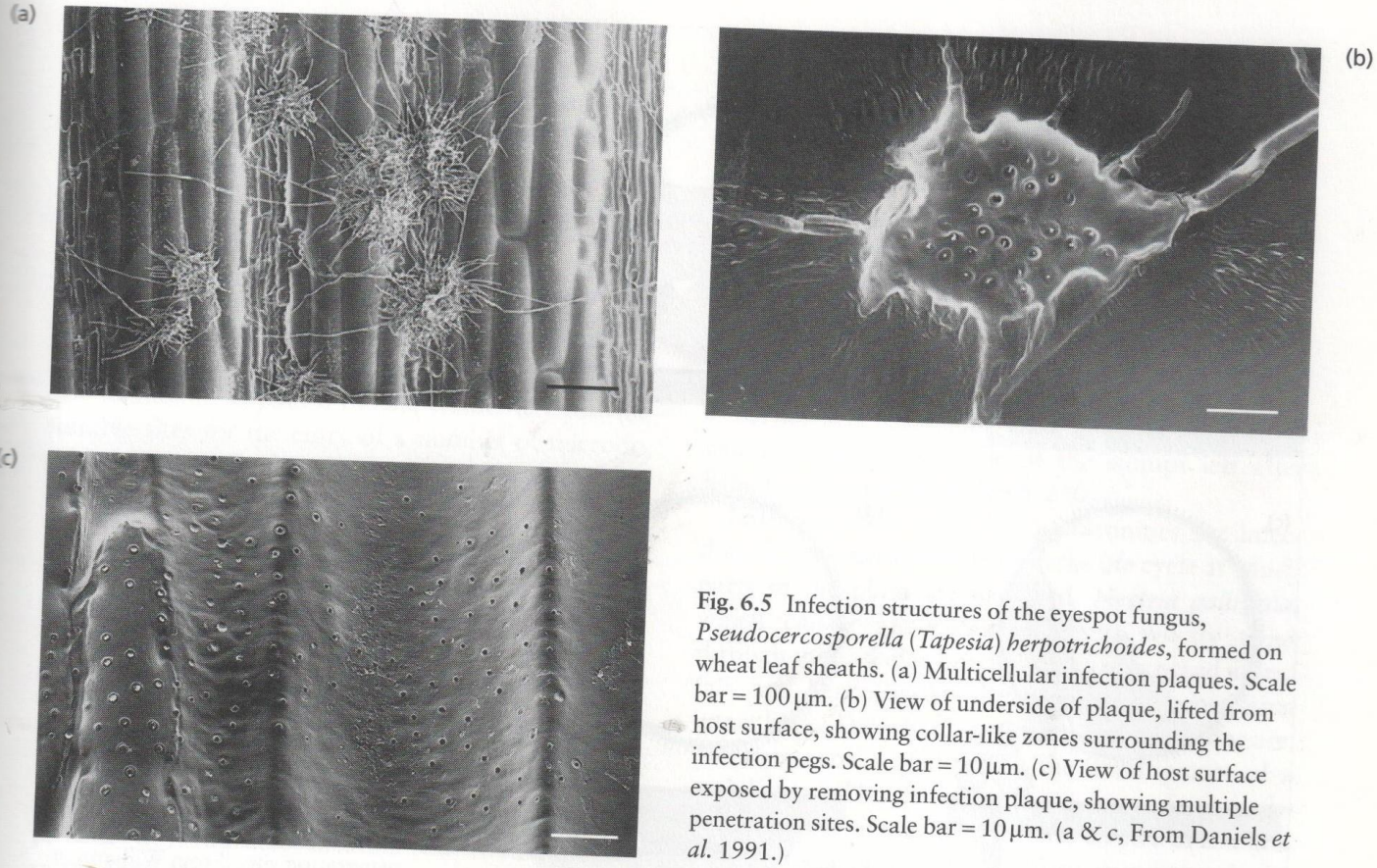


Fig. 6.5 Infection structures of the eyespot fungus, *Pseudocercosporella* (*Tapesia*) *herpotrichoides*, formed on wheat leaf sheaths. (a) Multicellular infection plaques. Scale bar = 100 µm. (b) View of underside of plaque, lifted from host surface, showing collar-like zones surrounding the infection pegs. Scale bar = 10 µm. (c) View of host surface exposed by removing infection plaque, showing multiple penetration sites. Scale bar = 10 µm. (a & c, From Daniels *et al.* 1991.)

appears to be unique. Zoospores of the pathogen encyst on the root hair wall. Entry begins when a bullet-shaped structure is suddenly forced from within the cyst through the wall into the root hair cell. The contents of the spore are then rapidly injected through the resulting puncture into the host cell (Fig. 6.6). This is a particularly dramatic example of mechanical penetration, as the actual infection process takes only about a second.

The bacterium *Rhizobium* also initiates infection through root hairs, one of the very few examples of direct penetration by a plant-infecting bacterium, although in this case the relationship is ultimately mutualistic, with the formation of nitrogen-fixing root nodules.

A further interesting example of a vulnerable site exploited by pathogens is the surface of the female organ, the stigma, which is adapted to trap pollen and permit penetration by pollen tubes to ensure fertilization. Several specialized pathogens, notably species of the ergot fungus *Claviceps*, produce airborne spores which germinate on the host stigma to form penetration hyphae which mimic pollen tubes and extend downwards to invade the ovary. The period of susceptibility to infection is brief, due to the short time during which the stigma is receptive to pollination. As was discussed in Chapter 3, some viruses, for example bean mosaic virus, may be transmitted to the ovules of healthy plants through infected pollen. This is a particularly interesting case as the virus takes advantage of a normal event in the life cycle of the plant to circumvent the structural defences of the host.

