Chapter 18 - Hitting the wall: Plant cell walls during *Botrytis cinerea* infections

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Abstract The cell wall is among the first structures that *Botrytis cinerea* encounters when colonizing plant tissues. From the perspective of *B. cinerea* as an infecting pathogen, host cell walls are potential sources of nutrients, but intact walls are also barriers that limit advancement of growing fungal hyphae beyond the initial sites of penetration. Plant cell walls are polysaccharide-rich extracellular matrices that surround individual cells. The architecture and composition of cell walls vary among plant species and organs. The shapes and attributes of organs are determined by the arrangements of the macromolecules that compose cell walls. Walls are synthesized, remodeled and disassembled as cells divide, differentiate, expand, and expire. Metabolic, developmental and external events, including infections by pathogens, alter the properties and components of plant cell walls. This chapter focuses on the cell walls of host plant tissues during infections by *B. cinerea*. The expression and the polysaccharide targets of *B. cinerea* and plant genes predicted to encode proteins that could modify plant cell walls as a consequence of infection are described. The impacts of these proteins on the properties of walls are discussed, noting potential alterations to extracellular anti-pathogen and pathogen-related defence proteins associated with the wall matrix.

keywords: Carbohydrate-Active enZymes (CAZymes), pectins, hemicellulose-cellulose network, pathogen perception, defence responses

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18.1 Introduction

Botrytis cinerea can enter host plant tissues deliberately by breaching the cuticle using enzymes and physical means, but stomata or incidental surface breaks provide options for the fungus to enter its hosts opportunistically (reviewed in Van Kan 2006; chapter 12). Regardless of the penetration strategy that *B. cinerea* uses,

the cell wall matrix is among the first plant structures encountered by the pathogen.

Plant cell walls are potentially rich sources of nutrients for the pathogen but walls are also barriers that can limit expansion of the pathogen into and through host tissues. *B. cinerea*'s access to the more diverse and readily metabolized nutrient compounds in the cellular cytoplasmic compartments can be thwarted by the host cell wall. Host cell walls that are recalcitrant to disassembly or walls that support appropriate anti-pathogen responses, limit growth of *B. cinerea* beyond the initial sites of infection (Cantu et al. 2008a). Therefore, it is not surprising that the breakdown of polysaccharide linkages within the plant cell wall is a prominent feature of the initial contact between the growing fungal hyphae and host cells. Digestion of host cell walls continues throughout infection, usually resulting in cell death and the extensive maceration of the host that are hallmarks of diseases caused by *B. cinerea*.

While it is clear that plant cell walls can contribute to resistance as well as susceptibility, the complexity of the architecture and composition of walls explains why confrontations between *B. cinerea* and plant hosts are diverse, dynamic and interactive. Determining which cell wall polysaccharides are important targets for disassembly by *B. cinerea* and the mechanisms it uses to advance within plant tissues, might lead to the development of resistant fruit and ornamental crops and guide strategies for efficient disease management.

18.2 The plant cell wall

The structures of plant cell walls have been extensively reviewed (Harris and Stone, 2008; Voragen et al. 2009; Burton et al. 2010; Scheller and Ulvskov 2010; Keegstra 2010; Cosgrove and Jarvis 2012). Primary cell walls are composed of polysaccharides, structural proteins and ions. Secondary cell walls have additional simple and polymerized phenylpropanoids that contribute to their rigidity, and the proportions of the major polysaccharides in secondary walls differ from those in primary cell walls. The heterogeneity of the walls of different cell types from diverse plant species makes it challenging to describe a general structure for all plant cell walls. Nevertheless, models based on comprehensive analyses of structural and compositional properties of leaf and fruit tissue cell walls provide an excellent starting point to study the characteristics of the host tissues that *B. cinerea* encounters (Keegstra et al. 1973; Carpita and Gibeaut 1993; McQueen-Mason and Cosgrove 1995; Scheller and Ulvskov 2010; Hayashi and Kaida, 2011; Park and Cosgrove 2012).

The most accepted model of the primary cell wall structure corresponds to the tethered-network model, which includes two "co-extensive" polysaccharide networks. These networks are 1) cellulose microfibrils cross-linked to one another via hemicelluloses (also called cross-linking glycans), embedded within 2) simple and branched pectin polysaccharides (Carpita and Gibeaut 1993; Cosgrove 2001; Hayashi and Kaida, 2011). The overall strength and rigidity of the plant cell wall

depends on the integrity of the hemicellulose-cellulose microfibril network (Harris and Stone 2008). The pectin network influences the wall's porosity and provides structural coherence (Ishii et al. 2001). The proportions of cellulose, hemicelluloses and pectins change during development and vary depending on the plant tissue and species. Fig. 18.1 illustrates the most common features of primary plant cell walls.

