3 Profiles in Pathogenesis and Mutualism: Powdery Mildews

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CONTENTS

I. The Importance of Powdery Mildews

Powdery mildews are parasitic fungi which infect and cause substantial economic losses on a wide range of agricultural and ornamental plants. There are nearly 700 species of powdery mildews, occurring on about 7600 species of angiosperms, including cereals, fruit crops, cucurbits and ornamentals (Braun et al. 2002). Infection can damage tissue or whole plants, causing defoliation, cosmetic damage and reducing yields and quality. Illustrations of the ranges of symptoms are shown in Fig. 3.1A–E. Further descriptions and suggested control measures are available from the extension service of Oregon State University (http://plant-disease.ippc.orst.edu/index.cfm) and in chapters 14–18 of the book 'The powdery mildews: a comprehensive treatise' (for details, see Braun et al. 2002).

All powdery mildews are obligate parasites, meaning that they require a live host to grow and reproduce. They obtain their nutrients through specialized feeding structures known as haustoria which grow inside living host cells. In early work, a wide range of species were studied (Harper 1905) and detailed cytology was performed on *Phyllactinia corylea*, the powdery mildew pathogen of hazel (*Corylus avellana*; Colson 1938). Barley powdery mildew (*Blumeria graminis* f. sp *hordei, Bgh*) is the most intensively studied of all powdery mildew fungi due to its economic importance and easily observed developmental biology (Both et al. 2005a, b; Zhang et al. 2005). Additional information on the biology of *Bgh* is available on the '*Blumeria graminis* Sequencing Project' website (http://www.blugen.org/). Powdery mildew of *Arabidopsis thaliana* caused by *Erysiphe cichoracearum* (*Ec*) has also received considerable attention because of the genetic tractability of the model host plant. In this chapter I cite mostly work from *Bgh* and *Ec*, whilst referring to work on other powdery mildews where appropriate.

Powdery mildews belong to the order Erysiphales within the Ascomycetes (Sac Fungi). There are about 18 genera currently described, which include the important plant pathogens: *Blumeria*, *Erysiphe*, *Sphaerotheca*, *Uncinula*, *Microsphaera*, *Phyllactinia*, *Podosphaera* and *Leveillula*. Characteristics of value for the taxonomy of powdery mildews include primary and secondary mycelia, the shape of appressoria and haustoria and the shape and fine structure of conidia. For those genera with teleomorphic states, the shape and structure of cleistothecia, asci and ascospores are important. The history of powdery mildew taxonomy and details of the important characteristics for their classification are provided by Braun et al. (2002). Illustrations of cleistothecia, asci and ascospores are shown in Fig. 3.1G–I.

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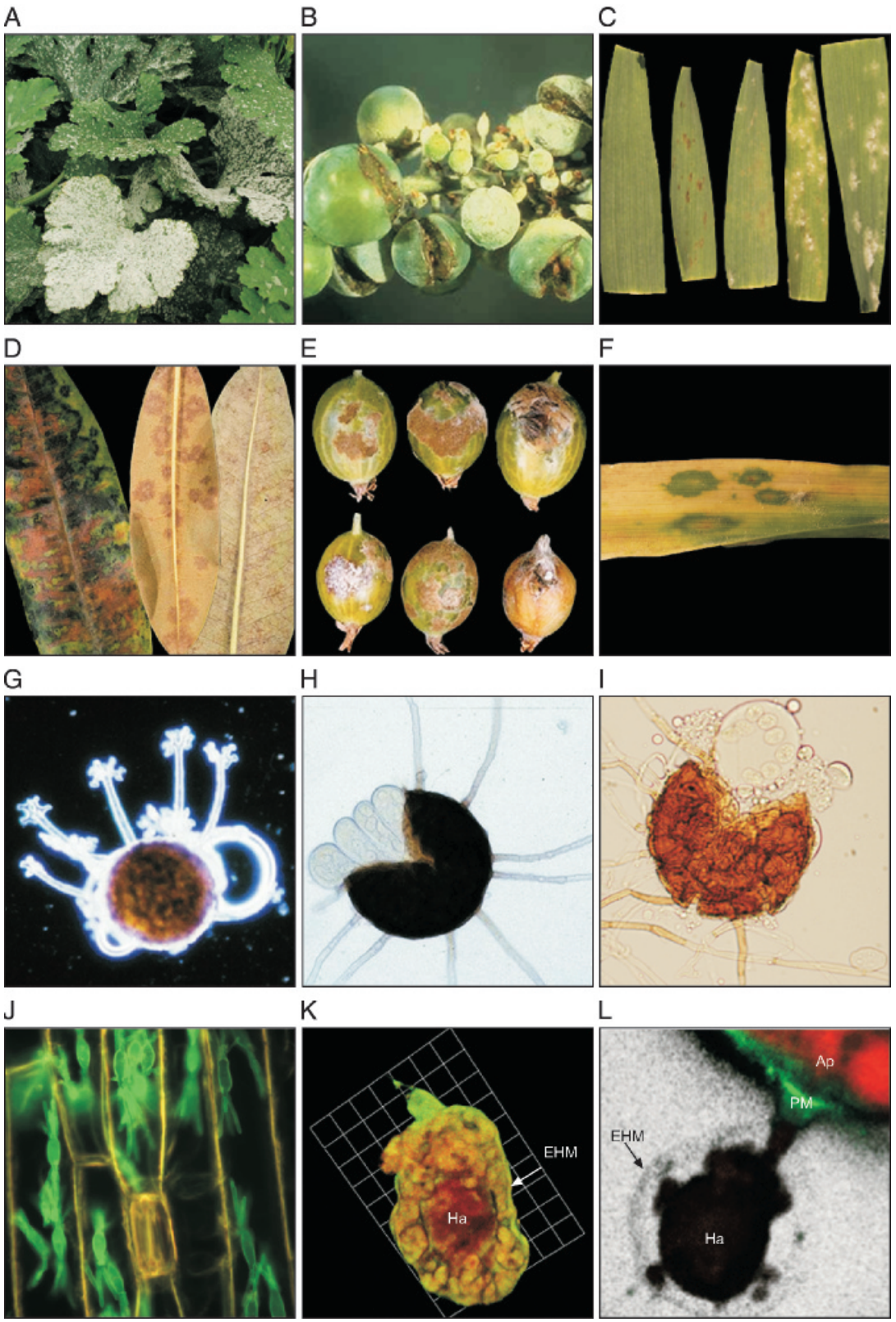
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II. Life Cycle of Powdery Mildews

A. Asexual Reproduction

The predominant mode of reproduction in powdery mildew fungi is the dispersal of asexual conidiospores, produced abundantly on leaf surfaces and other aerial parts of plants. These oval-shaped spores are produced in chains from conidiophores and are dispersed to plants by wind. Conidiospores are susceptible to desiccation and remain viable for only a few days at ambient temperatures although they can survive for 3–4 weeks below −4 °C (Cherewick 1944). They can be maintained in the laboratory by regularly transferring spores onto fresh, living host plant tissue, although a method of freezing spores at −80 °C for long-term storage has been reported (Pérez-Garcia et al. 2006). I describe here the infection process of *Bgh* as an example of that which occurs in other powdery mildew fungi and which is summarized in Fig. 3.2. The conidiospores of *Bgh* land on the leaf surface and within minutes release enzymes onto the leaf surface; these may prepare the infection court and facilitate subsequent fungal development (Carver et al. 1999). Within 1 h, a short primary germ tube (PGT) forms to only breach the epidermal cuticle. *Bgh* is unique in the formation of a PGT, but otherwise the infection process is similar in other powdery mildews. The PGT may contribute to the absorption of water and solutes present in the host cell wall for subsequent use by the conidiospore (Edwards 2002). Extracellular material is secreted beneath the PGT, which adheres to the leaf. A second appressorial germ tube (AGT) is then produced by 3–4 h after infection. The AGT develops into an appressorium and an infection peg forms beneath this to penetrate the host cell cuticle. In a successful infection, haustoria develop within the cell and are visible by 12–14 h. Nutrients are absorbed through the haustoria, enabling the fungus to grow and develop. Within 2 days, conidiophores bearing conidiospores start to be produced and within 4 days micro-colonies are visible on the leaf surface. The developing fungus eventually forms new conidia in chains by 4–6 days after inoculation.