Penetration through natural openings

Entry through stomata

The surface layers of field-grown plants are rarely free from minor wounds, but even if they were, there are still a number of natural openings through which microbes can enter. The most important of these are stomata, via which many pathogens enter their hosts. The detailed morphology of these structures may determine whether or not infection can occur, as in citrus fruits where the conformation of the cuticle

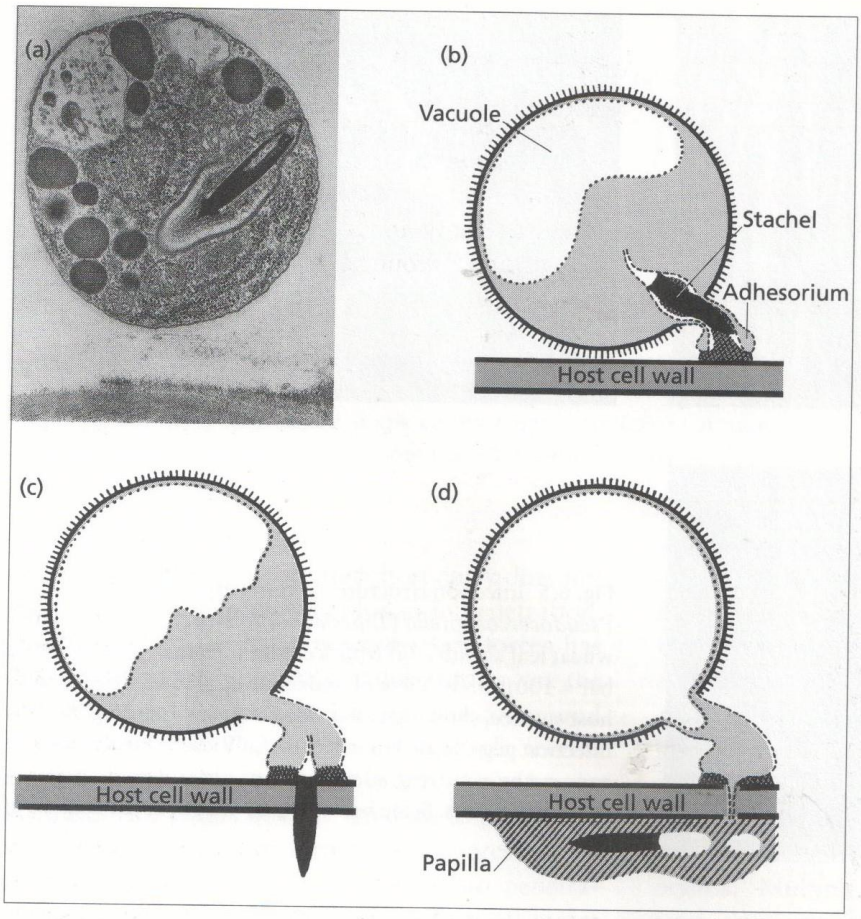


Fig. 6.6 (a) Electron micrograph section of a *Plasmodiophora brassicae* zoospore encysted on a root hair, showing bullet-like stachel (×26 500). (b–d) Diagrammatic summary of the penetration of a root hair: (b) vacuole enlarges and small adhesorium appears; (c) stachel punctures host wall; (d) penetration has occurred and the host protoplast has deposited a papilla at the penetration site. (From Williams *et al.* 1973.)

around the stoma either prevents or allows the passage of water droplets containing the bacterial pathogen *Pseudomonas syringae* pv. *citri*. Stomata are also the main site of entry of several important fungal pathogens. When a rust spore germinates on a cereal leaf the germ tube grows at right angles to the long axis of the leaf (Fig. 6.4b). This orientation of growth, which is an example of **thigmotropism**, is a contact response to the surface topography of cells, as similar tropisms occur on inert plastic replicas of leaves. Experiments with artificial surfaces etched or scratched with precise patterns suggest that the fungus recognizes a repetitive series of ridges spaced at intervals similar to the width of epidermal cells. Hyphal growth across the long axis of the cereal leaf ensures that the germ tube will sooner or later encounter a stoma, as these occur in longitudinal rows (Fig. 6.4b). Once a stoma is contacted the rust germ tube differentiates an appressorium and an infection hypha enters the substomatal cavity. With many rust fungi the signal for appressorium formation appears to be the shape of the stomatal guard cell, and in particular the stomatal lip. Bean rust, *Uromyces appendiculatus*, produces appressoria in response to small ridges about 0.5 µm in height, which corresponds closely to the dimensions of the stomatal lip of the host plant *Phaseolus*. On non-host leaves, extension growth of the germ tube continues indefinitely until the endogenous nutrient reserves are exhausted, and the germling dies. These precise morphogenetic responses to host surface features indicate that rust fungi possess a sophisticated contact-sensing system which aids location of natural openings for entry.

Several oomycete pathogens, including *Pseudoperonospora* on hops, *Plasmopara* on vines, and *Phytophthora* on potato, produce sporangia which germinate on host leaves by releasing motile zoospores. These zoospores are attracted to stomata where they encyst in a suitable position for their germ tubes to grow immediately between the guard cells. *Pseudoperonospora* zoospores are attracted to

open stomata, but not to closed stomata. This attraction is based in part on recognition of the morphology of the open apertures and partly on a chemical stimulus connected with gaseous photosynthetic metabolites.

Lenticels, hydathodes and nectaries

Lenticels allow gas exchange to occur through bark on woody stems and secondarily thickened roots. These loosely packed openings in the periderm are also abundant on potato tubers, where they provide suitable sites for the entry of a number of microorganisms, especially the common scab pathogen *Streptomyces scabies*.