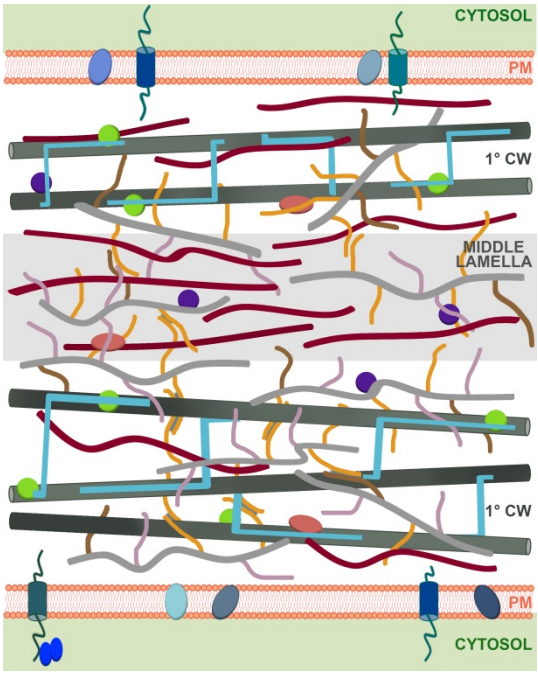

Fig. 18.1. Schematic model of the primary cell wall structure of a dicot plant based on the tethered-network model. The primary cell wall (1° CW) is composed mainly of cellulose, hemicelluloses, pectins and structural proteins. Cellulose microfibrils are represented as slate-gray rods, while hemicelluloses (i.e., xyloglucans and xylans) are the cyan-colored connectors that join the cellulose microfibrils together. The middle lamella is a pectin-rich matrix between two adjoining cells. Two major classes of pectin backbones are illustrated: homogalaturonan backbones are dark-red lines, and rhamnogalacturonan-I (RG-I) backbones are gray lines. Three types of RG-I side-branches are shown: branched arabinan (pink lines), linear galactan (orange lines) and branched, "type-I"- arabinogalactan (brown lines). Cell wall structural proteins (e.g., glycoproteins and extensins) are depicted as purple and green circles. Other proteins associated with the wall (e.g., PR-proteins) are shown as orange ovals. Trans-membrane wall-associated and receptor-like kinase proteins (WAKs and RLKs, respectively), which have parts that are in the plasma membrane (PM) and extensions into the cell wall, are depicted as blue ovals and cylinders.

Cellulose is composed of identical, long, unbranched glucans that are tightly bound together in microfibrils by multiple hydrogen (H)-bonds. Cellulose microfibrils are rigid and are the major strength-conferring elements of walls (Table 18.1; Harris and Stone 2008). Hemicellulose polysaccharides are H-bonded

to the surface of the cellulose microfibrils; they maintain the cellulose fibrils in fixed positions relative to one another. Two major types of hemicellulosic polymers are found in the primary cell walls of angiosperm plants: xyloglucans (XyGs) are found in dicots, while xylans are mostly detected in monocots and in the secondary cell walls of dicots (Table 18.1). The synthesis and disassembly of hemicelluloses is critical for plant cell growth and expansion (McNeil et al. 1984; Scheller and Ulvskov 2010). Other hemicelluloses, such as mannans (i.e., linear mannans, galactomannans, and galactoglucomannans) are important energy stores for seeds, but can be found in the cell walls of other plant tissues (Table 18.1; Scheller and Ulvskov 2010; Albersheim et al. 2011)