B. Sexual Reproduction

Many powdery mildews have a sexual phase resulting in the formation of ascospores within a cleistothecium, a closed spherical ascocarp (Fig. 3.1G–I). Formation of the pseudoantheridium and pseudoascogonium and events leading to ascus formation were described for some Erysiphales in the first half of the twentieth century using light microscopy (Harper 1905; Colson 1938). Wheat powdery mildew (*B. graminis* f. sp. *tritici*, *Bgt*) is heterothallic and hermaphroditic, so either of two compatible isolates can act as the maternal parent (Robinson et al. 2002); and this may indeed be the case for other powdery mildews. Details of the sexual cycle of most powdery mildews are, however, poorly understood. Cleistothecia remain dormant through periods of drought or temperature extremes when host plants are not present. When conditions become favourable, the cleitothecia absorb water and break open enabling ascospores to be ejected from the asci. For some powdery mildews, ascospores are the primary inoculum. For example the primary inoculum of

Fig. 3.1. Examples of powdery mildew diseases and illustrations of haustoria and cleistothecia. **A** Courgette (zucchini) powdery mildew (*Podosphaera fusca*). **B** Grape powdery mildew (*Erysiphe necator*). **C** Illustration of the range of infection phenotypes of barley powdery mildew (*Blumeria graminis* f. sp *hordei*) isolate CC148 on barley cultivars: *left to right* P08B (containing the resistance gene *Mla9*), Hordeum 1063 (*Mlk1*), Julia (*Ml/cp)*, Hordeum 1036 (*Mla3*), W37/36 (*Mlh*). These are described, respectively, as infection types 0 (fully avirulent), 1, 2, 3 and 4 (fully virulent). **D**, **E** Powdery mildew disease on *Rhododendron* spp. (*Erysiphe azaleae*; **D**) and gooseberry (*Podosphaera mors-uvae*; **E**). **F** Green islands underneath colonies of *Blumeria graminis* colonies growing on leaves of couch grass. **G** Cleistothecium of *Microsphaeria azaleae*. **H**, **I** Cleistothecial squash preparations to reveal asci and ascospores from *Erysiphe necator* (**H**) and *Podosphaera fusca* (**I**). **J** Haustoria of *B. graminis* f. sp. *hordei* growing in barley. **K**, **L** Morphology of *Arabidopsis thaliana* powdery mildew (*Erysiphe cichoracearum*) haustorial complexes. Methods used for the visualization of haustoria are described by Koh et al. (2005). **K** 3D volume-rendered isolated haustorial complex. Elaborated lobe-like structures are visible around the mature haustorium (*Ha*) within the extrahaustorial membrane (*EHM*). **L** 3D volume-rendered in vivo haustorial complex. The intact EHM (*arrow*) was also visible around the haustorium (*Ha*), but distinctive from the GFP-labelled plasma membrane (*PM*) of the *Arabidopsis* epidermal cell. The appressorium (*Ap*) is also shown on the leaf surface. Figures reproduced by permission of Alejandro Pérez, (**A**), David Gadoury (**B**), Jay Pscheidt (**D**, **E**, **G**), Melodie Putnam (**H**), Juan Antonio Torés (**I**), Pietro Spanu (**J**) and Serry Koh (**K**, **L**)