Glandular tissues which have especially thin surface barriers, such as hydathodes and nectaries, are also exploited by pathogens. Bacterial lesions on leaves often develop at the margin, at sites where water exudes through hydathodes. *Erwinia amylovora*, the bacterium responsible for the destructive fireblight disease of pears and apples, enters through nectaries at the base of flowers. In this case, the sugary secretions of nectar, when diluted by rain, provide a favourable medium for multiplication of the pathogen prior to penetration. Fireblight infections are also prevalent after severe thunderstorms, which suggests that the bacterium takes advantage of minor wounds caused by heavy rainstorms. Rainfall is an important predisposing factor in foliar infection by bacteria, as an external force sufficient to wash cells through natural openings into substomatal cavities and other internal tissues (see p. 51).

Penetration through wounds

For many pathogens, especially bacteria and viruses which are incapable of penetrating plants directly, wounds are the most frequent or only avenue of entry. Wounds are caused by human activities, as well as by natural agencies, including wind, hail, extremes of temperature and light, and by pests. The external barriers of the host may also be broken temporarily as a natural consequence of plant growth and development.

Many agricultural and horticultural practices involve accidental or even deliberate wounding. Grafting, pruning and picking spread pathogens through a crop or create wounds which can be exploited by opportunist fungi and bacteria. Mechanical harvesters often increase the incidence of wounding of plant produce. Post-harvest rots of apples (caused by *Penicillium expansum*) and citrus fruits (caused by *P. digitatum* (Plate 8, facing p. 12) and *P. italicum*) are only important if the fruits are mechanically wounded during harvesting, packing or transport. Many important forest pathogens also enter through wounds. *Heterobasidion annosum*, which is a destructive pathogen of conifers (see Fig. 13.9), normally colonizes wounds caused by high winds, snow or other natural agencies. It has become a particularly damaging pathogen in plantations where it takes advantage of the stumps left after felling as sites for entry (see p. 240).

Leaf abscission provides opportunities for infection, as does any other point in the life cycle at which parts of the plant are detached. *Nectria galligena*, which causes apple canker, enters woody twigs through the vascular bundles that are exposed at leaf fall, and hence avoids the problem of penetrating intact bark. The vascular bundles in the leaf scar are, however, soon sealed off by the development of a cork layer and hence the pathogen must take immediate advantage of the infection sites created at leaf fall. Lateral roots emerge by breaking out through the cortex of the parent root. Lesions caused by root pathogens, such as soil-borne *Phytophthora* species, are often initiated at these sites. Soft rot pathogens of potatoes commonly enter tubers through the scar left during separation of the tuber from the parent plant (see Fig. 7.11).

As well as providing entry sites, wounds may release solutions rich in carbohydrates and amino acids, which stimulate germination of spores, or attract motile bacteria and fungal zoospores. The crown gall bacterium, *Agrobacterium*, is dependent on wounds to initiate tumours; exudates leaking from wounded cells have been shown to contain phenolic compounds such as acetosyringone, which serve as molecular signals activating virulence genes on the Ti plasmid (see Fig. 8.12). This specific recognition-response system ensures that virulence functions are expressed only in the presence of susceptible host cells.

Senescent tissues which remain attached to plants also serve as an entry route, and facilitate the invasion of adjoining healthy tissues by opportunist pathogens. The grey mould pathogen *Botrytis cinerea* colonizes vegetables such as courgettes (Plate 7, facing p. 12) or tomatoes by vegetative growth from

the senescing remains of flowers, causing a disease known as blossom end rot.

Disease lesions may themselves allow the entry of other pathogens. In these instances the host is initially infected by a pathogen which may or may not itself cause serious damage. This pathogen, however, paves the way for more aggressive organisms. Potato late blight lesions in tubers may be exploited by soft rot bacteria such as *Erwinia carotovora* (see Fig. 8.2) which can destroy the tuber much more quickly than the blight fungus itself (see p. 125).

It was noted in Chapter 3 that many pests are also important vectors for plant pathogens. As well as dispersing the pathogen, their feeding activities cause wounds which serve as an entry route. The Dutch elm fungus, *Ophiostoma novo-ulmi*, is introduced directly into sapwood by its vector, the bark beetle. In this example the feeding tunnels not only breach the external protective layers of bark but also provide direct access to the vascular tissues in which the fungus can flourish. An even more elegant means of entry is provided by the aphid vectors of many viruses. The aphid stylet injects the virus into the sieve cells of the host with clinical efficiency, and subsequently the virus can spread freely via the phloem. Soil-borne viruses are often introduced via wounds caused by nematodes or fungal pathogens. The wide range of vectors exploited by viruses is paralleled by a similar variety of infection routes.

In some cases, wounds caused by animal pests can increase the incidence and severity of plant diseases, although the pest itself is not a vector. One of the best-known examples is the interaction between vascular wilt pathogens and nematodes. The fungus *Verticillium dahliae* causes a wilt disease in potatoes known as early dying, characterized by premature senescence of leaves and haulms. The pathogen survives in soil as microsclerotia, and there is a correlation between the number of pathogen propagules present in soil, and the incidence of early dying disease. If, however, the soil also contains significant numbers of nematodes capable of causing lesions on potato roots, the disease is much more severe. The most likely explanation for this synergistic effect is that feeding wounds caused by the nematodes provide enhanced access to the vascular tissues of host roots.

The host–pathogen interface

Once inside the plant, pathogens exhibit a wide variety of modes of growth within host tissues (Table 6.2). The site of contact between a pathogen and host cells is known as the host–pathogen interface. This zone is vital in understanding the nature of different host–pathogen interactions, as it is the site at which nutrient uptake by the parasite occurs, and also where molecular communication between the two partners takes place. It is likely, for instance, that recognition events determining active resistance or susceptibility to infection are initiated at this interface. Three main types of interface can be distinguished (Table 6.2):

1 intercellular, where the pathogen grows outside host cells;
2 partly intracellular, where limited penetration of cells by parasitic structures occurs;
3 intracellular, where growth and development takes place entirely within host cells.