The pectin polymers in plant cell walls are complex and diverse macromolecules that may contain as many as 17 different monosaccharide constituents joined by more than 20 different linkages (Voragen et al. 2009). Besides their roles in determining the porosity of the wall, pectins influence a variety of physiological and cellular processes including growth and expansion of cells and cell-to-cell adhesion. Pectins influence or are integral components of signaling, pH and ion balances in the apoplast, seed imbibition, leaf abscission, fruit softening and responses to pathogens (Ridley et al. 2001; Mohnen 2008). The major pectins are homogalacturonan (HG), rhamnogalacturonan (RG-I and RG-II; Table 18.1) and are present in the walls of dicots and non-graminaceous monocots. Pectins are particularly abundant in the middle lamella and at the corners of cells (Mohnen 2008). The cell walls of graminaceous monocots, such as cereals, have fewer pectins than dicot cell walls, and the complexity of these pectins is reduced (Jarvis et al. 1988). In general, *B. cinerea* is not an important pathogen of cereals, and it remains unclear whether the reduced pectin in cereal walls is one of the reasons why *B. cinerea* growth is not supported. Other monocots, such as onions, are infected by *B. allii*, *B. aclada, B. byssoidea*, and *B. squamosa* and tulips are infected by *B. tulipae* and *B. gesneriana*, but the role of the host tissue cell wall composition is not known. Lilies, another monocot, can be infected similarly by *B. cinerea* and *B. elliptica* (Hsieh et al. 2001).

Structural proteins, such as glycoproteins, contribute to the establishment and maintenance of the structural features of plant cell walls (Rose and Lee 2010, Fig. 18.1), although the details of their associations with the components of the wall and the ways they accomplish their structural roles are not well understood. Glycoproteins have been associated with wall strength, assembly of cell wall polysaccharides, regulation of cell growth, and responses to biotic and abiotic stresses (Showalter 1993; Nguema-Ona et al. 2013a). The major groups of these proteins are identified based on their amino acid contents; for example, glycine- and proline-rich proteins, hydroxyproline-rich glycoproteins (HRGPs), and arabinogalactan proteins (AGPs; Cassab 1998; Jamet et al. 2006). Characterizations of the chemical compositions and associations between constituents of cell walls are the primary approaches used to study how *B. cinerea* sequentially digests the wall polysaccharides of its hosts to successfully invade tissues.

Table 18.1. Chemical composition of the main polysaccharides in plant cell walls.

Cell wall Polysaccharides	Sugar components	Backbone	Side-groups and other features
Pectins			
Homogalacturonan (HG)	Galacturonic acid (GalA), Methyl ester (Me) to GalA carboxyl groups (variable frequency), Acetyl ester (Ac) to GalA 2- or 3-OH groups (variable), and Xylose (Xyl), rarely	Linear array of GalA residues α-1,4-linked to one another	Xyl α-1,3-linked to GalA residues of the backbone (rare side-chain)
Rhamnogalacturonan-I (RG-I)	GalA and Rhamnose (Rha) in backbone, GalA residues can be Me- and Ac-esterified (as for HG): Galactose (Gal) and Arabinose (Ara) in side chains associated with backbone Rha	Repeating disaccharide of Rha α-linked to the 4-OH of GalA, GalA, in turn, α-linked to the 2-OH group of Rha	Galactan (Gal) residues β-1,4-linked to one another; Arabinan (Ara) residues α-1,5-linked to each other with occasional branches α-1,3-linked; and Arabinogalactans, consisting of β-1,4-linked Gal residues occasionally substituted at the 3-OH with α-linked Ara residues
Rhamnogalacturonan-II (RG-II)	GalA with variable Me content (backbone) and GalA, Glucuronic acid (GlcA), Aceric acid, Rha, Ara, Fucose (Fuc) (some Fuc with a Me ether at the 2-OH), Gal, Apiose (Api), 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo) and 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha) in the four side branches	Linear array of GalA residues α-1,4-linked to one another	Numerous forms. The RG-II backbone is decorated with four different side branches, two of which are branched (O'Neill et al, 2004). Dimers of RG-II monomers associate when the Api residues of two RG-II monomers form a covalent diester cross-link with borate
Hemicelluloses			
Xyloglucans (XyGs)	Glucosyl residues (Glc) form the backbone. Single residues of Xyl, Gal, Fuc and Ara are side-groups. Acetyl esters have been identified at the 6-OH group of the Glc residues in the XyG backbone and at the 5-OH of Ara residues and the 6-OH of Gal residues in XyG side-branches	The fundamental XyG backbone consists of Glc that are β-linked to the 4-OH of the next backbone Glc residue	The main side-group is single Xyl residues that are α-linked to the 6-OH groups of >50% of Glc residues in the backbone. The Xyl side-groups can be substituted with Gal, β-linked to the Xyl 2-OH group. In some XyGs the Gal residue then is substituted with a Fuc residue, α-linked to its 2-OH group. In other XyGs, Ara residues may be α-linked to the 2-OH position of a Xyl