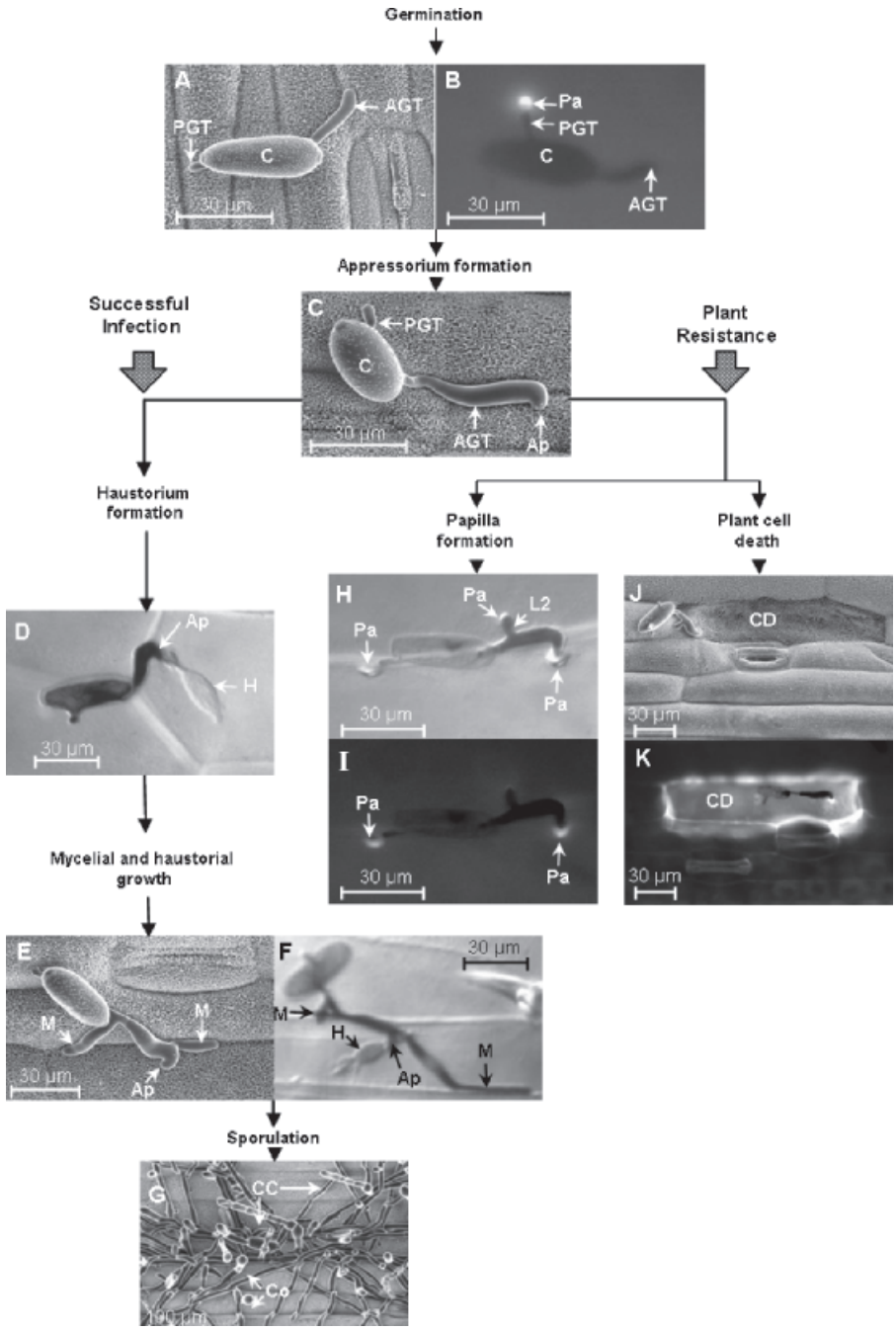

Fig. 3.2. Micrographs by cryo-SEM, transmitted light and fluorescence (blue light excitation) of key stages in *Blumeria gramins* f. sp. *hordei* development and barley cell responses. **A–C** Up to 10 h after inoculation (h.a.i.), fungal development is indistinguishable on susceptible and resistant plant genotypes such as Pallas, P01 and P22. **A** By 5 h.a.i., the primary germ tube (*PGT*) has emerged from the conidium (*C*) and adhered to the leaf surface. The appressorial germ tube (*AGT*) has elongated partially but not yet differentiated an apical lobe. **B** Fluorescence microscopy reveals autofluorogenic material accumulated within a small papilla (*Pa*) deposited as a plant epidermal cell response to the PGT. **C** By 10 h.a.i., the appressorium (*Ap*) has differentiated a hooked apical lobe. **D–G** Successful infection of a susceptible host. **D** By 15–18 h.a.i., a penetration peg emerging beneath the Ap has penetrated the plant cell and its tip has swollen to form a rudimentary haustorium (*H*) visible by light microscopy. Images by scanning electron (**E**) and light microscope (**F**) show developing colonies at 30 h.a.i. By 30 h.a.i., ectophytic mycelium (*M*) has grown from the AGT as the haustorium develops a digitate process from each end of its central body. **G** By 96 h.a.i., mycelial growth is extensive. Repeated penetration from hyphal appressoria results in the formation of further haustoria (not shown) and bulbous conidiophores

grape powdery mildew (*Erysiphe necator*, formerly *Uncinula necator*) can come from cleistothecia that overwinter primarily in bark crevices on the grapevine (Pearson and Gadoury 1987). Ascospores, released in the spring, are carried by wind and germinate on the leaf surface of developing vines. For other powdery mildews, the importance of the sexual phase is not so obvious or may depend on the local climatic conditions. For example, ascospores of *Bgt* are considered to be an important source of inoculum in the highlands of China, but less so in the lowland regions. This is because, in the lowlands, autumn wheat seedlings can become infected with conidiospores which subsequently function as the primary inoculum (Yu 2000). The occurrence of a sexual stage permits genetic analysis to be performed in some powdery mildew fungi (see Sect. V.B). Many powdery mildews, including *Ec*, do not have a sexual stage.

III. Interactions with the Host Plant

Early in the infection process, visible changes can be detected in the physiology and structure of the host cell. Upon attempted penetration, polarized cytoplasmic streaming occurs in the plant cell directed towards the site of attempted penetration (Kobayashi et al. 1997) and papillae develop at the point of first contact (Fig. 3.2B, I). Papillae function as physical and chemical barriers to resist infection and are comprised of inorganic and organic constituents, including callose and autofluorogenic phenolics. Their deposition involves: generation of nitric oxide (NO; Prats et al. 2005) and hydrogen peroxide (H_2O_2; Vanacker et al. 2000), cytoskeletal re-arrangement (Opalski et al. 2005) and redirected cytoplasmic streaming and aggregation (Zeyen et al. 2002). In barley/*Bgh* interactions, NO is generated at sites of papilla deposition commencing at around 10 h after infection (a.i.) and persisting until around 14–16 h a.i. NO may also be involved in the complex second step that involves synthesis, marshalling and assembly of papilla components (Prats et al. 2005). Although the layers of the papillae can appear heterogeneous, they may develop in a certain chronological order with compounds created at and/or delivered to the site of fungal penetration at specific times (Celio et al. 2004). Using GFP-tagged plasma membrane marker proteins, rings of fluorescence were observed around *Ec* penetration sites on *A. thaliana* plants, which extended across cell wall boundaries and into neighbouring cells. These rings, however, seem to be localized to those infection sites where papillae were deposited (Koh et al. 1995). Even in susceptible hosts, papillae formed in the plant cell may succeed in preventing infection.

Staining by 3,3-diaminobenzidine (DAB) reveals that H_2O_2 is produced by plant cells at infection sites. H_2O_2 can be detected at developing papillae in the epidermal cell subjacent to the primary germ tube from 6 h after inoculation, and underneath the appressorium after 15 h. The presence of H_2O_2 could catalyse the cross-linking of proteins in the papillae, resulting in a stronger physical barrier to invasion (Thordal-Christensen et al. 1997). During development of the fungal haustorium, multivesicular bodies, intravacuolar vesicle aggregates and paramural bodies develop in the penetrated epidermal cell. These structures also form at the periphery of intact cells adjacent to cells undergoing hypersensitive cell death (An et al. 2006). The implication from these investigations is that the vesicular compartments participate in secretion of chemical components required for cell wall appositions. They may also contribute to the internalization of damaged membranes, deleterious materials, nutrients, elicitors and elicitor receptors.

Although *Bgh* is an obligate parasite, it appears to have all the main metabolic pathways of a filamentous fungus; it has not lost metabolic capacity nor the ability to modulate its metabolism (Thomas et al. 2001, 2002; Soanes et al. 2002; Giles et al. 2003; Soanes and Talbot 2006). The distinct lifestyle of

(*Co*) have started generating chains of conidia (*CC*) for wind dispersal. **H–K** Resistance responses viewed 30 h.a.i. Images by transmitted light (**H**) and fluorescence (**I**) microscopy show a germling that failed to penetrate a living plant cell from its first appressorial lobe and therefore formed a second lobe (*L2*). Refractive, autofluorescent papillae subtend both appressorial lobes and the PGT, although fluorescence is weak in the smaller papilla subtending L2. **J**, **K** Epidermal cell death (*CD*) as a result of single gene-controlled hypersensitivity that prevents further pathogen growth. By SEM (**J**), dead epidermal cells are obviously collapsed while they show whole-cell autofluorescence viewed by fluorescence microscopy (**K**). In lines carrying *Mla1* attacked by an avirulent fungal isolate, most cells that do not form an effective papilla, collapse and become autofluorescent by 24 h.a.i. This figure and legend are reproduced from Prats et al. (2006) by permission of Dr. Elena Prats and Oxford University Press

B. graminis is reflected in its gene inventory based on EST analysis, having significantly fewer sequences in common with the genomes of filamentous ascomycetes than any of the other plant pathogenic fungi. Nearly half of the *B. graminis* unisequences currently available at NCBI have no homologues in the NCBI database of sequenced proteins or in the sequenced fungal genomes (Soanes and Talbot 2006). These observations suggest that the biotrophic life cycle of *B. graminis* necessitates a large number of gene products not found in necrotrophic and hemibiotrophic phytopathogens. Transcript profiling using cDNA microarrays indicates that wholesale changes in fungal gene expression occur during the switch from pre-infection development to biotrophic growth, including the co-ordinate regulation of entire suites of genes encoding enzymes in similar pathways of primary metabolism (Both et al. 2005a).