These categories are not absolute as many pathogens which initially grow between host cells subsequently invade them once tissues become moribund.

Intercellular relationships are characteristic of bacteria and fungi (e.g. *Cladosporium fulvum*) that grow between cell walls and through intercellular spaces. Soluble nutrients such as sugars and amino acids are scavenged from the apoplast or released from cell walls through the action of secreted hydrolytic enzymes (see p. 125). Hence, there is no intimate contact with living host protoplasts. Often, host cells are killed in advance of invasion, through the action of enzymes or toxins. With such necrotrophic pathogens some kind of structurally defined interface is short-lived as host cells rapidly disintegrate. However, not all intercellular pathogens are so destructive; fungi such as the apple scab pathogen, *Venturia inaequalis*, grow for an extended period beneath the cuticle of infected leaves or fruits without causing apparent tissue damage (see Fig. 2.2).

Intracellular relationships typically involve a more permanent contact between the partners, and penetrated host cells may remain viable for an extended period of time. In these cases the host–pathogen interface is a living and dynamic zone, often involving the formation of modified membranes or specialized parasitic structures such as haustoria.

Structure and function of haustoria

Many biotrophic fungi form modified hyphae,

Table 6.2 Modes of growth of parasites within host tissues, and interfaces, with examples shown in Fig. 6.9.

Type	Pathogen	Host
Subcuticular	*Rhynchosporium*	Barley
	Venturia	Apple
Intercellular	*Cladosporium fulvum*	Tomato
	Sclerotinia	Bean
	Monilinia	Pear
	Most bacteria	Various
Vascular	*Fusarium*	Various
	Verticillium	Various
	Ophiostoma	Elm
	Some bacteria, phytoplasmas	
Haustorial		
Epiphytic with haustoria	Powdery mildews	Various
Intercellular with haustoria	Rust fungi	Various
	Peronospora	Cruciferae
Intracellular vesicle, with intercellular hyphae and haustoria	*Bremia*	Lettuce
	Phytophthora	Potato
Intracellular		
Vesicle and intracellular hyphae	*Colletotrichum*	Bean
	Pyrenophora	Wheat
Wholly intracellular	*Plasmodiophora*	Cruciferae
	Polymyxa	Cereals, beet
	Viruses	Various

known as haustoria, which enter host cells. Haustoria typically develop from intercellular hyphae as narrow branches which penetrate through the plant cell wall and then expand inside the cell (Fig. 6.7b,c). They are diverse in morphology, ranging from small, club-shaped extensions to much larger, lobed or branched structures (see Fig. 3.2). Other fungi, such as hemibiotrophic species of *Colletotrichum*, form intracellular vesicles (Fig. 6.7a.) and hyphae within initially penetrated cells; these structures have some similarities with haustoria, as the host–pathogen interface is a fungal cell in intimate contact with the host protoplast.

Although haustoria and equivalent structures are formed within plant cells, the host plasma membrane is not penetrated and remains intact as an invagination surrounding the fungal cell (Fig. 6.7b). The interface between host and pathogen is therefore a complex zone comprising the fungal plasma membrane, the fungal cell wall, and an extrahaustorial membrane, or EHM (Fig. 6.8). In addition, there is often an amorphous matrix, probably secreted by the host, between the EHM and the fungal cell wall. Typically, where the haustorial neck breaches the host cell wall, a collar of callose-like material is deposited (Fig. 6.7c). In an incompatible host–pathogen combination this may extend to form a sheath completely encasing the haustorium. Finally, in the majority of haustoria, a discrete, electron-dense ring is visible in the fungal cell wall in the neck region (Figs 6.7b & 6.8). This is not observed in haustoria formed by oomycete pathogens such as the downy mildews and *Albugo* (Fig. 6.7c).

