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PLANT DEFENCES AGAINST PATHOGENS

Host-parasite relationships

Earlier chapters have described the diverse and constant threat pathogens pose to plant health. Yet, surprisingly, disease is the exception rather than the rule in natural plant communities. Put another way, most pathogens are unable to attack most plants; they have a restricted host range. Assuming environmental conditions favour pathogen development, the resistance or susceptibility of a plant to a particular pathogen depends on two interrelated factors: (i) the substrate requirements of the pathogen and (ii) the response of the plant to the pathogen.

In the previous chapter two broad groups of pathogens, necrotrophs and biotrophs, were distinguished by their different substrate requirements (Table 16.1). Necrotrophs are 'thugs' in the sense that they kill plant cells before parasitising them. Host and parasite cells cannot coexist harmoniously. Thus, an incompatible cellular relationship between the parasite and host is essential for disease development. If the toxins used to kill host cells are not released at the right time, place or concentration, or if a particular host genotype is insensitive to the toxin, host cells will not die. The necrotroph will be unable to colonise or reproduce and the plant will be resistant. Two types of necrotrophic pathogens exist: (i) those with a wide host range involving many plant species and (ii) those

with a host range restricted to a few plant species or even to cultivars within a species. The key difference between these two types of necrotroph is the specificity of the toxin(s) produced. Necrotrophs with a broad host range secrete toxins that act on metabolic targets common to many plants. In contrast, the pathogenic ability of necrotrophs that release host-specific toxins is conditioned by the gene that encodes the ability to produce the toxin and by a gene in susceptible cultivars of the host that encodes sensitivity to that toxin. Host-specific necrotrophs usually form a pathogenic race or pathotype structure where some races can attack some cultivars within a species but not others. If the gene that conditions sensitivity to a particular host-specific toxin is absent from a cultivar, that cultivar will be resistant to the disease caused by that pathogen.

Biotrophs on the other hand are obligate parasites that obtain nutrients from living cells. Consequently, they must establish a compatible cellular relationship with their hosts. Biotrophs act as 'sneaks'. They typically infect through natural openings or by directly penetrating their host's surface. They mostly then grow between the cells of their host and only penetrate host cell walls (but not host cell membranes) to form food-absorbing haustoria. The pathogen develops without eliciting the host's defence responses or by spreading in advance of the plant's ability to activate its defence responses. The level of specialisation required to establish this type of relationship usually means that biotrophs have a restricted host range and a well-defined pathogenic race structure. If host cells die in advance of invasion by a biotrophic pathogen, the plant will be resistant because the pathogen is unable to establish a parasitic relationship.

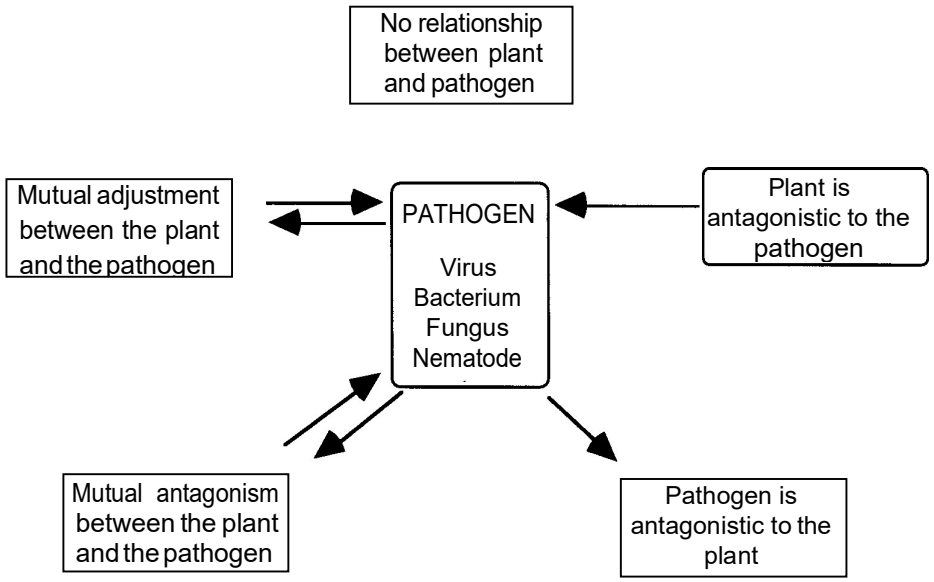
A second factor that influences whether a parasitic relationship will become established is the way that the plant under challenge responds. Some interactions between individual pathogen propagules and plant cells may lead to successful pathogen establishment, while others may not. In this chapter it will become evident that resistance or susceptibility of a whole plant and plant communities is the sum of many individual cellular interactions. Plants that are resistant restrict or retard the development and reproduction of an overwhelming majority of individual pathogen propagules that attack it. In this sense resistance is quantitative—resistant hosts prevent or slow the development and reproduction of a higher proportion of pathogen propagules than susceptible hosts. For the purposes of plant breeding, the response of a plant to pathogen inoculation is often categorised as either 'resistant' or 'susceptible', although from a cellular perspective this distinction is not always so clear. Resistance and susceptibility are more accurately portrayed as the extremes of a continuum upon which most host—parasite interactions sit. Resistance may be expressed in many ways, from the inhibition of propagule germination and penetration, the killing of pathogens before establishment, to the restriction or retardation of colony development and reproduction once the pathogen has established. For example, different genes for stem rust resistance in wheat act at different stages of the host—parasite interaction. Some cause the rapid death of the pathogen following attempted penetration, others allow initial infection, but prevent haustorial development and starve the pathogen, while the 'slow-rusting' genes allow parasitism and pathogen reproduction, but at a much slower rate than in susceptible cultivars. Each type of interaction provides useful resistance for plant breeders because they all delay the onset of epidemics and reduce yield losses.

The early steps involved in the establishment of a host—pathogen relationship are delicate and sensitive to environmental factors, including the presence of other micro-organisms. The host-parasite-environment interaction is mediated by a complex interchange of signals. Plants respond to pathogen attack by erecting a highly coordinated series of molecular, cellular and tissue-based defence barriers.

All plants have the capacity to activate these defences. However if they are activated too little, too late, or in the wrong place, they will fail to restrict the pathogen and the plant will be susceptible. Pathogens respond by escaping or suppressing plant defence responses or by rendering these responses impotent, for example by detoxifying plant antibiotics.

The interaction of pathogen nutrient requirements and host responses leads to five possible outcomes if environmental conditions favour infection (Fig. 17.1).

- No relationship is established when the plant and the pathogen ignore each other. For example, a spore of a fungus may germinate, but because the host does not provide essential requirements for pathogen development, the resulting hypha fails to penetrate or establish a parasitic relationship. The fungus dies when its energy reserves are exhausted. The plant does not react in any way and is resistant by default. It is a non-host.



Pigure 17.1 Five possible relationships between plants and potential pathogens.

- A plant is antagonistic to the pathogen when it secretes inhibitory compounds into its environment that prevents pathogen development. For example, the stubble of some brassicas releases 'biofumigants' into the soil that prevent the hatching of nematode eggs and inhibit the growth of some root-infecting fungi. Asparagus and marigolds *(Tag ete* s spp.) secrete substances into the rhizosphere that are toxic to nematodes and provide useful protection against nematodes when interplanted with nematode- susceptible plants like tomato. Many plants secrete phenolic compounds onto their leaf surfaces that not only discourage herbivore feeding, but also inhibit many micro-organisms, including potential pathogens. In this relationship, the pathogen fails to develop and has no observable effect on the metabolism of the host plant. In some cases, such as in the quiescent infection of ripening avocado fruit with Colletotrichiim *gtoeosporioides,* plant antagonists only temporarily inhibit pathogen development. Spores germinate to form appressoria, but their development is arrested by fungistatic substances in the peel. After harvest, these substances are enzymically degraded and the appressorium germinates to form infection hyphae. Eventually, anthracnose lesions develop. This type of interaction involving a quiescent stage in

pathogen development is common among the stem end rot pathogens of avocado and mango (e.g. Dothiorelia dominicnnn, Lnstodiploctin theobromne, *Phornopsis* spp. and Coiletotrichiim *gloeosporioides!.*

- The pathogen is antagonistic to the plant when it secretes compounds that

damage the plant. For example, Periconin circinata, infects the roots of sorghum, but only those strains of the fungus that produce the host-specific toxin, periconin, induce symptoms of milo disease, but only in cultivars that are sensitive to this toxin. Similarly, some strains of A lternnrin ntternntn release host-specific toxins that kill cells of susceptible host species and cultivars. For example, a strain of the fungus that is pathogenic on tomato produces AAL-toxin, to which tomato is uniquely sensitive. Strains producing AAM-toxic attack apples, AAK-toxin producing strains affect Japanese pears, AAC toxin-producing strains affect citrus and so on. The tomato, apple and Japanese pear strains are not pathogenic to citrus because citrus is only sensitive to the AAC-toxin. Cochtiobolus *uictoriae* produces the toxin victorin that causes severe seedling blight on susceptible cultivars of oats, but has little effect on resistant cultivars or on other plant species. Resistance is the result of insensitivity to the toxin produced by the pathogen. If this insensitivity is common to all cultivars within a plant species, that species is said to be a non-host.