A. Influence on Host Metabolism

Powdery mildews influence host cell metabolism in many ways. One striking manifestion of powdery mildew infection is the appearance of 'green islands' underneath and around developing colonies (Fig. 3.1F). Green islands become apparent during the later stages of infection when the remainder of the leaf has senesced. Cytokinins are known to delay senescence and play a role in the synthesis and maintenance of chlorophyll and are known to influence chloroplast development and metabolism. Indeed, cytokinins have also been shown to promote re-greening of senescent leaf tissue (Zavatleta-Manchera et al. 1999). Therefore it is not surprising that cytokinins are implicated in green island formation (Brian 1967; Dekhuijzen 1976). It is not clear whether the cytokinins are of fungal origin, or whether they are formed by the plant as a direct result of the infection or by manipulation of host metabolism by the fungus (Walters and Mc Roberts 2006).

Leaves infected with obligately biotrophic fungal pathogens, including powdery mildews, often exhibit reduced rates of net photosynthesis. It is not known whether the fungus directly causes this or whether it is a response of the plant to infection (Walters and Mc Roberts 2006). Powdery mildews can, however, influence the expression of some genes of their host plants, which may help to establish successful infection. Single epidermal cells containing haustoria show reduced accumulation of transcripts from several pathogenesis-related genes, including peroxidase and an oxalate oxidase-like gene. These genes are normally up-regulated in whole-leaf tissue in both compatible and incompatible interactions, so these results indicate that the fungus can suppress defence gene activation in physical proximity to haustoria (Gregersen et al. 1997). Suppression of basal defence-related transcripts including those genes involved in the shikimate pathway, signal transduction and defence can be measured 16 h after infection in compatible interactions (Caldo et al. 2006).

B. Induced Susceptibility and Resistance

The formation of a haustorium during a compatible interaction influences the ability of the host cell to resist subsequent attack, further illustrating that powdery mildews can influence host metabolism to assist infection (Lyngkjær and Carver 1999). Even in compatible interactions, not all infection attempts are successful. For example, 67% of infections of compatible *Bgh* isolate GE3 on barley line Risø 5678S went on to produce a haustorium. Using a double-inoculation procedure, cells were first attacked by an 'inducer', followed by a second inoculation with a 'challenger'. Successful infections by the challenger increased to over 90% where cells already had an inducer haustorium in them. Conversely, if the inducer attack failed, attacked cells were rendered highly inaccessible to subsequent attack. Inaccessibility was also induced in cells immediately adjacent to the attacked cell, but not in more distant cells. Induced accessibility appeared to be associated with suppression of localized autofluorescence characteristic of host cell death. Inaccessibility was, however, associated with increased frequency and intensity of cell death responses. The results from these experiments indicate that induced changes may relate to modification in a host cell's ability to synthesize phenolic compounds. These observations are also consistent with the transcript changes during infection described earlier.

The phenomenon of induced susceptibility extends to powdery mildew interactions that are normally incompatible. When *Bgh* isolates virulent on barley varieties carrying the resistance gene *Mla1* formed haustoria, the cells became highly susceptible to isolates that are normally avirulent (i.e. cannot grow) on these barley lines (Lyngkjær et al. 2001). The conclusion from this work is that factors

released by the fungus are able to suppress defence responses. However, the suppressive effect was confined to the epidermis and defence responses were observed as normal in underlying mesophyll cells. Thus, the suppressive effect only occurs in the immediate vicinity of the infected cell. *Blumeria graminis* exists as a number of formae speciales (ff. spp.), each adapted to different grass hosts. For example, *B. graminis* f. sp. *hordei* normally grows on barley, but cannot infect oats. If, however, grasses are first infected with compatible ff. spp., infection by non-compatible ff. spp. can occur (Moseman et al. 1964). In subsequent work, double-inoculation experiments, as described above, showed that infection by the inducer f. sp. enhanced infection by an incompatible f. sp. (Olesen et al. 2003). Enhanced infection correlated with suppression of defence responses within epidermal cells containing the inducer haustorium. The suppressive effect extended to adjacent cells, but did not occur at two cells distance. Suppression of penetration resistance allowed most challenger attacks by inappropriate ff. spp. to form haustoria, so enabling the fungus to survive and develop a colony. Induced susceptibility even extends beyond related powdery mildew, since barley coleoptile cells penetrated by *Bgh* and containing haustoria are more susceptibile to subsequent attack by the non-host pathogen, *Erysiphe pisi* (Kunoh et al. 1985, 1986).

IV. Establishment of Haustoria in Host Cells and Structure of the Haustorium/Plant Interface

Haustoria are specialized structures that are formed inside living host cells and which are likely to be involved in the exchange of substances between host and fungus. In fungal and oomycete diseases of higher plants, haustoria are formed by downy mildews, white rusts (peronosporales), powdery mildews and rusts. They are also formed by other fungi, including lichens, endotropic mycorrhizae, some specialized filamentous fungi in the order Mucorales and aquatic phycomycetes in the orders Chytridiales, Hyphochytridiales and Saprolegniales (Bushnell 1972). Considerable attention has been focused on investigations into the formation, structure and function of haustoria in rusts (see Chap. 4) and powdery mildews which are reviewed in this chapter.

Haustoria are considered to be the location for exchange of nutrients, and there is evidence for this in other biotrophic plant pathogens. cDNA libraries prepared from haustoria of bean rust (*Uromyces fabae*) reveal the presence of many in planta induced genes, including hexose transporters, invertase, amino acid transporters and H^+ ATPase, all consistent with the increased nutrient uptake (Hahn and Mendgen 1997; Struck et al. 2002; Voegele et al. 2001, 2006; Jakubovic 2006). In both *Bgh* and *U. fabae*, transcript profiling using cDNA microarrays indicates that wholesale changes in fungal gene expression occur during the switch from pre-infection development to biotrophic growth (Both et al. 2005a; Jakupovic et al. 2006). The failure to detect plant wall components in powdery mildew extrahaustorial matrix (EHMAT) led to the suggestion that either the pathogen suppresses their synthesis and secretion at the interface or that they become degraded by fungal hydrolytic enzymes after secretion into the matrix, perhaps providing a source of nutrition to the pathogen (Green et al. 2002). Effectors which suppress defence responses may also be released from haustoria, although so far there is no direct evidence for this.