The structure and location of haustoria, which

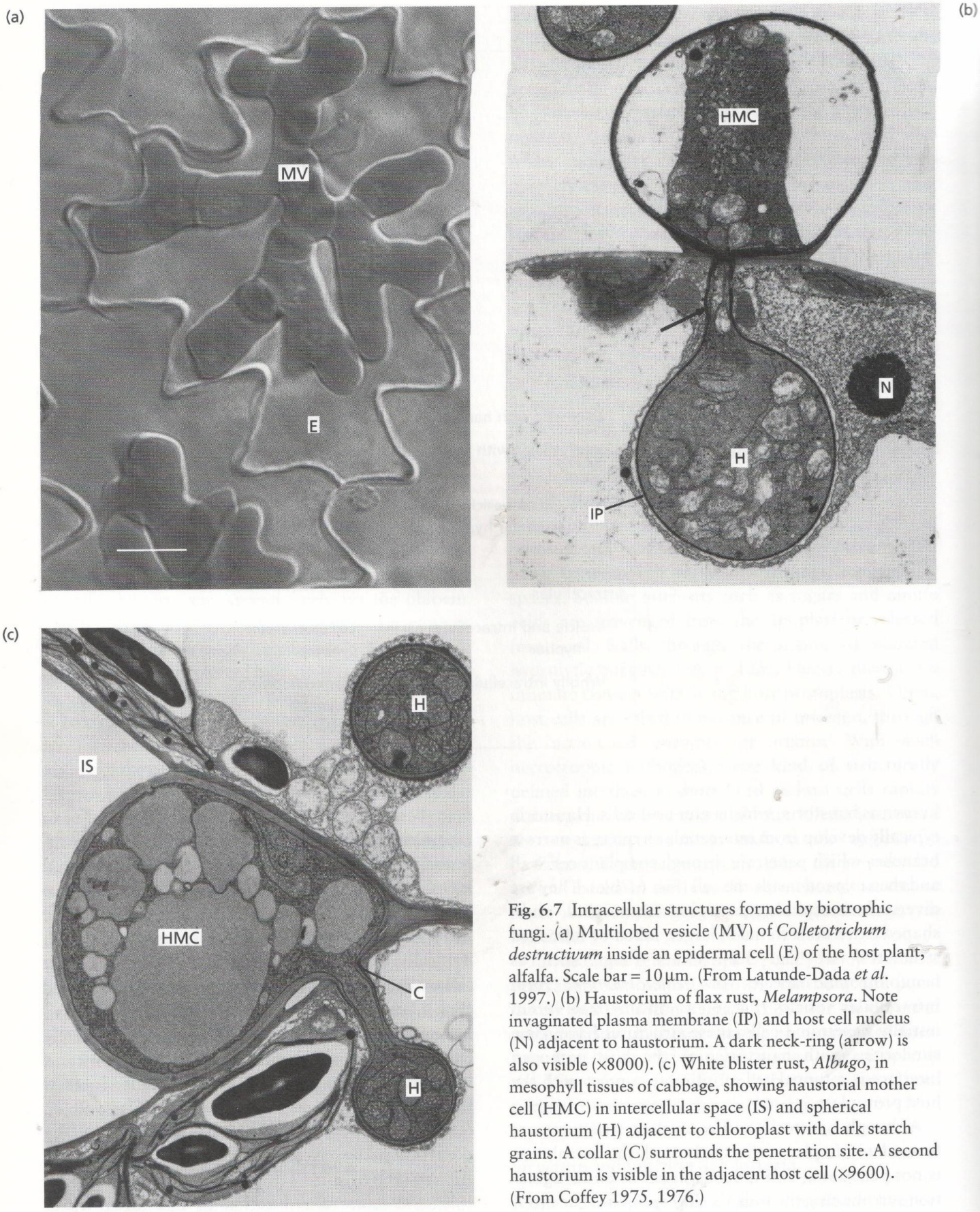


Fig. 6.7 Intracellular structures formed by biotrophic fungi. (a) Multilobed vesicle (MV) of *Colletotrichum destructivum* inside an epidermal cell (E) of the host plant, alfalfa. Scale bar = 10 μm. (From Latunde-Dada *et al.* 1997.) (b) Haustorium of flax rust, *Melampsora*. Note invaginated plasma membrane (IP) and host cell nucleus (N) adjacent to haustorium. A dark neck-ring (arrow) is also visible (×8000). (c) White blister rust, *Albugo,* in mesophyll tissues of cabbage, showing haustorial mother cell (HMC) in intercellular space (IS) and spherical haustorium (H) adjacent to chloroplast with dark starch grains. A collar (C) surrounds the penetration site. A second haustorium is visible in the adjacent host cell (×9600). (From Coffey 1975, 1976.)