- Mutual antagonism between plant and pathogen results in the inhibition or death of both the host tissue and pathogen. For example, an incompatible interaction between the stem rust pathogen, *Pucciriin* praminis f. sp. trittci and resistant cultivars of wheat causes the death of both host and pathogen cells.
- Mutual adjustment leads to a compatible cellular relationship between the host and pathogen. Symbiotic relationships between mycorrhizal fungi and plant roots and between nitrogen-fixing prokaryotes and plant roots, are examples of mutually beneficial interactions. Endophytic fungi and bacteria colonise the intercellular spaces of plant tissue, apparently without damaging their host cells. Many stem end rot pathogens have an endophytic phase in leaves and twigs before they infect fruits. Biotrophic pathogens, like the mildews and rusts, grow and reproduce on living host tissue. However, the diversion of nutrients to the invading pathogen adversely affects the growth of the host, even though host cells are not killed.

In this chapter, plant defence mechanisms will be discussed in the order they are usually confronted by pathogens. Broadly speaking, passive defence mechanisms are those that are present before contact with the pathogen, while active defence mechanisms are activated only after pathogen recognition (Fig. 17.2). In reality this distinction is not always clear, as many pre-existing defences are modified after infection.

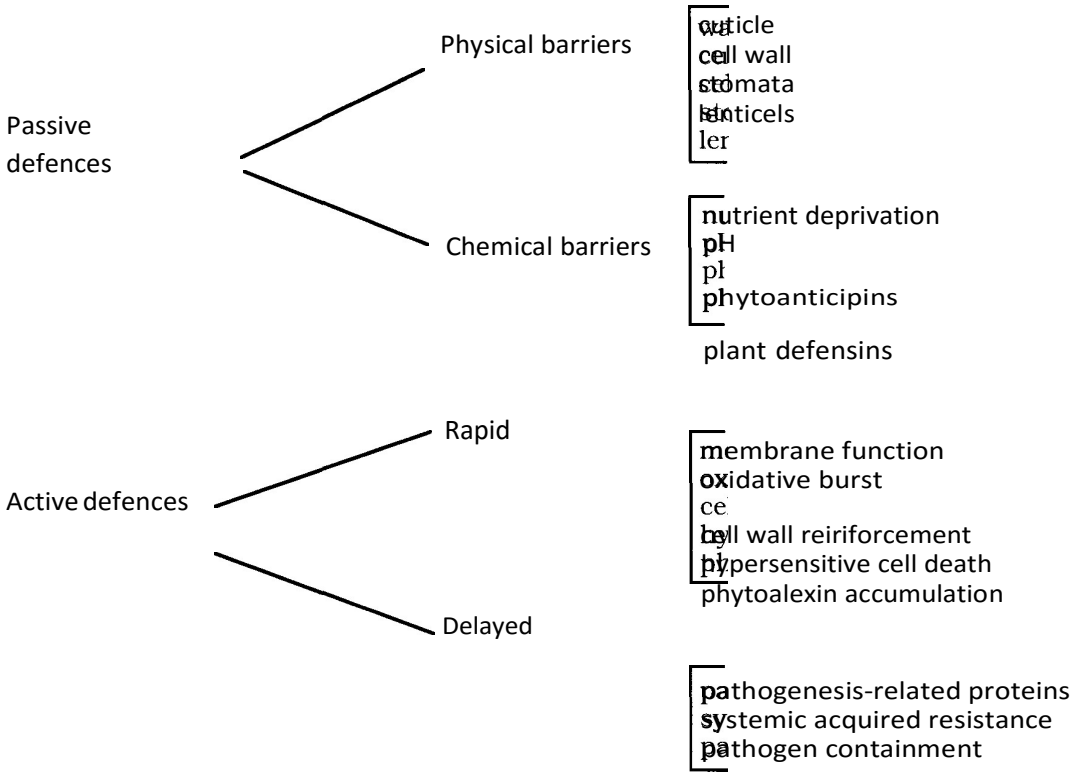
Passive defences

To gain access to the nutrients or replication machinery available within the host cell, pathogens must first breach the natural barriers presented by healthy plants. These barriers may be physical (the cuticle, cell wall, stomatal aperture or lenticel) or chemical (including inhibitory compounds or the absence of stimulatory compounds needed for pathogen development). Saprophytes lack the ability to penetrate these natural barriers.

Physical barriers

The importance of the cuticle as a barrier to penetration has been demonstrated by the dependence of many pathogens on adhesion and the subsequent release of

cutin-degrading enzymes at the time of penetration. Although cutin-degrading enzymes are also secreted by many saprophytic fungi and bacteria, their primary activity is to allow access to cellulose in plant cell walls as a nutritional substrate. Different forms of cutin-degrading enzymes are used by pathogens to puncture the cell wall (Chapter 16). The activity of this type of cutinolytic enzyme in isolates of Ftisariiim solani f. sp. *pier* is directly related to their aggressiveness on pea stems, indicating that pathogens unable to dissolve the cuticle at the point of penetration are excluded.



Pigure k7.2 Some defence mechanisms in plants.

Cuticle and cell wall thickness may influence resistance to certain pathogens. Some types of 'adult plant resistance' could be associated with a reduced ability of pathogens to enter through thicker, tougher cell walls. Some pathogens such as Puccinia praminis only infect young barberry leaves with thin cuticles and the germ tubes emerging from basidiospores do not penetrate thicker cuticles on mature leaves. Similarly, the ability of Taphrinn de/ormnris to infect only young, newly unfolded leaves has been attributed to the inability of germ tubes to penetrate the thicker cuticles of older leaves. The presence of secondary cell walls in sclerenchyma, xylem or older plant tissue often retards pathogen development, leading, for example, to angular leaf spots where pathogen spread is restricted by leaf veins. Thick cuticles may physically prevent the eruption of sporophores and release of spores. However, most experimental evidence suggests that toughened cuticles and cell walls are just one of the many factors that contribute to resistance.

Waxy cuticles and vertically oriented leaves may prevent the formation of moisture films on leaf surfaces. Dry leaf surfaces inhibit infection by pathogens such as bacteria, nematodes and fungal zoospores that require a film of water for motility. Fungal spores might also be inhibited because most require moisture for germination. This must be balanced with the fact that vertically oriented leaves

are more prone to impaction by wind-borne pathogen propagules and are likely to face higher inoculum levels compared with those that are horizontally oriented.

Many pathogens enter through wounds, natural openings or are introduced by vectors. In these cases it is difficult to see how natural barriers such as the cuticle and cell wall could be involved in resistance. Some researchers have proposed that plants that have stomatal apertures that are the wrong shape or size for pathogen infection structures to enter or that have stomata that close at the time of day that pathogen spores normally germinate, may be more resistant to pathogen attack. The black pod pathogen, Phptophthorn paimtoora, enters cocoa pods through stomata. Cocoa genotypes that produce pods with few, relatively smaller stomata, allow fewer lesions to establish than genotypes with more numerous, larger stomata. Not surprisingly, as the pathogen enters through stomatal pores, there is no correlation between cuticle thickness or pod case hardness and resistance to black pod. The bacterium that causes citrus canker, Aanthomonns campestris pv. citri, enters grapefruit through open stomata. Mandarins are resistant because their stomata are too small to allow entry of the bacterium. Similarly, lenticels that suberise rapidly so that their size is reduced may physically exclude pathogens such as *Streptoingces* scnbies, the cause of common scab of potato.

Chemical harriers

Exudates or the surfaces of plants or compounds in plant cells may stimulate or inhibit the development of pathogens. Sometimes, plants resist infection because they do not provide the pathogen with its required nutrients. Resting spores of pathogens such as *Spongospora* siibterranen (powdery scab of potato), *Urocgstis* aproppri (flag or leaf smut of wheat) and Ptnsmoctiophorn brassicoe (club root of crucifers) and eggs of the potato cyst nematode, Globodern rostochiensis, require specific substances to stimulate germination or hatching. These are provided in secretions from certain plants, including potential hosts. Plants that fail to secrete these stimulators are resistant by default.

Other plant secretions may simply not support the pre-penetration growth of the pathogen. Experimental depletion of iron availability using binding agents (siderophores) inhibits the growth of certain fruit-rotting bacteria. Host cultivars that secrete lower than normal levels of iron onto their surface may deprive pathogens of essential nutrients, inhibiting their growth. Similarly, micro- organisms that sequester available iron on leaf surfaces have potential as biocontrol agents (Chapter 27).

Plants sometimes produce compounds during normal growth that inhibit the development of pathogens. Phytoanticipins maybe excreted into the external environment (e.g. rhizosphere or phylloplane), accumulate in dead cells or they may be sequestered in vacuoles in an inactive form. The dead cells of brown onion skins contain the quinones catechol and prOtocatechuic acid, which inhibit germination of spores of the smudge pathogen, Colietotrichiim circirinns, and the neck rot pathogen, Botrptis *cinerea.* White onions do not produce these compounds and are susceptible to smudge. *Aspergill us* niper is insensitive to these inhibitors and attacks both white and brown onions. Avocado rootstocks resistant to root rot caused by Phptophthora cinnnmomi secrete borbinol, an antimicrobial phenolic compound, into the rhizosphere. The secretion of nematode-inhibiting substances into the rhizosphere surrounding asparagus and marigold roots has already been mentioned. Symptoms of anthracnose of

avocado, caused by *Colletotrichuin* ploeosporioides, only develop on ripe fruit. The peel of unripe avocado fruit contains antifungal lipids called dienes that prevent

appressorial germination. As these dienes are gradually metabolised during fruit ripening to less toxic compounds, quiescent appressoria germinate and susceptibility to anthracnose increases. In anthracnose-resistant cultivars, diene breakdown is blocked following infection, so that antifungal levels are sustained for longer periods. The resistance of immature apples and pears to scab, caused by Venturin innequnlis and V. *pirirtn* respectively, correlates with the presence of the phenolic compounds chlorogenic acid, phloridzin, arbutin and iso-chlorogenic acid in the outer layers of the fruit. These compounds also contribute to the bitter taste of unripe apples and pears and, as the fruit ripens and sweetens, it also becomes more susceptible to scab.