The primary haustorium of *Bgh* starts to form in an epidermal cell during the first day after infection and continues to develop for five of six days growing at a rate of about 250 µm/day (Hirata 1967; for a review, see Bushnell 1972). Haustoria of *B. graminis* ff. spp. are unique among powdery mildew fungi in that they characteristically have finger-like branches (Fig. 3.1J), other species typically having a more spherical structure. The extrahaustorial membrane (EHM) encases the haustorium, separating it from the host cytoplasm. The EHM generally appears liquid or gel-like in consistency (Manners and Gay 1983) and is considered to be a specialized membrane derived from the host although the pathogen may also contribute components (Koh et al. 2005). The EHMAT (Green et al. 2002) lies between the EHM and the fungal haustorial wall and is a gel-like layer enriched in carbohydrates of both fungal and host origin (Koh et al. 2005). To move from plant cytoplasm to haustorial cytoplasm, substances must pass sequentially through the EHM, EHMAT, the haustorial wall and the haustorial plasma membrane. The EHM, as well as the EHMAT, is thought to act as a molecular sieve and there is evidence for pore-like structures rendering the EHM permeable to molecules up to 40 kDa (Gil and Gay 1977). Despite its appar-

ent continuity, the EHM differs from the host cell plasma membrane both physically and chemically. The use of high-pressure frozen and freeze-substituted electron microscopy provides exceptional preservation of the host pathogen interface to reveal the fine detail of haustoria EHM and papillae (Fig. 3.3).

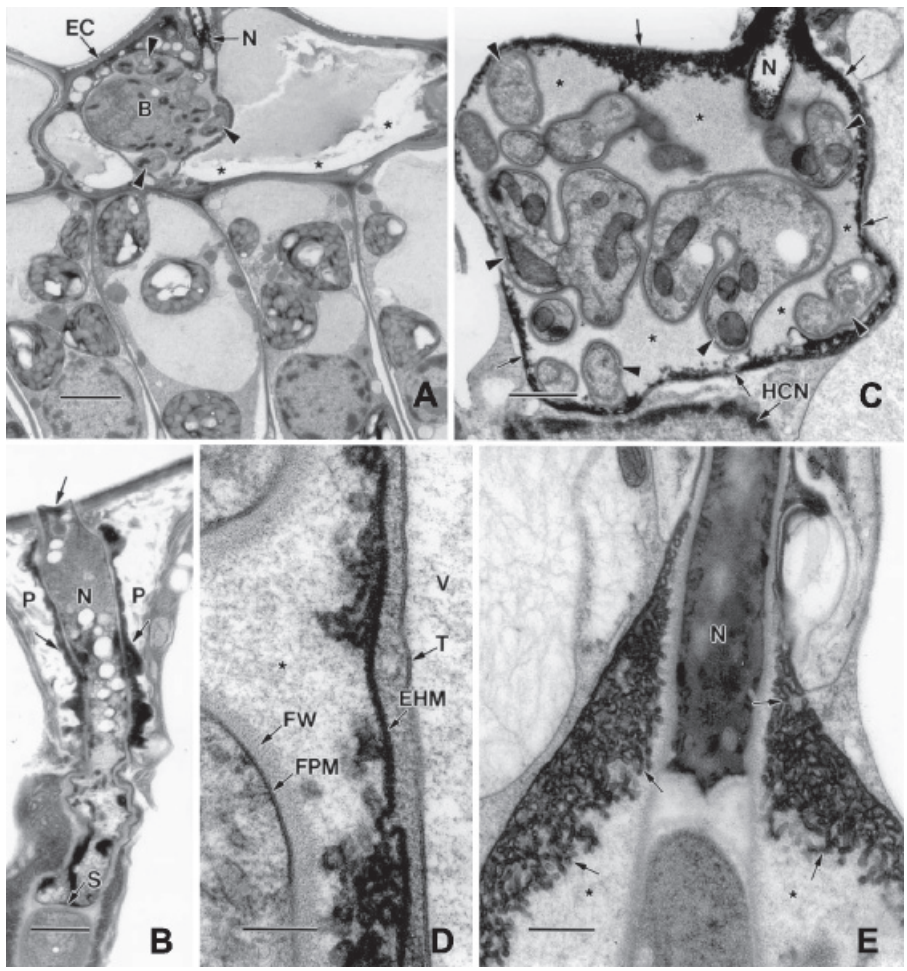

Fig. 3.3. TEMs of high-pressure frozen and freeze-substituted samples of poinsettia leaves infected with *Oidium* sp. **A** Portion of a leaf showing an epidermal cell (*EC*) containing a haustorium. Both the neck (*N*) and body (*B*) of the haustorium are visible as well as numerous slender, coiled lobes (*arrowheads*) surrounding the body. The large vacuole of the epidermal cell shows considerable mechanical damage (*asterisks*). Note the well preserved nature of the palisade cells visible below the epidermal cells. *Bar* 2.5 µm. **B** Example of the neck (*N*) of a haustorium surrounded by a papilla (*P*). Note the layer of electron-dense material (*arrows*) coating the neck of the haustorium. A septum (*S*) is visible at the base of the neck. The appressorium that formed this haustorium was lost during freezing. The sheared end of the neck of the haustorium that was continuous with the appressorium is shown at the arrow. *Bar* 0.5 µm. (**C** Section showing the highly convoluted extrahaustorial membrane (*arrows*) surrounding a haustorium. A portion of the haustorium neck is visible at *N*. Although most of the body of the haustorium is out of the plane of the section, numerous lobes of the haustorium are visible at the arrowheads. Note the extrahaustorial matrix material (*asterisks*) surrounding the lobes. A portion of the host cell nucleus is visible at HCN. *Bar* 0.5 µm.(**D** Highly magnified view of the host–pathogen interface. Visible are a portion of a host cell vacuole (*V*) and its membrane or tonoplast (*T*), the thickened and highly convoluted extrahaustorial membrane (*EHM*) and parts of the fungal plasma membrane (*FPM*) and wall (*FW*) of a lobe of the haustorium. Extrahaustorial matrix material is visible at the *asterisk*. *Bar* 0.15 µm. **E** Section showing the highly convoluted nature of the extrahaustorial membrane (*arrows*) near the base of a haustorium neck (*N*). Extrahaustorial matrix material is visible at the *asterisks*. *Bar* 0.3 µm. This figure is reproduced from Figs. 1–5 of Celio et al. (2004) with permission of Dr. Gail Celio and the National Research Council, Canada

Microscopic investigation of the barley/*Bgh* interaction reveals that multivesicular bodies (MVBs) of host plant origin proliferate near to haustoria in the penetrated epidermal cell (An et al. 2006). MVBs probably participate in the secretion of building blocks for cell wall appositions to arrest fungal penetration. They may also participate in the internalization of damaged membranes, deleterious materials, nutrients, elicitors and elicitor receptors. They may also be involved in plasma membrane extension during haustorium development if they fuse with the plasma membrane. Large bubble-like structures resembling MBVs have also been observed adjacent to the plasma membrane near to young haustoria of *Ec* in *Arabidopsis thaliana* (Koh et al. 2005).

V. Susceptibility and Resistance in Powdery Mildew Interactions

Investigations into the basis of susceptibility and resistance in powdery mildew interactions have preoccupied scientists for decades. The practical basis for these studies is to understand the nature of resistance for the development and breeding of plant varieties for agriculture and horticulture. The establishment of powdery mildew fungi on the host plant is particularly suited to microscopic investigations, and investigations of this kind have advanced our understanding of infection processes in both mildews and in other parasitic micro-organisms. The ability to perform genetic analysis in powdery mildews has advanced our understanding of the molecular basis of resistance, especially gene for gene interactions.