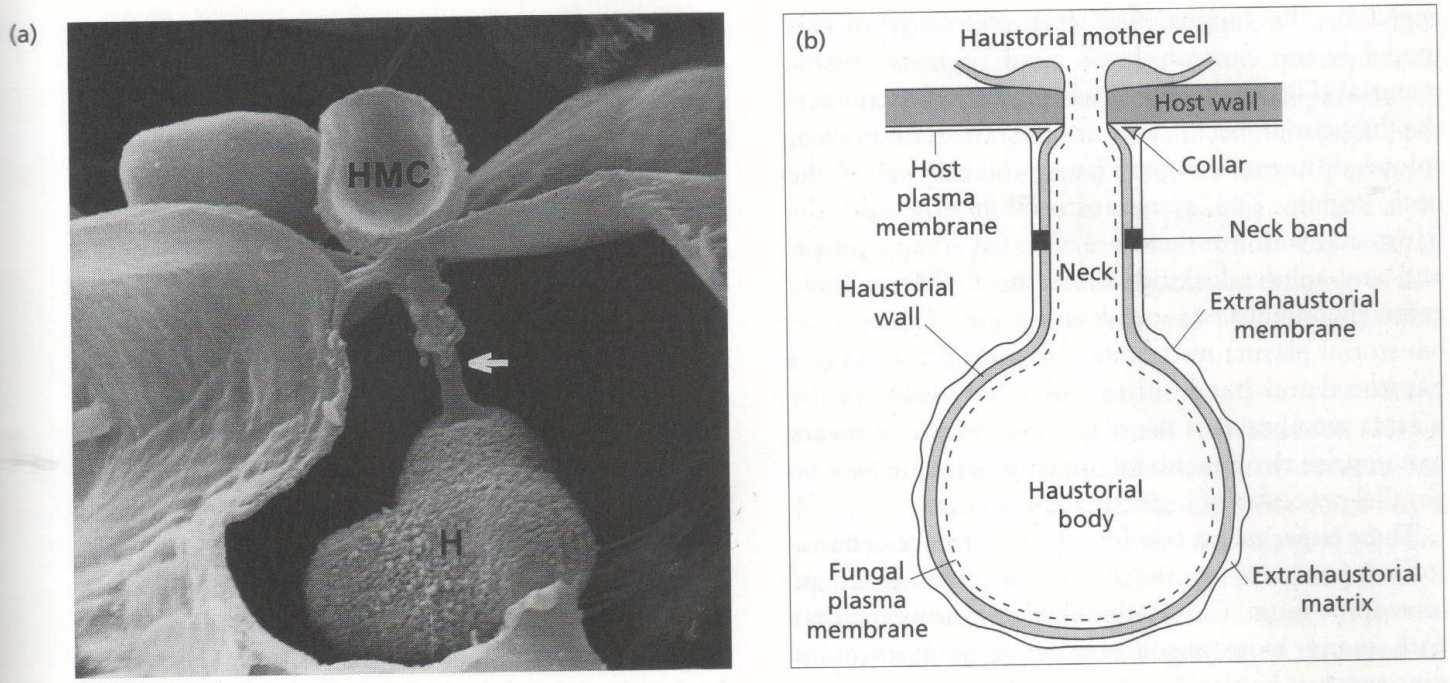


Fig. 6.8 The structure of haustoria. (a) Scanning electron micrograph of coffee leaf tissue infected by rust, *Hemileia vastatrix*. The tissue has been frozen and fractured to reveal a haustorium (H) within a mesophyll cell. Note a slight swelling (arrow) in the haustorial neck at the position of the neck band, and the haustorial mother cell (HMC) external to the penetrated host cell (×5000). (Courtesy of Rosemarie Honneger.) (b) Diagrammatic interpretation of haustorial structure, showing the main interfacial components.

provide an enlarged surface area of the parasite directly adjacent to nutrient sources such as chloroplasts (Fig. 6.7c), suggests that they play a role in nutrient uptake. Obtaining direct physiological evidence to confirm this idea has proved difficult. To date, most of the work on haustorial function has been conducted with powdery mildew fungi, as these epiphytic parasites form haustoria only in epidermal cells, and are therefore a convenient experimental system for analysis. Most of the fungal biomass can be stripped off the leaf and separated from the host tissues. If plants infected by powdery mildew are fed radiolabelled carbon as $^{14}CO_2$ a proportion of the carbon fixed in photosynthesis travels to the epiphytic hyphae and spores of the fungus. No significant uptake of radiolabelled solute occurs until after formation of the first haustoria. It has also proved possible to isolate intact powdery mildew haustoria from epidermal cells; such structures comprise the haustorial neck and body with the EHM still attached. Experiments with labelled sugars and amino acids have shown that solutes cross the EHM, and that the epidermal cell cytoplasm plays an essential role in transporting assimilates into haustoria. The main compound initially moving from the host to the fungus is sucrose. Thus, with powdery mildew fungi, the pathway of carbon flow is from the source (chloroplasts in mesophyll cells) to the sink (epidermal cells which lack chloroplasts), and then into a secondary sink, the fungus, via haustoria in epidermal cells. The plant sugars are eventually converted into fungal metabolites such as mannitol and glycogen.

What is the actual mechanism by which solutes are removed from host cells? Electron micrographs of stained or freeze-fractured haustoria suggest that the invaginated region of the host membrane, the EHM, is altered in structure and composition by comparison with the rest of the host plasma membrane. In particular, the EHM lacks intramembrane particles, and ATPase, an enzyme involved in the active transport of solutes. ATPase activity can be detected in the host membrane where it lines the plant cell wall, and also in the fungal plasma membrane inside the haustorium, but not in the EHM. It appears therefore that both the host cell protoplast and the fungal protoplast are actively importing solutes, while the membrane enclosing the haustorium has diminished control of solute transport, and leaks nutrients into the extrahaustorial matrix, from where they are scav-

enged by the fungus. One further feature of this model is the electron-dense band of impermeable material (Figs 6.7b & 6.8) where the EHM contacts the haustorial neck. This is presumed to prevent solutes diffusing along the haustorial cell wall in the neck region. The extrahaustorial matrix and the haustorial wall are therefore a sealed compartment, and any solutes leaking across the EHM can only enter the fungus via active transport through the haustorial plasma membrane. In biotrophs lacking a haustorial neck band, such as the downy mildews, the plasma membrane of the penetrated cell still appears to comprise two functional domains, so there may be parallel processes of nutrient acquisition.

These experiments confirm the importance of haustoria in nutrient uptake by at least one group of biotrophic fungi. Calculations suggest, however, that with many biotrophs a significant proportion of nutrients can be also acquired from the host apoplast via intercellular hyphae. A detailed three-dimensional analysis of colonies of brown rust (*Puccinia recondita*) on barley estimated that the total length of intercellular hyphae in a colony is approximately 1 m. Haustoria occur at a frequency of one every 70 μm, giving a total number of more than 10 000 per colony. However, haustoria accounted for less than 20% of the total colony surface area, and the major area of contact between host and pathogen was therefore between intercellular hyphae and host cell walls. There may of course be other possible functions for haustoria, such as a regulatory role in manipulating host metabolism, or in the maintenance of compatibility between the two partners (see Chapter 10).

Further insights into haustorial function are now being gained by studies using monoclonal antibodies raised against isolated haustorial complexes, or the infection vesicles of hemibiotrophs such as *Colletotrichum* (Fig. 6.7a). Such antibodies have detected specific antigens which appear to be located only at the host–pathogen interface. This confirms that novel proteins or glycoproteins are produced at specific stages in the development of biotrophic fungi within their hosts. Identification of such stage-specific antigens, and the genes encoding them, should ultimately provide insights into the nature and regulation of biotrophic parasitism by fungi.

Some pathogens appear to grow preferentially at sites in the plant where nutrient transfer is occurring. A good example of this type of relationship is the ergot fungus, *Claviceps purpurea*, which colonizes ovary tissues and diverts nutrients passing from transfer cells to the developing embryo. The pathogen competes with the embryo and ultimately replaces it with a fungal structure, the sclerotium. It has also been noted that many biotrophic fungi, such as rusts, invade host tissues adjacent to vascular elements, where loading or unloading of sugars into or from phloem cells is occurring. The term **transfer-intercept** infection has been used to describe such behaviour.