One group of phytoanticipins, the saponins, are plant glycosides with surfactant (wetting agent) properties. Saponins bind sterols in pathogen cell membranes, destroying membrane integrity and function. In this way saponins are toxic to organisms containing sterols in their membranes (e.g. plants and fungi, but not Oomycota). Inactive saponin precursor molecules appear to be stored in vacuoles of intact plant cells, but hydrolase enzymes released following wounding or infection convert these precursors to active, antimicrobial forms. Several lines of evidence suggest that saponins are involved in disease resistance and host range determination. It appears that the ability of some pathogens to detoxify specific saponins matches their host range. For example, a strain of the take-all pathogenthat attacksoats as well as wheat and barley (Gneiirnannompces prnminis var. nuenae), releases the enzyme avenacinase. Avenacinase detoxifies the triterpenoid saponin, avenacin, found in epidermal cells of the roots of oat plants. Mutants in which the gene for avenacinase production has been deleted are sensitive to avenacin in vitro and are not pathogenic on oats, but remain pathogenic to wheat and barley. Gaeiimannomyces prnrnirtis var. tritici lacks avenacinase and attacks wheat and barley, but not oat species containing avenacin. An oat species that does not produce avenacin, Arena loripiqliimis, is susceptible to Gneiiman nomyces prnmirtis var. tritici. Another saponin, tomatine, contributes to the resistance of tomato leavesto Bot tis ctnerea.

Some plant peptides also inhibit the development of fungi, bacteria, viruses and insects. They act as proteinase and polygalacturonase-inhibitors, as ribosome inhibitors or lectins. These inhibitors interfere with pathogen nutrition and retard their development, thus contributing to disease resistance. Because of their similarity to peptides called defensins found in insects and mammals, they have been termed plant defensins. Secreted defensins provide an important

defence against damping-off pathogens. While only 0.5% of the total protein found in ungerminated radish seeds is defensin, it makes up 30^{0}/ of the proteins released from germinating seeds. It provides an antimicrobial micro-environment around the emerging radicle. Defensins may constitute up to 10^{0H} of the total proteins in cereal, legume and solanaceous seeds. Similar studies have shown defensins are also present in the outer cell layers of other plant organs such as flowers, leaves and tubers. While many defensins accumulate during normal

plant development, others are induced, or their accumulation is enhanced, after wounding. Defensins, because of their anti-feeding activity against insects, provide a defence against insect-transmitted viruses.

Pathogen recognition

The ability of plants to respond to challenge by potential pathogens implies that plants recognise these potential pathogens as ‘non-self. While mammals use antigen-antibody interactions to recognise non-self, plants recognise a vast array

of signals originating from micro-organisms and the environment to elicit defence responses.

Non-specific eiicitors

Many signals of abiotic and biotic origin induce defence responses in a range of cultivars and host species that bear little relationship to pathogen host ranges. The magnitude of the response depends on the amount of elicitor present. Abiotic elicitors, including heavy metal ions, UV light and some metabolic inhibitors, precipitate physiological stress responses, some of which contribute to resistance. Their effect is generally transitory and non-specific. The significance in host— parasite interactions of abiotic elicitors is not always obvious as they are rarely present at the infection court. However solar UV radiation may elicit stress responses in exposed plant tissues, providing an additional barrier for invading pathogens. On the other hand, environmental stresses usually increase the susceptibility of plants to necrotrophic pathogens.

Cell wall fragments released from fungi and bacteria elicit defence responses in plants. Cell wall fragments fr ITl Phytophthorn *inegasperma* f. sp. plpcirten are potent elicitors of defence responses in soybeans. The smallest active fragment is a heptabetaglucan (seven glucose units) that is found in cell walls of many pathogenic and non-pathogenic races and species of oomycetes. Recently, a receptor was identified in the plasma membrane of soybean cells. This, together with its potency, suggests a role for heptabetaglucan and related oligosaccharins, in pathogen recognition.

Hydrolytic enzymes of plant or pathogen origin also catalyse the release of plant cell wall fragments (endogenous elicitors) that elicit defence responses. For example, polygalacturonase enzymes released by fruit decay fungi and bacteria dissolve the middle lamella of plant tissues. While this facilitates pathogen colonisation, it also causes the release of pectic fragments, oligosaccharides consisting of nine to thirteen polygalacturonate units, that are potent elicitors.

A number of peptides and glycoproteins that elicit defence responses in plants have been isolated from culture filtrates of bacterial and fungal pathogens. A 46 ID glycoprotein extracted from culture filtrates of the black shank pathogen, Phptophthorn nicotiartne var. nicotionne and from tobacco leaves infected with this pathogen, is a potent elicitor. There is some evidence that Ppn 46E, a 46 kD glycoprotein, has endoxylanase activity, suggesting that it may also elicit through the release of cell wall fragments. A 42 kD glycoprotein with glucanase activity has been isolated from Phptophthora *inegasperrna* f. sp. *gtyciiiea.* The active fragment of this glycoprotein is a thirteen-amino acid peptide that binds to a receptor on the host plasma membrane. These elicitors are found in both avirulent and virulent isolates, suggesting that their activity does not determine resistance.

A family of 10kD peptides called elieitins' has been isolated from culture filtrates of Phptophthorn spp. and a number of related oomycetes. There are two groups of elicitins (i) the acidic n -elicitins such as parasiticein produced by

P. nicotinrtne var. *parasitica,* and capsicein produced by *P. capsici* and (ii) the basic b-elicitins such as cryptogein, produced by P. *c ptogea,* melonin produced by *P.* melonis and cinnamomin produced by *P.* cirtnamomi. All elicit systemic necrosis in tobacco. Elicitins are translocated when applied to the plant, but they

have yet to be found at the infection court. They are not known to have any metabolic function in the fungi that produce them. Highly aggressive isolates of *P.* riicotionae var. nicotinnne do not release an elicitin and do not elicit host

defence responses. However, less aggressive isolates and isolates from hosts other than tobacco, release parasiticein. This evidence indicates that elicitin release may limit the host range of certain oomycetes. The black shank pathogen is a biotroph in the early stages of infection and aggressive mutants with low elicitin levels may have been selected during co-evolution with its host, tobacco.

Polyunsaturated fatty acids like arachidonic and eicosapentaenoic acid from cell membranes of Phptophthorn in/estnris elicit defence responses in potato slices. Although they have lower elicitor activity in other plants when applied on their own, these fatty acids enhance the elicitor activity of glucans when applied in combinations. This, and other evidence, indicates that the complex responses of some infected plants may depend on the recognition of a combination of elicitors.

Gene-spec/fic *elicitors*

Gene-specific elicitors are those conditioned by avirulence genes in the pathogen. Their activity precisely matches the gene-for-gene hypothesis. Only recently has the application of molecular techniques allowed the characterisation of a few gene-specific elicitors, although their presence has been inferred for many years. A series of race-specific peptide products of the avirulence genes of Fttlria Inn, a biotrophic pathogen of tomato, has been identified. These peptides were first isolated from intercellular fluids of infected leaves and have since been found around the infection site.

A heat labile exudate from germinating basidiospores of incompatible races of cowpea rust (*Uromyces uignae!* elicits defence responses only in cowpeas with the corresponding resistance gene. Similarly, a 6.4 kD peptide from the barley leaf scald pathogen, Hhpnchosporium secatis, specifically elicits resistance in cultivars with the corresponding resistance gene. Host receptors for these peptides have yet to be identified.

A number of avirulence genes have been identified in plant pathogenic bacteria, although their gene products are yet to be characterised. Avirulence (orr) genes determine host range (species /pathovar and cultivar/race interactions) according to the gene-for-gene hypothesis. However, studies with genetically-transformed bacteria show that nor genes only appear to function in the presence of another set of genes, the hrp (hypersensitive response and pathogenicity) gene cluster. Hrp genes are found among a wide range of pathogenic and non-pathogenic Gram-negative bacteria. They function as pathogenicity genes in the absence of the nor gene and hypersensitive response- eliciting genes in their presence. One of these hrp genes encodes a heat stable protein, harpin, that is involved in membrane transport. Clusters of harpin subunits apparently line a pore allowing secretion of apr gene products. *Hrp* gene products are also involved in the secretion of the extracellular polysaccharides that disguise the pathogen from host recognition, thus functioning in both virulence and avirulence.