A. Gene for Gene Interactions

The gene for gene (GFG) relationship between resistance in a host plant and avirulence in a pathogen was first described in the interaction between flax rust (*Melampsora lini*) and flax (*Linum usitatissimum*; Flor 1955). Following Flor's work, the powdery mildews of wheat and barley were among the next diseases for which the GFG relationship was demonstrated (Moseman 1959; Powers and Sando 1960). Since then, the GFG relationship has been demonstrated or inferred in many other plant diseases, including those caused by fungi, oomycetes, bacteria, viruses, nematodes, insects and parasitic plants (Thompson and Burdon 1992; Crute et al. 1997). In GFG interactions, avirulence (AVR) molecules of pathogens and pests are recognized in plants by race-specific resistance (R) proteins. Specificity is a crucial feature of GFG relationships, with the plant mounting an effective defence against infection only if it has a resistance allele matching a specific avirulence allele in the pathogen.

GFG resistance is usually associated with a hypersensitive response (HR) in the attacked host cell. In this, recognition of the AVR gene product by the R protein causes the attacked cell to die quickly. It is often assumed that this rapid destruction of host cells is directly responsible for arrest of pathogen growth, and this is probably true in many cases. Cell death can result in a visible manifestation of the defence response that is relatively easy to observe and measure. There are also examples where HR is not associated with pathogen arrest. It has been proposed that HR could function in signaling to neighbouring cells rather than a direct defence mechanism (Heath 2000). Two examples of this type of response which occur in powdery mildew interactions are described later in this section.

B. Genetic Analysis of Avirulence Genes

Crosses between *Bgh* isolates can be made, enabling the inheritance and linkage of over 30 *AVR* loci to be investigated (Jørgensen 1988; Brown and Simpson 1994; Brown and Jessop 1995; Jensen et al. 1995; Brown et al. 1996; Caffier et al. 1996; Pedersen et al. 2002). Most *Bgh AVR* genes map as single loci as expected for the standard GFG pattern. There is also evidence for more than one avirulence gene matching a single barley resistance gene, indicating that there may be modifier and inhibitor genes which affect AVR recognition or expression (for a review, see Brown 2002). Two regions of the *Bgh* genome are known to contain clusters of linked *AVR* genes. The AVR_{a10} cluster comprises AVR_{a10}, AVR_{k1}, AVR_{a22}, AVR_{a9}, AVR_{a13}-1 (one of two *AVR* genes recognized by *Mla13*), AVR_{g} and possibly AVR_{a6} and AVR_{a7} (Jørgensen 1988; Christiansen and Giese 1990; Brown and Simpson 1994; Jensen et al. 1995; Caffier et al. 1996; Pedersen et al. 2002; Ridout et al. 2006). Another possible cluster occurs at the AVR_{a12} locus, and was identified in a cross

between the *Bgh* isolates CC146 and DH14 (Brown et al. 1996). In this, AVR_{a12} was linked to AVR_{P17} and AVR_{a6} by 21.2 cM and 22.0 cM, respectively, and AVR_{La} was linked to AVR_{P17} at a distance of 21.2 cM. However, in all other crosses studied, no linkage of AVR_{a12} to any other *AVR* gene was established (Brown and Simpson 1994; Jensen et al. 1995; Caffier et al. 1996).

In addition to cereal powdery mildews, there is evidence of GFG resistance in the interaction between other powdery mildews and their hosts. However, these investigations were observed with naturally occurring isolates and were not subjected to rigorous genetic analysis. The most comprehensive study of the genetics of GFG interactions in powdery mildews, apart from that of cereals, was with *Golovinomyces cichoracearum* var. *fischeri* (syn. *Erysiphe cichoracearum* var. *fischeri*) on groundsel (*Senecio vulgaris*; for a review, see Clarke 1997). The patterns of interaction were more complex than those between *B. graminis* and barley or wheat varieties (Harry and Clarke 1986; Bevan et al. 1993a, b). Some interactions between particular pairs of *G. cichoracearum* var. *fischeri* isolates and groundsel lines could be classified as compatible or incompatible, but in others a clear classification was not possible. Difficulties in ascribing the nature of the interaction were due to partial resistance in the host or because the pathogen was not very aggressive. Six races of *Podosphaeria fuliginea* were defined on the basis of their specific virulence on varieties of melon (Bardin et al. 1997; Hosoya et al. 1999). In addition, pathotypes of the fungus have been defined by their ability to infect different cucurbit species. There is some evidence for specific interactions between isolates of *Erysiphe pisi* lines of the host plant, pea. (Tiwari et al. 1997).

C. Identification of Powdery Mildew Avirulence Genes

Two *AVR* genes have been isolated from *Bgh*, AVR_{k1} and AVR_{a10}, recognized by barley varieties containing Ml_{k1} and *Mla10*, respectively (Ridout et al. 2006). AVR_{k1} and AVR_{a10} belong to a large family with >30 paralogues in the genome of *Bgh* and homologous sequences are present in other ff. spp. of the fungus which infect other grasses (Ridout et al. 2006). The central core of the AVR_{k1} protein is highly conserved, whereas the amino acid sequences towards the N- and C-terminal regions are divergent. No signal peptide is predicted in the proteins, suggesting that they are not delivered to the host cell in a conventional secretion pathway. The proteins are rich in the amino acid residues lysine and arginine and are very basic. There are no homologues of AVR_{k1} or the related paralogues in any sequence database. AVR_{k1} and AVR_{a10} were demonstrated to have a dual function, both as: (a) elicitors of the resistance response and (b) as effectors which enhance infection on susceptible hosts. These findings suggest that *Bgh* and other ff. spp. of *B. graminis* might have a repertoire of related effectors, some of which may encode AVR proteins. This would explain why individual *AVR* genes can be lost without apparent loss of fitness, so enabling the fungus to overcome plant *R* genes (Ridout et al. 2006). Whether other members of the gene family may there encode AVR proteins remains to be determined.

D. Race-Specific Resistance (*R*) Genes

R genes encode receptors which recognize specific AVR elicitors in certain races of an adapted pathogen. More than 85 barley *R* genes, each conferring resistance to specific *Bgh* AVR elicitors, have been described, including over 28 alleles at the *Mla* locus on barley chromosome 5 (Jørgensen 1994). The *Mla* locus encodes allelic receptors containing an N-terminal coiled-coil (CC) structure, a central nucleotide-binding (NB) site and a leucine-rich repeat (LRR) region. The six isolated *Mla* alleles (*Mla1*, *Mla6*, *Mla7*, *Mla10*, *Mla12*, *Mla13*) are predicted to encode proteins that share >90% amino acid sequence identity (Zhou et al. 2001; Halterman et al. 2003; Shen et al. 2003; Halterman and Wise 2004). Analysis of *Mla1/Mla6* chimeras revealed that recognition specificity is determined by different but overlapping LRRs and a C-terminal non-LRR region (CT; Shen et al. 2003). Mla protein steady-state levels are critical for effective resistance and are subject to control by cytosolic heat-shock protein 90 (Hsp90) and the co-chaperone-like proteins RAR1 and SGT1 (Bieri et al. 2004; Hein et al. 2005). Details of the molecular functioning of chaperones in Mla resistance protein function are reviewed by Shirasu and Schulze-Lefert (2000). Co-expression of the *Bgh* AVR_{a10} effector induces nuclear associations between the Mla10 receptor and a WRKY-2 transcription factor, which could initiate downstream defence responses (Shen et al. 2007). It is proposed that the WRKY proteins repress defence

responses and that activation of Mla proteins by pathogens blocks this repressor activity allowing for rapid and efficient disease resistance.