Intracellular pathogens

Intracellular relationships are typical of some mutualistic associations, for example the root nodule bacterium, *Rhizobium*, and some types of mycorrhizal fungi. In this context it is interesting to recall the theory that the chloroplasts and mitochondria of eukaryotic cells may have arisen from endosymbiotic microorganisms. A few pathogenic fungi also live inside host cells. The club root pathogen *Plasmodiophora* exists in the form of a naked cell, or **plasmodium**, and the interface consists simply of the plasmodial cell membrane surrounded by a second membrane which is presumed to originate from the host. An even more intimate contact is found in the parasitic chytrids such as *Olpidium*, where the fungal cell is not surrounded by a host membrane and is therefore in direct contact with the host cytoplasm. A similar type of relationship has been found in cells infected by phytoplasmas.

The ultimate examples of intracellular pathogens are the viruses and viroids. Virus particles occur within the cytoplasm, plastids and nuclei. Hybridization techniques used to locate and visualize specific nucleic acid sequences have recently shown that viroids accumulate in the nucleolus. Due to their unique properties viruses and viroids are not comparable to cellular pathogens regarding the nature of the host–pathogen interface. Successful replication of viruses requires removal of the coat protein, so that the interface during multiplication is between a nucleic acid molecule and the synthetic machinery of the host cell.

Development following infection

Following entry there are wide variations in the extent and pattern of colonization of host tissues (Fig.

6.9). Further development is related both to the nature of the parasitic relationship between the two partners, and to the relative success of host resistance mechanisms in limiting pathogen invasion (see p. 140). Broadly speaking, two main patterns of colonization occur, **localized** or **systemic**. In localized infection the pathogen multiplies or grows within a particular tissue or organ to give discrete lesions. In a systemic infection the pathogen spreads widely throughout the plant, and in extreme cases occurs in every part of the root and shoot system. Complete systemic colonization, in which literally every cell is infected, probably never occurs, as even viruses which spread efficiently from cell to cell are usually absent from meristems and from gametophyte tissue (see below).

Within these two broad categories there are numerous subtle variations in pathogen behaviour, and in the ultimate extent of damage to the host. Many pathogens exhibit **tissue specificity**; in other words they grow preferentially in certain host tissues. Vascular pathogens, for instance, including both bacterial and fungal examples, grow within the xylem, while phytoplasmas are usually confined to the phloem. The reasons for such behaviour are poorly understood, although the mode of nutrition of the pathogen is clearly important. The less specialized necrotrophic pathogens tend to spread indiscriminately through plant organs, while biotrophs, in keeping with their more benign form of parasitism, grow selectively within certain well-defined host tissues. A further special case is where a pathogen induces major changes in the organization and morphology of host tissues, for instance tumours, in which it subsequently lives; the classic example of this mode of colonization is provided by *Agrobacterium* (see Fig. 8.11).

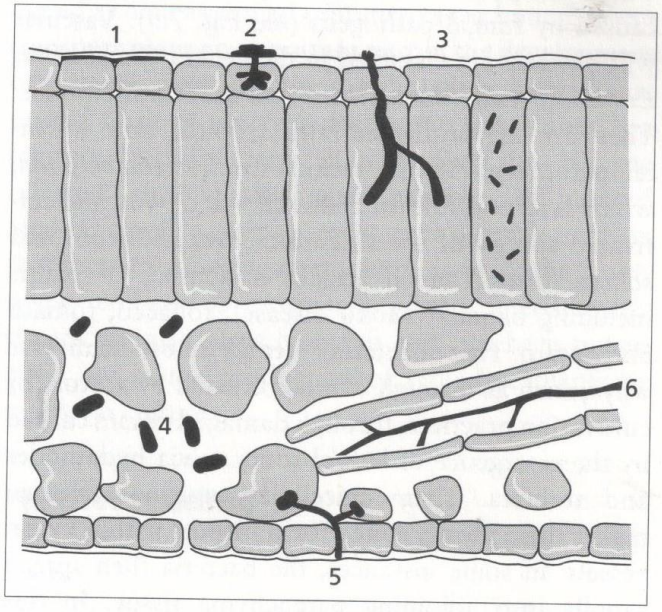


Fig. 6.9 Some patterns of pathogenic invasion of plant tissues. 1, Subcuticular; 2, epiphytic with haustoria; 3, intracellular; 4, intercellular; 5, intercellular with haustoria; and 6, vascular.

One might assume that there is a correlation between the extent of host colonization by a pathogen, and the eventual severity of disease, but this is by no means always the case. For instance, a localized pathogen may disrupt an essential physiological function, such as water transport, or produce a diffusible toxin which can act at a distance from the lesion itself. In an extreme example of this type of behaviour, the pathogen causing choke disease of grasses, *Epichloe typhina*, is restricted to a short section of leaf sheath tissue but its growth in this strategic position prevents the emergence of the flowering axis and hence its effect on the life cycle of the host is dramatic. Conversely, it is quite common to encounter systemic virus or viroid infections in which the host is asymptomatic. Such cryptic infections pose a particular problem when attempting to eradicate a pathogen from a crop.

The pattern of colonization can be determined by the infection route. Downy mildew fungi, such as *Peronospora* and *Plasmopara* species, typically infect leaf tissues, where growth is restricted by large veins, resulting in angular, localized lesions. Infection of the stem apex of young seedlings, however, leads to a more systemic mode of growth below the dividing meristem, causing severe stunting of the host. Some modern pea cultivars are more susceptible to this type of infection by *Peronospora pisi*, simply because they lack large stipules — leaf-like structures which normally enclose and protect the stem apex.