Suppressors *and compatibility factors*

It has been proposed that compatibility factors operate at two levels. All biotrophs must establish basic compatibility with their hosts. Virulent races might also produce specific compatibility factors that delay, avoid or negate recognition by normally resistant cultivars of a host species. Experiments using a range of host— parasite interactions have demonstrated that co-inoculation of a host with compatible and incompatible strains of a pathogen allows the normally avirulent strain to infect, colonise and reproduce (Fig. 17.3). These results suggest that the

virulent isolate somehow suppresses the resistance mechanisms of the host. However, if the virulent strain is inoculated some hours after the avirulent strain, the host is resistant to both, indicating that suppressors are unable to switch off resistance responses once they are activated. Water-soluble molecules found on the surface of virulent, but not avirulent, isolates of Phptophthorn irr/estnns suppress defence responses in potato tuber slices. Glycopeptides produced by Ascochqfn rnbiet and *Mycosphaerelta pier* suppress defence responses in their respective hosts, chickpea and pea. Such interactions may be common in nature.

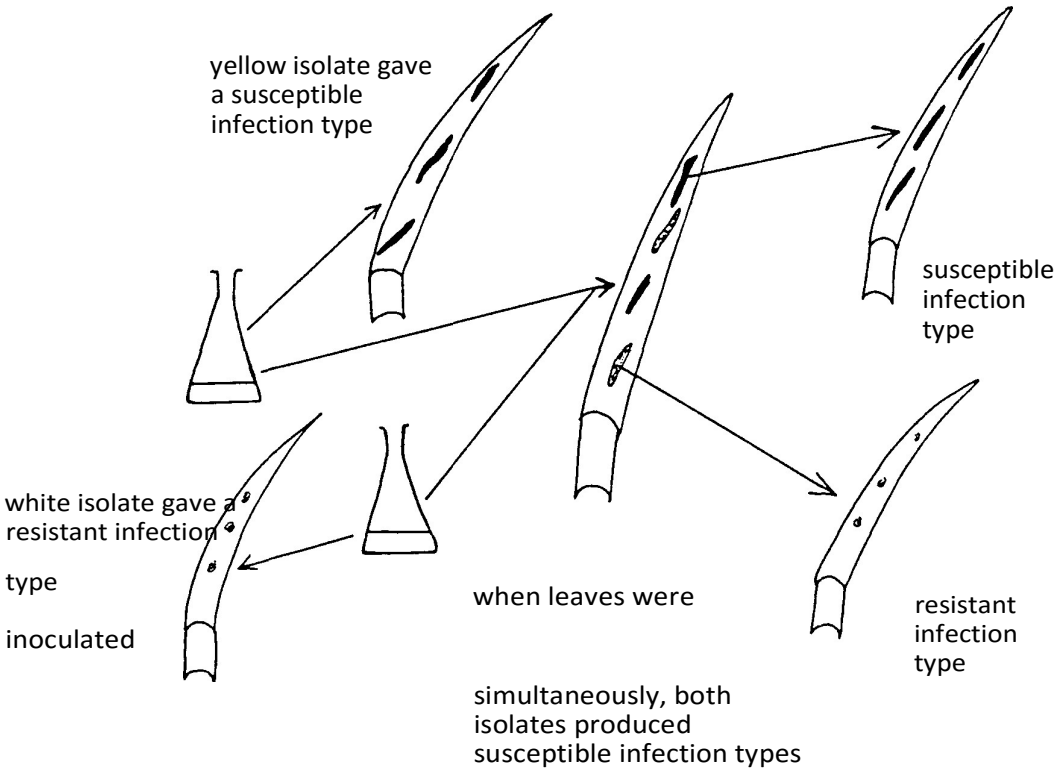


Figure 17.3 Induced susceptibility to stripe rust of wheat induced by simultaneous inoculation of plants with compatible and incompatible isolates of Pttccinin *striiformis.* (After Brown and Sharp, 1970.}

Physiological role of elicitors

To understand fully how the discriminatory expression of active defence responses determines resistance or susceptibility, we must understand the basis of specificity. In other words, why do incompatible pathogens trigger plant defence responses, while compatible pathogens do not?

The simplest prediction from the gene-for-gene hypothesis would be that avirulence and resistance gene products recognise each other, triggering a race- specific response. Only recently have molecules been identified that elicit plant defence responses according to the gene-for-gene hypothesis. These molecules are peptides encoded by avirulence genes, and some, perhaps all, bind to receptor peptides encoded by host resistance genes. Of the half-dozen or so resistance genes sequenced, most have some homology to genes encoding proteins involved in protein-protein interactions in cells, such as protein kinases and polygalacturonase-inhibiting proteins. Some are

membrane-bound, while others are cytoplasmic. Activation of these proteins following the recognition of avirulence gene products triggers a cellular alarm mechanism, involving signal transduction pathways that lead to a massive shift in gene transcription and

plant cell metabolism. As well, local and systemic signals are released that prime the plant against further infection.

Specific recognition takes place against a background of non-specific events triggered by the multitude of molecules produced by pathogens that are recognised by plants as non-self. The lack of race-specificity of these elicitors makes their role in disease resistance unclear. Furthermore, the relevance of many non-specific elicitors to recognition in host—parasite interactions is questionable as they have been isolated from cultures of the pathogen rather than from the infection court. For example, culture filtrates of Monitinia cticoln contain a small peptide, monilicolin A, that elicits defence responses in pea pod cavities. However, M. /ructicota is not a pathogen of pea and monilicolin A is inactive on natural hosts of the pathogen such as peach.

Non-specific elicitors present at the infection court may simply function to amplify the defence response. Cell wall fragments, released from both the host and pathogen in increasing quantities as colonisation is attempted, activate responses that are amplified in a positive feedback loop. Combined signals from the pathogen and host could help the plant differentiate between damage and infection.

Evolution of host-parasite specificity

Clearly, pathogens produce a diverse range of molecules able to elicit host defence mechanisms. Only a few of these elicitors define the pathotype—cultivar, or even species, specificity characteristic of the hosts they were extracted from. If one assumes that disease resistance in plants is due to an active response, this is not surprising. There would have been a heavy selection pressure on individual plants with the ability to recognise and resist pathogens with the potential either to kill them or to reduce their fitness. Thus, any molecule released by a potential pathogen could function as an elicitor, whether or not that molecule has anything to do with virulence. Examples might include cell-wall fragments, membrane lipids or extracellular enzymes, none of which is specific to avirulent races of a pathogen. On the other hand, gene-for-gene or pathotype-specific resistance is determined by the interaction between products of pathogen avirulence genes, gene-specific elicitors, and products of host resistance genes.

What do elicitors do? Where do they bind? Where do they act? The defence responses of plants are very rapid. Host gene expression begins within minutes or even seconds of exposure to elicitors or pathogens. Elicitors may act directly on host genes as regulators. However, the diversity of elicitors that activate a common suite of responses suggests that second messengers are involved and that elicitors induce a range of responses through complementary action. This notion is supported by the recent identification of resistance gene products that appear to be membrane-bound proteins involved in signal transduction, by the involvement of active oxygen as a second messenger and by the identification of salicylic acid as a common mediator of systemic defence responses.

An alternative explanation for the evolution of host—parasite specificity proposes that random mutations might confer the ability to produce host-specific toxins. The dependence of formae speciales of Altemnrin *atternata* on host-specific toxins for virulence on their respective hosts described in the previous section supports this explanation. These toxins allow an otherwise saprophytic organism to necrotrophically colonise a previously unavailable host. These mutants, being able to occupy a new ecological niche, have an evolutionary advantage in the presence of their hosts. Here, virulence, rather than host resistance, is the active phenomenon that was selected under evolutionary pressure.

Rapid active defences

Plant responses to infection are complex and there is no universal model or sequence of events that accurately describes the dynamics of resistance in the few interactions studied, let alone the vast majority of undescribed interactions. Almost every host—parasite interaction is unique in the details of the activation, localisation, timing and magnitude of each component of the defence response. As previously stated, resistance is rarely absolute and whether a plant ends up being resistant or susceptible depends on the sum of many individual responses.

Changes in membrane function

Most studies on the earliest stages of the host—parasite interaction conclude that the host membrane is involved in pathogen recognition and signal transduction. Membrane permeability changes rapidly following the exposure of plant cell suspension cultures to fungal and bacterial elicitors, usually leading to a loss of cellular electrolytes such as K“ and an uptake of H +. At the same time, there is often an influx of Ca^{2+} , a key intracellular signal in plants that is involved in the activation of enzymes and gene expression. The experimental blocking of Ca^{2+} transport across membranes in inoculated bean cells also inhibits gene activation and subsequent defence responses.

The oxidative burst

Membranes are also the sites where the oxidative burst occurs. The term ‘oxidative burst’ was first used to describe a rapid increase in respiration observed in neutrophils involved in the immune response of mammals. This increased level of respiration is now known to be due to the generation of reactive oxygen species, especially hydrogen peroxide and the superoxide anion ( ). through the addition of electrons to 2 catalysed by the membrane-bound enzyme, NADPH oxidoreductase. Reactive oxygen species are also produced by errors in electron transport during respiratory and photosynthetic reactions in plant cells. Cells are normally protected from the damaging effects of reactive oxygen by superoxide dismutase, various peroxidases and catalase and by natural antioxidants such as carotenes. The pioneering work of Dole and his colleagues at Nagoya University in Japan revealed that slices of potato tuber exposed to compatible and incompatible races of the late blight pathogen, Phptophthorn in/estans, undergo a two-step oxidative burst. The first burst rapidly follows wounding and inoculation, while a much larger burst in

incompatible interactions immediately precedes hypersensitive cell death. Since then, an oxidative burst has been described in a range of plant-fungal and plant- bacterial interactions. The rapid oxidative burst generates levels of reactive oxygen species that initiate membrane lipid peroxidation and cell death. The oxidative burst in plants is associated with the release of local and systemic signals that trigger gene expression and the oxidative cross-linking of host cell wall components. Levels of reactive oxygen species accumulate at the infection court that are sufficient to kill micro-organisms in vitro. Experimental suppression of the oxidative burst shows that it is involved in initiating later defence responses. On the other hand, colonisation of avocado fruit by the necrotroph, Not tis cinereo, apparently exploits the oxidative burst to kill host cells in advance of invasion.