Resistance to *Bgh* controlled by a GFG interaction is generally associated with a strong HR. However, a halt in cytoplasmic streaming is the first visible sign of incompatibility in Mla/AVR interactions and preceeds hypersensitive cell death by 1–3h (Bushnell 1981). Resistance phenotypes from AVR/Mla interactions on barley can vary considerably ranging from complete sensitivity to complete resistance (Fig. 3.1C). The extent of necrosis and sporulation varies for resistant reactions on varieties with the same *Mla* gene but with different genetic backgrounds. There is even variation between individual colonies on the same leaf and the phenotypes can be influenced by spore density and environmental conditions. Thus, consistency in inoculation and growth conditions is required for accurate scoring of phenotypes. Resistance responses for each *Mla* allele can be defined as slow or fast acting, on a continuous scale between the two extremes. For example, on *Mla1* and *Mla6* plants, a rapid HR was associated with a higher percentage of germlings arrested in their development of the haustorial stage. There was also a significant papilla response corresponding to a larger number of germlings arrested in *Mla6* plants (Boyd et al. 1995). On *Mla3* and *Mla7* plants, a slower hypersensitive response was associated with more haustoria and elongating secondary hyphae were formed, indicating that some fungal colonies were starting to form. However, in the latter cases there was a more extensive combined epidermal and mesophyll HR. It was proposed that the differences in speed on *Mla* reaction could result from different stage specific delivery of AVR molecules (Shen et al. 2003). The varying responses could also result from different amounts of each type of Mla protein, which are present in only small amounts in the cell. Evidence for this was the alteration of the reaction kinetics to produce a more rapid response by over-expressing the *Mla12* resistance gene (Shen et al. 2003).

Wheat powdery mildew (*Pm*) resistance genes follow the GFG model and the resistance reaction is associated with rapid host cell death. Major host resistance genes have been identified at 33 loci in wheat (Huang and Röder 2004; Zhu et al. 2005). Five of these loci (*Pm1*, *Pm3*, *Pm4*, *Pm5*, *Pm8*) have more than one allele conferring resistance, making a total of 49 named *Pm* resistance alleles. *Pm3* was one of the first described loci among the *Pm* genes. *Pm3* is a single, dominant locus on the short arm of wheat chromosome 1A, and carries ten different resistance specificities (*Pm3a–Pm3j*). *Pm3* resistance alleles were generated in agricultural ecosystems after domestication of wheat 10 000 years ago (Yahiaoui et al. 2006). Since that time, *Pm3* alleles have been widely and successfully employed in breeding programmes. Some of these alleles remain effective in conferring resistance. Phylogenetic analysis of the Pm3B protein indicates that it is more similar to rice disease resistance-like proteins rather than Mla (Yahiaoui et al. 2004).

GFG resistance not associated with HR has been identified in powdery mildew interactions. Three lines of evidence strongly suggest that HR is not required for the *Mlg*-associated resistance to *Bgh*:

1. The inhibition of the HR by addition of the transcriptional inhibitor, cordycepin did not result in the release of fungal growth arrest.
2. The growth arrest of a *Bgh* isolate occurred in the absence of a detectable HR in two barley genetic backgrounds.
3. Gene dosage experiments showed that heterozygous *Mlg/mlg* plants, in comparison to the *Mlg/Mlg* genotype, show a drastic reduction of single-cell HR frequency without a proportional increase of haustorium formation (Görg et al. 1993; Schiffer et al. 1997).

Again in *Bgh*, the AVR_{Ab} gene segregates as a single Mendelian locus and follows classic GFG interaction. In this case, however, resistance is manifest as a reduction in the number of colonies formed by approximately 85% (Brown and Jessop 1995). No microscopic investigations were made in this study, so it is not known whether a reduction in colony number correlates with increased HR. In wheat, the powdery mildew resistance gene *Pm2* also governs penetration success, but not HR. Since effective papillae and HR did not occur in the same cells, papilla deposition may be independent of the HR response despite the fact that both defence mechanisms were associated with high H_2O_2 accumulation (Li et al. 2005). HR may act as a second line of defence to contain infection when the papilla defence fails.

E. Race-Non-Specific Resistance Genes

Genes conferring resistance to all powdery mildew races have been identified. *RPW8.1* and *RPW8.2*

in *A. thaliana* are naturally occurring dominant alleles closely linked to each other and are required for defences associated with the hypersensitive response. Unlike *R* genes, however, they confer resistance to a range of powdery mildew pathogens, apparently not through a GFG interaction. The *RPW8* resistance locus is unusual because it mediates dominant resistance to diverse powdery mildew species, including 15 tested isolates of *Erysiphe cichoracearum*, *E. cruciferearum*, *E. orontii* and *Oidium lycopersici* (Xiao et al. 1997, 2001).

A transmembrane protein in barley known as Mlo is a pre-requisite for successful colonization by *Bgh* (Büschges et al. 1997). Plants carrying loss-of-function alleles (*mlo*) of the *Mlo* locus are resistant against all known isolates of *Bgh* (Piffanelli et al. 2006). The *mlo* mutation does not, however, affect a range of other foliar pathogens. In the absence of Mlo protein function (such as in barley *mlo* mutants), barley plants are resistant because germinated fungal spores fail to enter epidermal host cells. A characteristic feature of *mlo* resistance to *Bgh* is an early cessation of penetration through the epidermal cell wall that is not accompanied by the HR, a typical response of most *R* gene-triggered resistance (Wolter et al. 1993; Shirasu and Schulze-Lefert 2000). Since the first identification of *mlo* mutant barley, *mlo* resistance has been widely used in barley cultivation (Jørgensen 1992). Resistance to powdery mildew equivalent to *mlo* is not known in other crop species. However, non-host resistance and mlo-based immunity in *Arabidopsis thaliana* and barley respectively share similar features (see Sect. V.F). No naturally occurring broad-spectrum resistance against powdery mildew attack has been demonstrated in wheat (*Triticum aestivum*) against *B. graminis* f. sp. *tritici* and no *mlo* mutants have been detected. This may be because of the hexaploid nature of bread wheat and the likelihood that mutations may have to occur in all six copies of presumptive *Mlo* orthologues. Homologues of barley *Mlo* are, however, found in syntenic positions in all three genomes of bread wheat and also present in rice, *Oryza sativa* (Elliott et al. 2002). The Mlo protein also has homologues in *Arabidopsis*, indicating that a common host cell entry mechanism of powdery mildew fungi evolved once and at least 200 million years ago, suggesting that, within the powdery mildews, the ability to cause disease has been a stable trait throughout phylogenesis (Consonni et al. 2006).

The Mlo protein resides in the plasma membrane and has seven transmembrane domains reminiscent of the transmembrane receptors in fungi and animals. In animals, these are known as G-protein-coupled receptors and exist in three main families, lacking sequence similarity. A domain in Mlo mediates a calcium-dependent interaction with calmodulin in vitro. Loss of calmodulin binding reduces the ability of Mlo to negatively regulate defence against powdery mildew in vivo. Based on these investigations, a sensor role for Mlo in the modulation of defence reactions was proposed (Kim et al. 2002). Using non-invasive fluorescence-based imaging techniques, the Mlo protein was shown to be redistributed in the plasma membrane and accumulate beneath fungal appressoria coincident with pathogen entry into host cells (Bhat et al. 2005). Polarized Mlo accumulation occurs upon fungal attack and is independent of actin cytoskeleton function. Since *mlo* resistance is effective only against barley powdery mildew, the fungus may be targeting Mlo to achieve defence suppression (Kim et al. 2002). There are also reports that *mlo* resistance to powdery mildew increases susceptibility to other barley pathogens, including *Magnaporthe grisea* (Jarosch et al. 1999).