Only a few fungi, notably the smuts, are truly systemic. *Ustilago nuda*, causing loose smut of barley, occurs as a dormant mycelium in the embryo of infected grain. During germination of the seed the pathogen also resumes activity and grows intercellularly within the young seedling. As the plant matures the pathogen keeps pace just behind the apical meristem, and eventually invades the developing flower head to form a mass of black teliospores which replace the grain. The older

mycelium in the stem may break down as the host matures, but it often persists in the nodes. One interesting feature of smut diseases is that visible symptoms are not obviously manifest until the pathogen sporulates. Infected plants may, however, be slightly taller than normal.

Amongst those fungi which are specific to particular host tissues, the vascular wilt pathogens have a particularly interesting mode of spread within the host. Fungi such as *Fusarium oxysporum* and *Verticillium albo-atrum* enter in the apical region of the root. In this region the endodermis is not fully differentiated and the fungi are able to grow through it and reach the developing protoxylem. Further colonization of the living host is restricted to the xylem (see Fig. 7.9). Hyphae grow through the vessels and tracheids and pass from cell to cell via pit pairs. In addition, long-distance movement is accomplished by the production of microconidia, which are carried in the transpiration stream. This mode of spread is much more rapid than would be possible by mycelial growth; the Panama disease wilt pathogen, *Fusarium oxysporum* f.sp. *cubense*, can migrate from the bottom to the top of an 8 m-tall banana plant in less than two weeks. Because xylem tissues ramify throughout the plant, wilt pathogens can migrate into every part of their host. Thus although they are tissue specific these pathogens can become virtually systemic.

Colonization by bacteria

The morphology of bacterial cells limits their capacity for widespread growth through compact tissues. Thus, spread within the host is often accomplished by maceration of tissues or by the exploitation of natural channels.

For example, the bacterium *Erwinia carotovora* causes a common storage soft rot of potato tubers (see Fig. 8.2). The pathogen is unable to pass through intact periderm and therefore gains entry via lenticels or wounds, including those caused by other agents (see p. 99). Once inside the tuber, the bacterium spreads rapidly through the parenchyma giving rise to a soft, slimy, putrid lesion, and under favourable conditions it can quickly destroy the tuber. At all stages of colonization the bacterium occupies intercellular spaces, and its spread is facilitated by the production of pectolytic enzymes which degrade middle lamellae and thus macerate the host tissues. However, under aerobic storage conditions at low relative humidities, the rate of invasion is slower and the bacterium may be localized by a black oxidation zone. This is partly an anatomical barrier, involving the deposition of suberin in tuber cell walls, and partly a chemical barrier, consisting of oxidized polyphenols. Similar histological defence reactions are important in restricting the spread of many other relatively unspecialized pathogens within storage tissues.

Not all plant-pathogenic bacteria macerate tissues. *Agrobacterium tumefaciens* grows biotrophically within crown gall tumours without separating or killing cells, while the bean halo blight pathogen, *Pseudomonas syringae* pv. *phaseolicola*, proliferates in intercellular spaces, causing a water-soaked lesion in which host cells initially remain alive. Chlorotic symptoms in this case are associated with the production of a toxin (see p. 133), but toxin-deficient strains of the bacterium are equally capable of multiplying in bean tissues. Fireblight, *Erwinia amylovora*, invades flowers, leaves and stem tissues, spreading through vascular elements causing a necrotic die-back. The bacterium produces only low amounts of cell-wall-degrading enzymes, and host cell death appears to be due instead to a toxin.

A number of bacteria can cause serious vascular wilt diseases, in which the symptoms parallel those caused by fungal pathogens (see Fig. 7.9). Vascular wilt bacteria are classified in the genera *Clavibacter*, *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas*. They are important in both tropical and warm-temperate regions, and include *Erwinia tracheiphila*, which is responsible for bacterial wilt of wild and cultivated species of the Cucurbitaceae, and *Ralstonia solanacearum*, which attacks a number of plants including banana (Moko disease), tobacco, tomato and potato. These bacteria enter their hosts in diverse ways, such as through wounds created by vectors or cultivation practices, through damaged tissues caused by the emergence of lateral roots or via hydathodes and stomata. Their unicellular morphology then makes them ideally suited for transport within xylem vessels. In some instances, the bacteria then spread rapidly into adjoining parenchyma tissue. In this respect several of these bacteria differ from the typical vascular wilt fungi, which do not grow out from the xylem until after the host has died.

Colonization by viruses

The spread of viruses within their hosts is unique in that they can only multiply within cells and are small enough to behave as subcellular particles. As such they are able to move directly from cell to cell through plasmodesmata; virus particles have been observed within plasmodesmata by electron microscopy. This short-distance cell-to-cell spread is fairly slow, with the virus taking four or five hours to move from one cell to the next. There is now evidence that the movement of viruses through plasmodesmata is mediated by 'movement proteins' encoded by the virus itself, for instance the P30 protein of tobacco mosaic virus (TMV) (see p. 37). Antibodies specific for the movement protein show that it becomes localized to plasmodesmata; this has been recently confirmed by experiments in which a green fluorescent marker protein, derived originally from jellyfish, was linked to the virus protein, and the subcellular distribution of fluorescence studied by microscopy. Highly localized bright-green sites were seen associated with host cell walls, coinciding with pit fields where plasmodesmata perforate the wall. The exact mechanism involved is not yet known, although these proteins may modify plasmodesmata to alter the exclusion size and hence allow free passage of the virus.

Much faster long-distance spread takes place via the phloem; here the rate of movement has been estimated as high as several centimetres per hour. Phloem transport plays an important part in the development of systemic virus infections, although how the transported form of the virus leaves the sieve tubes is a mystery. Literally every cell in the plant may become infected, although the small numbers of infected seeds and pollen grains in most virus diseases suggests that movement into gametophyte tissue of the developing embryo is restricted. Often, meristematic tissues are also virus free; this fact has been put to good use in the production of virus-free plants by meristem culture.

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