Cell wall reinforcement

The first visible response to attempted penetration of plant cell walls by pathogens is often the intensification of cytoplasmic streaming followed by the accumulation of host cytoplasm under the site of attempted penetration. These cytoplasmic aggregates are thought to contain the cellular apparatus for the synthesis of cell wall fortifications. Most pathogens must penetrate host cell walls at some stage, either as germ tubes, hyphae or haustoria. If the cell can respond quickly enough to repair or reinforce the cell wall, penetration efficiency may be reduced and pathogen development retarded.

A number of different types of cell wall fortifications are produced in response to the attempted penetration of plant cell walls. Some pathogens induce the deposition of a papilla, a reinforcement composed of a branched §-1, 3 glucan, callose, along with silicon, lignin and proteins, between the host cell wall and plasma membrane, directly under the penetration peg. The rapid deposition of papillae is a common response of cereals to attempted penetration of epidermal cells by the powdery mildew fungus *iBtumeria* prnminis). Papillae in resistant cultivars form more rapidly and are more difficult to penetrate, than those formed by susceptible cultivars. As a result, haustorial development is prevented. Lignitubers are lignified callose deposits that ensheath invading hyphal tips (Fig. 17.4A). Lignitubers have been observed in both resistant and susceptible cereals following challenge by the take-all pathogen, Gaeiimnnrtompces qraminis, demonstrating again the importance of timing—the more rapid the response, the more likely it is to succeed.

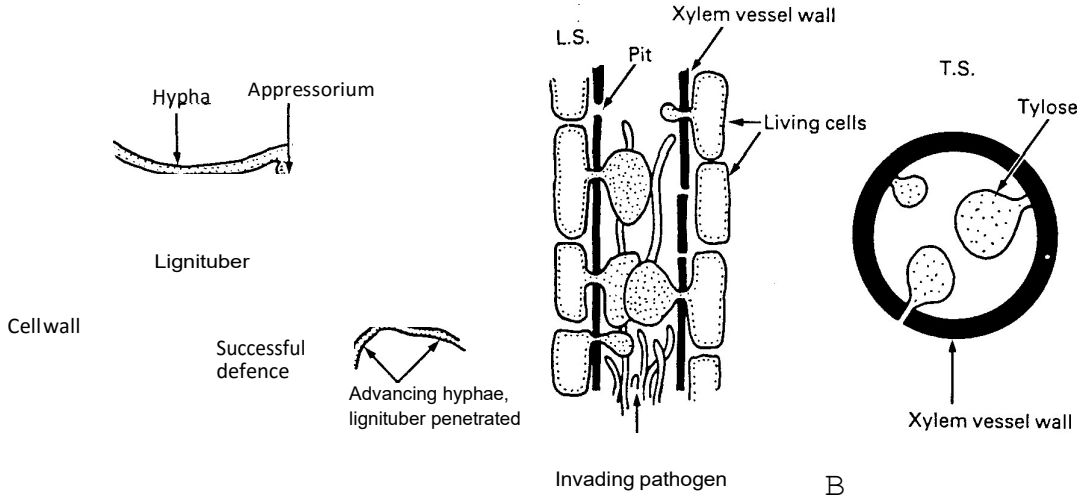
Hydroxyproline-rich glycoproteins are structural proteins in plant cell walls involved in the organisation of secondary cell wall thickening. Genes encoding hydroxyproline-rich glycoprotein biosynthesis are transcribed in advance of invading hyphae, making cell walls tougher. Hydrogen peroxide, released during the oxidative burst following pathogen challenge, causes extensive cross-linking between hydroxyproline-rich glycoproteins and other cell wall components, making the walls even more resistant to microbial digestion.

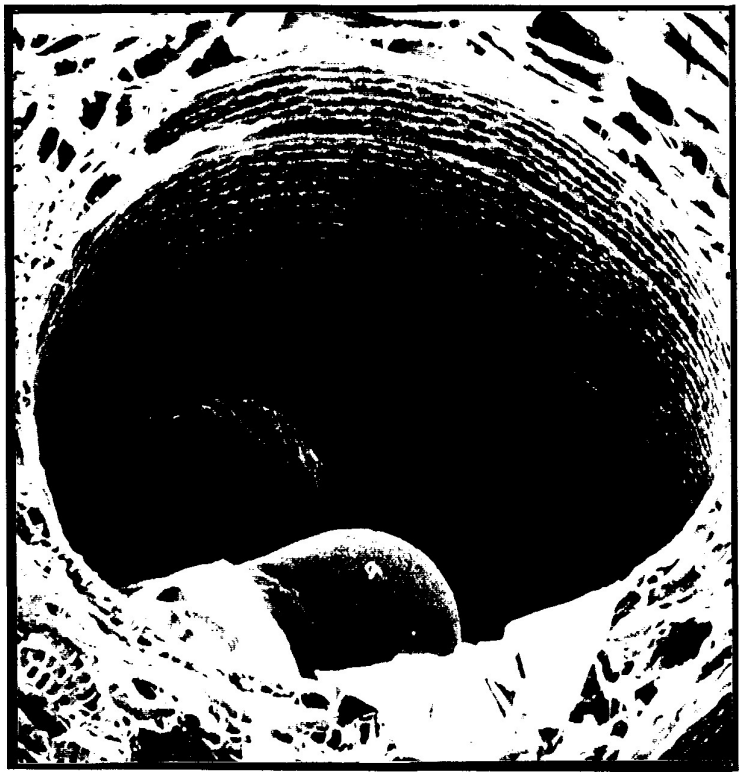
Cross-linked hydroxyproline-rich glycoproteins also provide a focus for lignin deposition on the plant cell wall. The rapid deposition of lignin and suberin following infection is associated with resistance to non-pathogens and to avirulent pathogens in many plants, including cereals, Solanaceae, brassicas, melons and carrots. Lignin deposited on plant cell walls ahead of invading hyphae increase their resistance to fungal penetration. Lignin also binds to hyphal tips and bacterial cells, preventing further growth and movement and restricting the diffusion of pathogen enzymes and toxins and the uptake of water and nutrients by the pathogen. Furthermore, precursor molecules and free radicals produced during lignin biosynthesis are toxic to pathogens and inactivate pathogen enzymes, toxins, elicitors or suppressors. The effect of lignin can be further enhanced by the release of reactive oxygen species and the activation of phenol oxidase enzymes that convert phenolic compounds to more toxic complex polymerised phenolics and quinones during the defence response.

The evidence that cell wall reinforcements are important components of plant disease resistance can be summarised as follows:

- Their deposition often coincides with failed penetration and sometimes precedes the cessation of pathogen growth.
- Reinforcements in resistant hosts are larger, form more quickly (often before penetration) and are more dense than those formed by susceptible hosts.
- Experimental attempts to re-penetrate induced reinforcements usually fail.
- Inhibition of lignin or callose biosynthesis enhances penetration efficiency.

However, the deposition of cell wall reinforcements is not always associated with disease resistance. Clearly, cell wall reinforcements contribute to reslstance and cell repair but are not always sufficient on their own to prevent infection.





C

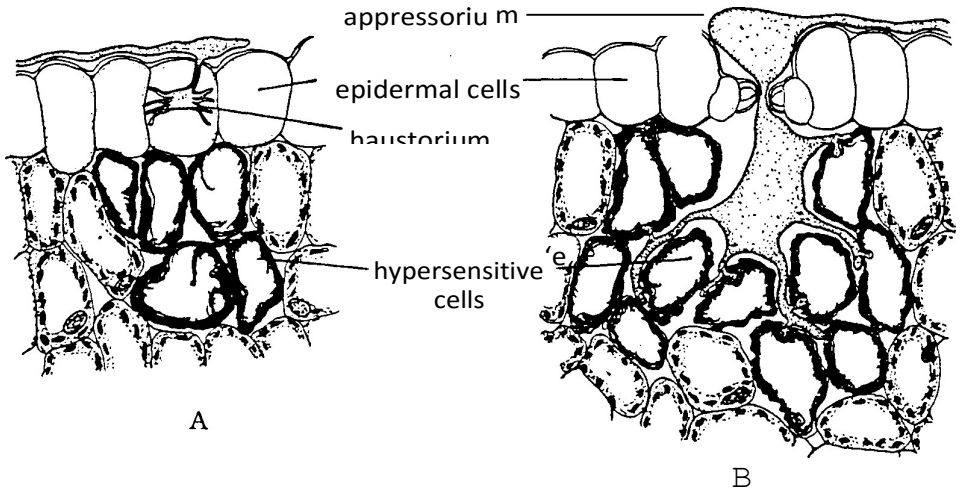
ylgure 17.4 Induced mechanical barriers to infection. (A) Lignituber formation in a root of a wheat seedling in response to infection by Gneiirnnnnom9ces *graminis.* (From Parry, 1990.) (B) Diagrammatic representation of tyloses in response to invasion of the xylem by a vascular wilt fungus. (From Parry, 199O.)