F. Cell Entry Control and Non-Host Resistance

Attack by *E. cichoracearum* usually results in successful penetration and rapid proliferation of the fungus on *A. thaliana*. By contrast, the non-host pathogen *Bgh* typically fails to penetrate *A. thaliana* epidermal cells (this is defined as non-host resistance). Genetic screens for mutations that result in increased penetration of *Bgh* on *A. thaliana* enabled the identification of penetration (pen) mutants. PEN1 is a syntaxin and has a close homologue SYP122 (Assaad et al. 2004). Both of these proteins are members of a large family of SNAREs (soluble N-ethylmaleimide-sensitive factor adaptorprotein receptors), present in the *A. thaliana* genome. Host proteins exhibit focal accumulation (local aggregation) at powdery mildew entry sites. Localization and genetic studies suggest that PEN1 plays an active role in the polarized secretion events that give rise to the formation of papillae during fungal attack. The *pen1* phenotype can therefore be described as the converse of the *mlo* phenotype;

mlo mutants have an increased penetration resistance whereas *pen1* mutants have a decreased penetration resistance (Collins et al. 2003). The barley orthologue of *pen1*, *ror2*, was identified as a locus '*r*equired for ml*o* *r*esistance' in screens for suppressors of *mlo* (Freialdenhoven et al. 1996; Collins et al. 2003). During the establishment of a compatible interaction, the timing of papilla formation is potentially critical and may affect the frequency of fungal penetration. SYP122 may have a general function in secretion, including a role in cell wall deposition. These investigations illustrate that there are multiple layers of resistance in the context of the non-host resistance and provide evidence for the existence of a vesicle-associated resistance mechanism preventing powdery mildew infection.

Pen2 encoding a glycosyl hydrolase was identified and characterized in *A. thaliana* and shown to act as a component of an inducible pre-invasion resistance mechanism (Lipka et al. 2005). Impairment of pre- and post-invasion resistance results in *A. thaliana* becoming a host for non-adapted fungi. *Pen3* encodes a putative ATP binding cassette transporter and *pen3* mutant plants permitted both increased invasion into epidermal cells and initiation of hyphae by non-host *Bgh* (Stein et al. 2006). The Pen3 protein is concentrated at infection sites and probably contributes to defences at the cell wall by exporting toxic materials to attempted invasion sites. Although *pen* mutants identified in such screens enable efficient entry of non-host powdery mildews, post-invasive fungal growth invariably ceases, coincident with a cell death response of epidermal cells containing haustoria. Thus, other factors are responsible for maintaining a compatible interaction. Inoculating *Bgt* onto a defence-related mutant *eds1* (*e*nhanced *d*isease *s*usceptibility) of non-host *A. thaliana* resulted in partial development of the fungus (Yun et al. 2003). Bilateral haustoria were also observed, which resembled those typically produced in the compatible host, wheat. A similar decrease in non-host resistance was observed following treatment with cytochalasin E which inhibits microfilament polymerization. In *eds1* mutants, inhibition of actin polymerization severely compromised non-host resistance in *A. thaliana* against *Bgt*. Results from these investigations reveal that cytoskeletal function and *eds1* activity contribute to non-host resistance in *A. thaliana*.

VI. Significance in Agriculture: Breakdown of Resistance in the Field

When left uncontrolled, powdery mildew infections can have a significant effect on crop yield and quality. Understanding the physiology of powdery mildews and their interaction with the host plant can help to improve disease management strategies, assisting in the deployment of resistance genes and establishing when chemical control measures can be used most successfully. For example, the duration of surface wetness is important for infection by several powdery mildew species, and a model for control of glasshouse rose powdery mildew based on this has been developed. However, excessive wetness is generally detrimental to powdery mildew infection. Soil conditions and crop nutrition also significantly affect the development of powdery mildew infections. In particular, nitrogen fertilizer can be directly correlated with the amount of infection in *Bgh*. A comprehensive summary of the effects of temperature, moisture, soil conditions and light on powdery mildew epidemiology is given by Jarvis et al. (2002).

Varietal resistance is an important control measure that can be used in the management of powdery mildew diseases. However, powdery mildews can rapidly evolve to overcome resistance. When a new resistance gene is introduced, the population of the pathogen may respond by rapid growth of a few virulent clones, which spread quickly to become predominant leading to field breakdown of resistance. This has been well described in the GFG interactions of *Bgh* with barley, where barley varieties lost their *Mla* resistance within a few years of being introduced (Brown 1994; Hovmøller et al. 2000). Breakdown of resistance has been reported in other powdery mildew interactions. Pl2 is a major resistance gene used in apple breeding programmes. Virulent isolates appeared within six years after planting apple P12 genotypes resulting in a breakdown of the resistance. Ten years after planting, the percentage of genotypes that were still resistant to powdery mildew varied between 2% and 56% (Caffier and Laurens 2005). Long-term cultivation of varieties with widely used resistance genes results in significant shifts in virulence frequencies of *Bgt* on wheat (for a review, see Hsam and Zeller 2002). To delay

the development of virulence, the pyramiding of several resistance genes into a single cultivar has been proposed, since the pathogen would need to undergo multiple simultaneous changes to become virulent (McIntosh and Brown 1997). The availability of molecular markers linked to resistance genes in wheat breeding could assist in this process.

There have been reports of breakdown of *mlo* resistance, but *mlo* virulence has not developed in field populations and does not seem to present a problem for barley growing. The breakdown of *mlo* resistance is associated with the relief of water stress following a period of drought (Baker et al. 2000). In these situations, *Bgh* infection increases on both *Mlo*-susceptible and *mlo*-resistant spring barley cultivars. The breakdown of *mlo* resistance is temporary and is determined by the genetic background of the host barley plant rather than the specific resistance allele.

Investigations into the molecular mechanism of resistance described earlier could eventually provide leads for the development of more durable disease control. Powdery mildews are highly adapted pathogens with a limited host range, and infection does not succeed on non-host plants. Some of the molecular components of non-host resistance are starting to be identified. Selective breeding for natural variants in non-host resistance components could provide a basis for the development of broad-spectrum resistance. The apparent inverse relationship between *pen1* non-host resistance and *mlo* illustrates that more needs to be learnt about control of pathogen entry before such resistance can be exploited in agriculture. Barley *Mlo* homologues are present in other cereals and in *A. thaliana*, illustrating that this molecule, associated with mildew resistance, could potentially be used in a range of crops. It has been proposed that R proteins guard essential virulence targets in host plant cells, which pathogens attack to establish infection (Mackey et al. 2002). If powdery mildew effectors and their host targets can be identified, this principle can be exploited in agriculture to develop durable disease control. Selecting crop varieties with natural polymorphisms in such targets could prevent attack by adapted powdery mildews, so bypassing the reliance on R proteins which are easily defeated.

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