(C) Scanning electron micrograph of tyloses formed in the xylem of maize in

response to infection by Verticitlium nlboatrum. (From Troughton and Sampson, 1973.)

Hypersensitive cell death

In 1902 Harry Marshall Ward, Professor of Botany at Cambridge University in England, observed an association between necrotic mesophyll cells in *Browne* sp. and attempted infection of resistant cultivars by the leaf rust fungus, Puccinin reconditn. Later E. C. Stakman at the University of Minnesota reported similar observations in resistant wheat cultivars infected with the stem rust pathogen, P. praminis, and in 1915 he introduced the term hypersensitivity to describe this necrotic host reaction. Stakman contended that the more resistant the cultivar, the more rapid was the collapse of host cells and the sooner the fungus was inactivated. The term hypersensitivity indicates that the host cells are 'over- (hyper-) sensitive' to the presence of the pathogen. Host cells suicide in the presence of the pathogen, preventing further spread of the infection (Fig. 17.5). In some cases hypersensitive cell death kills the invading pathogen (e.g. Hhizoctonin soioni) while in others it is fungistatic (e.g. Puccirtin praminis). Hypersensitive cell death is a widespread, but not universal, response to incompatible viral, bacterial, fungal and insect attack in the plant kingdom.



rigure 17.5 Wheat leaves showing the hypersensitive reaction in response to infection by (A) *Blumeria* Srnmints and (B) Puccinin *graininis.* (From Brown, 1980.)

Typically, hypersensitive cell death is preceded by a rapid oxidative burst, an increase in cytoplasmic streaming, cytoplasmic aggregation followed by granulation, membrane disruption, cellular decompartmentalisation and browning usually within 12—24 hours of attempted penetration (Fig. 17.6). Hypersensitive cell death in plant cells shares many features in common with apoptosis, or programmed cell death, observed during development of defence against disease in animals. Apoptosis is a distinct form of cell suicide directed by the dying cell and regulated by a number of identified genes. Animal cells undergoing apoptosis shrink, their DNA is digested into fragments of 180 base pairs and multiples of 180 base pairs and these fragments are organised into apoptotic bodies, seen as 'blebs' on the nuclear membrane. These orderly fragments of DNA are resolved as 'DNA ladders' by gel electrophoresis. The emerging similarities between hypersensitive cell death in plants and apoptosis in animal cells suggest that cell suicide is an ancient defence response.

It is not always easy to conclude from research data whether host cell death is a consequence of murder or suicide. Recent experiments have shown that in many host—parasite interactions hypersensitive cell death precedes pathogen

death, regardless of whether biotrophic or necrotrophic pathogens were involved. In some interactions however, disease resistance does not depend on hypersensitive cell death. The success of hypersensitive cell death as a resistance mechanism in individual host-parasite interactions depends on the nutritional requirements of the pathogen and on the timing, location and magnitude of the host response in relation to pathogen development. In some interactions the rapid suicide of challenged host cells undoubtedly restricts pathogen development, contributing to the overall defence response.

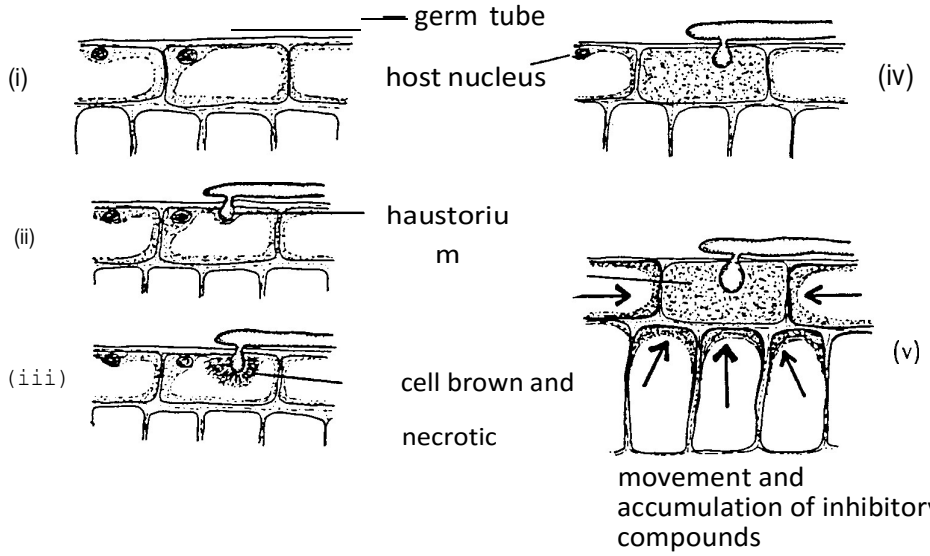


Figure 17.6 Sequence of events leading to the hypersensitive reaction in plants infected by incompatible pathogens. (From Brown, 1980.)

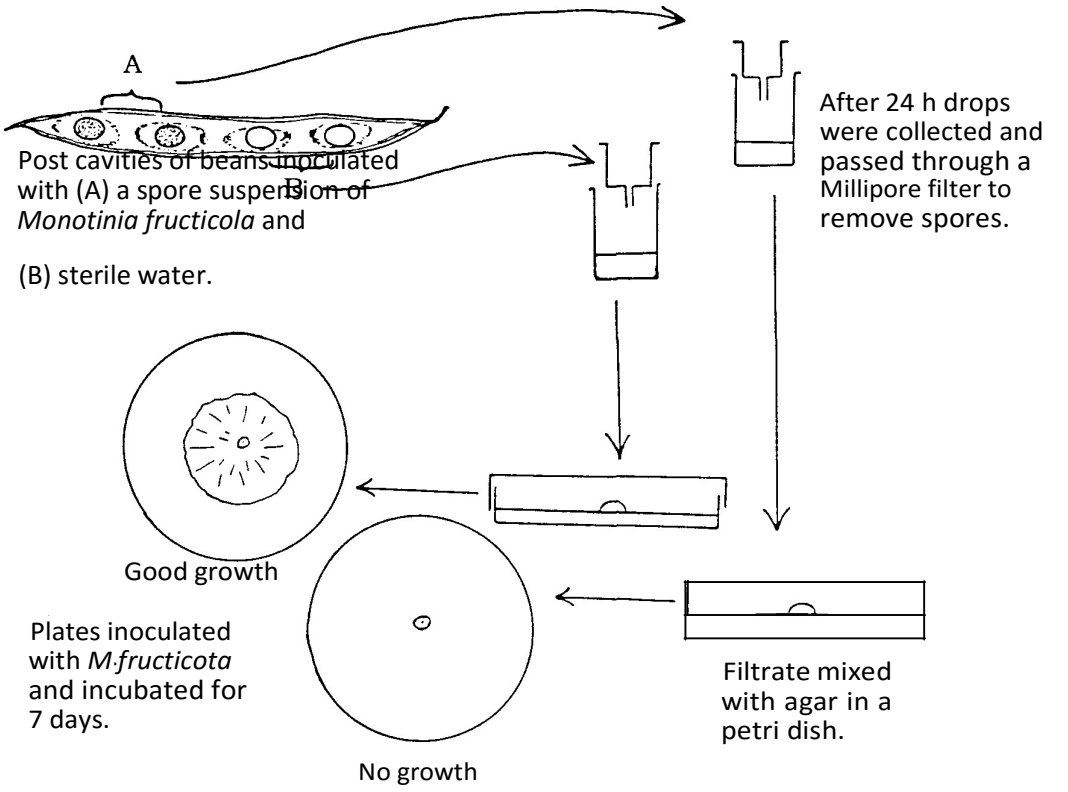
Phytoalexins

Phytoalexins are low molecular weight antibiotics produced by plants in response to infection. Their toxicity is non-selective and the chemical affinity of most phytoalexins for lipids suggests that they accumulate in cell membranes. For phytoalexins to play a role in disease resistance, they must accumulate to inhibitory levels at the infection court and restrict further development of the pathogen.

Evidence for the synthesis of antibiotics in infected plants has been accumulating for much of the twentieth century. In 1909 Bernard found that some fungi rotted ungerminated orchid seeds, others penetrated several layers of cells before stopping and disintegrating, while others colonised the seed and established a successful mycorrhizal association with the seedling. Fungi that penetrated a few cell layers, but were then destroyed, induced resistance to subsequent infections by seed-rotting fungi. Nobécourt, in 1923, showed that this induced resistance was due to the synthesis of antibiotics by the seed. In 1945 Ernst Gaumann working in Switzerland identified these inhibitors as two phenolic compounds, orchinol and hircinol. At about the same time Muller and Borger in Germany found that slices of potato tuber reacting hypersensitively to Phptophthora in/estnns produced antibiotics that protected the tissue against subsequent infection by normally virulent strains of the pathogen.

After World War II, K. O. Muller moved to the CSIRO in Canberra, where he studied responses of the seed cavity of french bean pods to spores of the peach pathogen, Monitinia *fructicota.* While water droplets from uninoculated cavities

stimulated fungal grOwth, inoculated cavities became necrotic and diffusates became inhibitory to fungal growth within 24 h of inoculation (Fig. 17.7). The unidentified inhibitor was extracted with organic solvents and was termed a phytoalexin (from the Greek words meaning plant defender). This inhibitor was subsequently purified by a team led by Ian Cruickshank at the CSIRO, found to be a phenylpropanoid compound and named phaseollin. A related compound, pisatin, was identified in pea pods inoculated with M. *fructicota* or in pod cavities exposed to a peptide, morrilicolin A, extracted from this fungus.



Since then over 350 phytoalexins have been found in over 100 plant species from 30 families of dicotyledons and monocotyledons (Table 17.1). Phytoalexins have been isolated from all parts of plants but different organs may accumulate different phytoalexins. The cherrñcal structure of phytoalexins is diverse but, with one exception, they are small organic compounds synthesised from one of three secondary metabolic pathways—the acetate-mevalonate, actetate-malonate or shikirnic acid pathways. The notable exception is the recent report of elemental sulphur accumulating in and around xylem vessels of cocoa infected with the vascular wilt pathogen, Uerticilliitm dnh rue. In general, related plant species synthesise chemically-related phytoalexins. Most plant species produce several, chemically related phytoalexins, presenting a toxic cocktail to any invading pathogen. For example, many legumes synthesise phenylpropanoid phytoalexins via the shikimic acid and acetate—malonate pathways, while most solanaceous plants produce terpenoid phytoalexins via the acetate—mevalonate pathway.

French bean produces at least five phenylpropanoid phytoalexins, while potato synthesises at least four terpenoids.

Table 17.1 Examples of phytoalexins produced by higher plants.

Structure	Name	Plants involved
Inorganic	sulphur	cocoa
Phenolic	chlorogenic acid	potato, tobacco, apple
	avenalumins	some cereals
Terpenoid	capsidiol	capsicum, tobacco
	rishitin	potato, tobacco, tomato ipomeamarone sweet potato
	gossypol	cotton
Phenylpropanoid	pisatin	pea
	phaseollin	french bean, cowpea
	kievitone	french bean, cowpea
	glyceollins	soybean
	medicarpin	alfalfa, clover, broad bean, chickpea
	scoparone	citrus
Acetylenic	wyerone	broad bean
	safynol	safflower
Stilbene	resveratrol	grape, peanut
	batatasins	yarn
Indole-sulphur	camalexin	*Arnbidopsis*
	brassinins	cabbage, rape, turnip

Phytoalexins are thought to be synthesised in cells adjacent to the infection site, in response to a signal produced either by the invading pathogen or by infected host cells. They are packaged in lipid vesicles and exported to the infected cell. Consequently, the infected cell becomes a toxic micro-environment for the invading pathogen. Phytoalexin accumulation is often associated with hypersensitive cell death. However, phytoalexin biosynthesis requires gene expression and the activation of complex biochemical pathways involving perhaps 20 enzymes, which must occur in living cells. Many steps in their biosynthesis are sensitive to regulation by the host and the pathogen. Some plants, such as soybean and chickpea, synthesise phytoalexins upon infection, but convert a proportion into inactive sugar conjugates held in reserve in vacuoles. If the initial defence response fails to check pathogen growth, enzymes that cleave the sugar molecule are activated and the phytoalexin reserves are rapidly released.

Life other active defence responses, the success of phytoalexin accumulation depends on the speed, location and magnitude of the response. There is a good experimental correlation between resistance and rapid, localised phytoalexin accumulation in many host—parasite interactions. There is evidence that:

- phytoalexins accumulate faster and to higher concentrations in resistant cultivars. In resistant plants, gene transcription begins within one hour of recognition, phytoalexins appear within four hours and concentrations peak to fungitoxic levels about 18—24 hours after challenge (Fig. 17.8). These events are delayed and more diffuse in susceptible plants.
- phytoalexin biosynthesis is localised in cells immediately surrounding the infection court. There is no evidence that they disperse in the plant. Experiments using laser microprobe analysis, radioimmunoassay,

hybridisation histochemistry and immunocytochemistry of the phytoalexin biosynthesis pathway have confirmed this in several host—pathogen interactions.

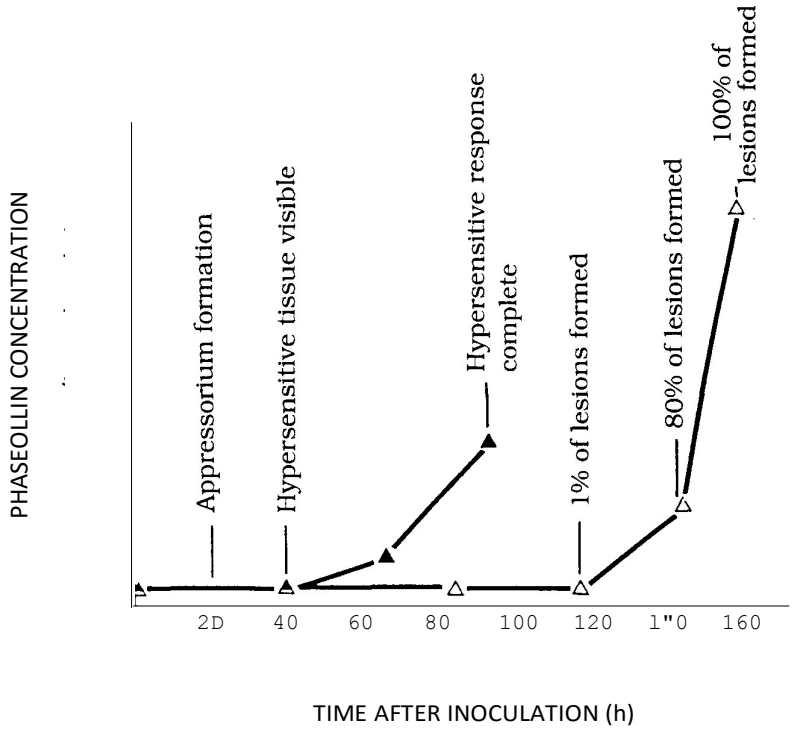


Figure 17.8 Accumulation of phaseollin in beans inoculated with compatible (A) and incompatible strains (A) of *Colletotriciium* lindemiithinnum. (From Bailey and Deverall, 197 l .)

- in a number of interactions, resistance is lost if phytoalexin biosynthesis is blocked by inhibitors of enzymes involved in the process of phytoalexin biosynthesis and is reduced in mutants that are slow to accumulate phytoalexins.
- resistance is increased in plants transformed to express novel phytoalexins or if exogenous phytoalexins are applied. For example, although the biochemical precursor of resveratrol is widely distributed in the plant kingdom, only grapevine and peanut have the enzyme required to complete its synthesls. When the genes encoding this enzyme are transformed into tobacco, resveratrol is synthesised in response to infection.

Phytoalexin synthesis is not universal among plants. Wheat and cucumber apparently do not produce phytoalexins, yet effectively resist most pathogenic fungi and bacteria. Nevertheless, in many interactions the rapid accumulation of toxic concentrations of phytoalexins at the infection court plays a decisive role in the expression of resistance.

Delayed active defences

Pathogen containment and wound repair

While earlier responses retard the development of pathogens, later responses restrict their spread and contain the damage to host tissues. The ability of a plant to repair tissue damage may contribute to its ability to fight off secondary

infections by opportunistic pathogens. Infected areas of fleshy tissues, roots, fruits and bark are sealed by layers of cork cells with thick, suberised walls. Wound cork is produced by a secondary meristem, the cork cambium, formed from mature parenchyma tissue in response to the damage caused by infection. In some cases, such as in the response of potato tuber tissue to the powdery scab pathogen *i!Spongospora* subterraneo), cork barriers appear to seal the infected area and prevent further colonisation by the pathogen. However in other interactions, including the response of brassicas to the leaf spot pathogen, Altemarin brassicne, cork layers do not restrict infection. Some pathogens induce plants to form abscission layers in which cork cambium develops around the infected area and extends from the upper to lower surface of the infected leaf. The infected areas fall out, leaving the classical 'shothole' symptom. Such pathogens include Stipminn *carpophila* and Pseiidomonns sprinpne pv. morsprunorum on plum and Cercosporn beticoln on silverbeet. Wounded tree trunks often secrete gums that effectively seal the wound from opportunistic pathogens.

If pathogen growth is retarded by environmental conditions or other disease resistance mechanisms, induced barriers may also prevent further colonisation by the pathogen or by secondary invaders. However, there is little direct evidence to support a decisive role in resistance for wound repair. It has been said that these barriers are 'of no greater significance than a monument on a battlefield; it merely marked the place where an issue was decided' (William Brown, 1955).

Tyloses are ingrowths of the protoplasts of xylem parenchyma through xylem vessel pits into the lumen of xylem vessels (Fig. 17.4B and C). They are thought to impede the progress of fungal and bacterial vascular wilt pathogens such as Ftisariiim *oxigsporurn, Verticitliuin* dahline and Halstonin solnnnceariim. If tyloses form rapidly enough ahead of the advancing pathogen they may restrict colonisation or the spread of propagules in the xylem. The formation of tyloses involves a cost to the plant, as they not only block the spread of the pathogen, but reduce the translocation of water, possibly causing wilt symptoms.

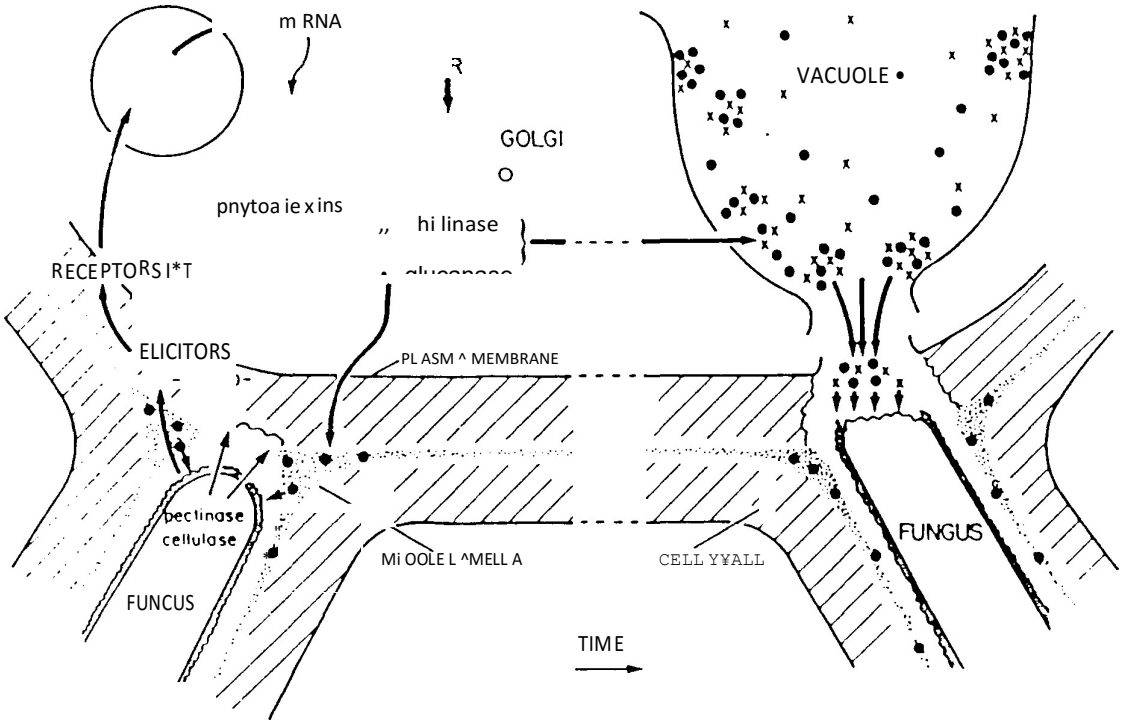
Pathogenesis-related proteins

During the massive shift in cellular metabolism and gene expression referred to earlier, plants synthesise many novel proteins following infection. Some of these novel proteins may be enzymes involved in phytoalexin biosynthesis and some may have no role in disease resistance at all. However, the pathogenesis-related proteins' have §-glucanase, chitinase or lysozyme activity. Some are related to plant defensins while others are proteinase inhibitors that disrupt pathogen nutrition. Pathogenesis-related proteins are sometimes present in low levels before infection and are induced following stress, wounding or flowering, indicating that they may have a wider function in plant growth and development than just disease resistance.

Sixteen novel proteins have been identified in tobacco mosaic virus-infected tobacco, making up 5—10% of the total leaf protein. These include four chitinases and four glucanases that are small, monomeric, stable at low pH and resistant to protease digestion. Potato leaves infected with Phqtophthorn tn/estans accumulate two §-1,3-glucanases and six chitinases.

Chitinase and glucanase accumulate in vacuoles, although some glucanase is secreted to the intercellular space (Fig. 17.9). These enzymes dissolve fungal cell walls and the fragments released elicit hypersensitive cell death and phytoalexin biosynthesis. Cellular decompartmentalisation during hypersensitive cell death leads to an ambush of the pathogen by a flood of hydrolytic enzymes released from the vacuole. Hydrolytic enzymes have antiviral, antibacterial and antifungal

activity. Plants genetically transformed to overproduce glucanases, chitinases and ribozyme-inactivating proteins show about a 50% reduction in disease severity. Paradoxically, some pathogens exploit the lytic activity of pathogenesis-related proteins to increase their virulence. Glucanases elicited by some viruses increase the porosity of plant cell walls, thus facilitating the movement of viral particles between cells.



Pathogenesis-related proteins accumulate over several days, reaching a maximum about seven to ten days after initial infection. In contrast, gene-for-gene resistance is determined within hours of the initial attack. These results show that hydrolytic enzymes reduce disease susceptibility if they are present at the time of challenge, as in plants with systemic acquired resistance, a response that protects plants against re-infection.

Systemic *acquired resistance*

It has been known since Bernard and Nobécourt's work in the early twentieth century that plants surviving an attack by a pathogen become systemically protected against subsequent infections. In the 1970s, Kuc and his co-workers in the United States showed that inoculation of one cucumber leaf with the anthracnose pathogen, Cotletotrichum *tagenarium.,* protects the entire plant against subsequent infection with the same and other pathogens. Systemic acquired (also called induced) resistance protects against a wide range of pathogens, not just the pathogen that induced the response. In this way systemic acquired resistance fundamentally differs from the specific antigen-antlbody mediated immune response of mammals. The expression of systemic acquired resistance reduces disease severity rather than providing immunity.

There are three steps involved in the development of systemic acquired resistance:

- The induction of systemic acquired resistance usually requires the development of a slowly expanding necrotic lesion. Induction of systemic resistance may be associated with other localised responses such as hypersensitive cell death, phytoalexin accumulation, papilla deposition and lignification.

- Two or three days after the inducing lesion first appears, a signal is released that is systemically translocated in the phloem. This signal is graft- transmissible and is not cultivar, species or genus specific, but is not active once plants have begun flowering. All of the signal originates from the induction site.
- The systemic signal primes the rest of the plant against further pathogen challenge. Defence responses such as the rapid release of reactive oxygen species, hypersensitive cell death, phytoalexin accumulation, and enhanced levels of pathogenesis-related proteins are expressed more rapidly and intensely than in uninduced plants.

The identity of the signal that triggers systemic acquired resistance is the subject of intense study, but remains unresolved. There are several molecules that can induce features characteristic of systemic acquired resistance, including salicylic acid, §-ionone and jasmonic acid. The entire response is, however, apparently mediated by a complex signal transduction pathway regulated by a number of stress signals.

Salicylic acid, a precursor of aspirin widely distributed in the plant kingdom, plays a key role in systemic acquired resistance. Salicylic acid binds to at least two proteins found in plant cell membranes. One salicylic acid-binding protein has catalase activity that is inhibited upon binding, causing a localised build-up of hydrogen peroxide. This form of reactive oxygen, as previously mentioned, causes a number of changes in plant cells that increase their resistance to pathogens. A second, high affinity, salicylic acid-binding protein appears to directly activate gene expression. Levels of salicylic acid rise rapidly around neurotic lesions in plants and remain high in plants that have acquired resistance. However, a series of experiments show that it is a local, rather than a systemically translocated, signal. Although it must be present for systemic acquired resistance to be expressed, salicylic acid is not translocated over long distances in plants and presumably interacts with another systemic signal. Synthetic analogues of salicylic acid, such as dichloroisonicotinic acid (INA) and the benzothiazoles, induce similar responses to those induced by salicylic acid and have potential use as practical disease-protectants. Although INA induces resistance in field and glasshouse trials, the effective dose is sometimes phytotoxic and this risk will probably prevent its commercialisation. A more promising benzothiazole, benzo(1, 2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), is similarly effective but less phytotoxic.

The dynamics and coordination of defence responses

Disease resistance mechanisms may be conveniently classified as either passive or active mechanisms. Passive mechanisms, such as the barriers presented by the cuticle, cell wall and phytoanticipins, exclude saprophytic and epiphytic micro-organisms. Active mechanisms, those activated only upon pathogen challenge, restrict the invading pathogen. Wound repair mechanisms, such as cork layers, papillae, lignitubers and the expression of systemic acquired

resistance, exclude secondary invaders and opportunists and retard colonisation and spread of pathogens that survive or escape initial defence responses.

Active defence responses are most likely to be effective if they are expressed in combination (Table 17. 2). The rapid release of reactive oxygen species and the deposition of papillae, lignin and cross-linked hydroxyproline-rich glycoproteins at the point of penetration of the cell wall are followed by rapid hypersensitive cell death and phytoalexin accumulation. Lytic enzymes accumulate in the intercellular spaces and vacuoles, systemic acquired resistance is activated and wounds and tissue damage are repaired. Plants coordinate these weapons to form a potent arsenal against invading pathogens. The failure of these responses, or their delayed employment, invariably leads to susceptibility. Disease resistance depends on the speed, localisation and magnitude of these responses.

Table 17.2 Events involved in the coordination of defence responses in plants to challenge by pathogens.

Time	Event
Minutes	Membrane depolarisation and electrolyte leakage
	Reactive oxygen generation
	Expression of genes involved in phytoalexin biosynthesis
Hours	Oxidative burst
	Membrane lipid peroxidation
	Rise in salicylic acid levels
	Cytoplasmic aggregation, cell collapse and hypersensitive cell death
	Phytoalexin accumulation
Days	Cell wall reinforcements
	Accumulation of pathogenesis-related proteins Systemic acquired resistance

A pattern is emerging that indicates that the outcome of many, if not all, host— parasite interactions depends on complex interactions between host and pathogen cells. These interactions are conditioned by host and pathogen gene expression, are mediated by chemical signal transduction pathways and involve dynamic interactions between elicitors, enhancers, suppressors, receptors and secondary signals. The dynamics of the interaction is sensitive to environmental fluctuations and is regulated by feedback from both host and pathogen. It is the complexity of plant—pathogen interactions that defines the multitude of possible outcomes.