			side-group or to an unsubstituted Glc residue
Xylans	The backbones are composed of Xyl. Single Ara or GlcA residues are found as side-groups.	Xyl residues b-linked to the 4-OH of the next backbone residue	Most side-branches are Ara residues that are α-linked to the 3-OH groups of Xyl residues in the backbone, as in monocots, or to the 2-OH groups, as in dicots. An additional xylan side-group is a single GlcA residue α-linked to a few of the 2-OH groups of backbone Xyl residues
Mannans, Galactomannans and Galactoglucomannans	Mannose (Man) and Glc may compose the backbones. Gal is present in the side-branches.	Man residues b-linked to the 4-OH of the next sugar residue in the backbone. Galactoglucomannans with backbones composed of alternating stretches of b-linked Man and Glc residues	Galactomannans can have single Gal residues that are α-linked to the 6-OH of backbone Man residues. Side-groups in Galactoglucomannans are single Gal residue side units that are α-linked to the 6-OH groups of Man residues
Cellulose	Glc residues	Backbone is Glc residues b-1,4-linked to one another	Multiple backbones are H-bonded and assembled into microfibrils

18.3 Decomposition of plant cell walls by *B. cinerea*

Botrytis cinerea produces and secretes proteins and enzymes, which modify host cell walls, in order to overcome the physical constraints imposed by the wall and to release sugars to sustain its own energy needs (chapters 12 & 16). The enzymatic cleavage of relatively few linkages between cell wall polysaccharides, which are important for maintaining the integrity of the polysaccharide networks, might facilitate the intercellular expansion of hyphae and, thus, enhance the pathogen's ability to access cellular resources. On the other hand, use of the wall's sugar constituents for energy might require the complete breakdown of a variety of polysaccharides into monosaccharides. Here we provide details about the proteins and enzymes that *B. cinerea* expresses when infecting various host tissues.

18.3.1 Botrytis cinerea CAZy proteins

The diverse group of enzymes and proteins that affect cell walls are usually referred to as cell wall modifying proteins. The CAZy (Carbohydrate-Active enZymes; http://www.cazy.org) database "describes the families of structurally-related catalytic and carbohydrate-binding modules (or functional domains) of enzymes that degrade, modify, or create glycosidic bonds" (Cantarel et al. 2009). Therefore, CAZy proteins are associated with modifications and breakdown of cell wall polysaccharides.

Among the approximately 16,000 genes in the genome of *B. cinerea* (strain B05.10; according to Amselem et al. 2011), 1,155 are predicted to encode CAZy proteins, and 275 of these have secretion signal peptide sequences that suggest that they function in the apoplast (Blanco-Ulate et al. 2014). Most of these putative secreted *B. cinerea* CAZy proteins (49%) are glycoside hydrolases (GHs) and the most abundant GH subfamily is GH28, which includes the polygalacturonases (PGs) that target pectins in plant cell walls (14% of all GHs). The next most abundant CAZy family (23%) is the carbohydrate-binding proteins (CBMs). Carbohydrate esterases (CEs, 17%), glycolsyltransferases (GTs, 8%) and polysaccharide lyases (PLs, 3%) are less abundant (Blanco-Ulate et al. 2014). The potentially secreted proteins encoded by 88 of these CAZy genes have been detected in *B. cinerea*-infected tomato fruit and/or *B. cinerea* grown in culture (Shah et al. 2009a and 2009b; Espino et al. 2010; Fernández-Acero et al. 2010; Shah et al. 2012; Li et al. 2012). The activities and potential roles of most of these enzymes during *B. cinerea*-plant interactions have not been confirmed.

A core set of 229 CAZy genes encoding proteins with secretion signals was detected in *B. cinerea*-infected lettuce leaves, ripe tomato fruit and ripe grape

berries (Blanco-Ulate et al. 2014). Nine PGs and eight pectin/pectate lyases (PLs/PELs) are in this core set and are the most abundantly expressed genes in all hosts evaluated. Because *B. cinerea* is a generalist pathogen with a broad host-range, these proteins are likely to be the primary enzymatic machinery utilized by the fungus as it penetrates and invades diverse host tissues. Other cell wall modifying proteins might be produced only in specific conditions. These proteins might provide adaptations for diverse host tissues and enable different stages of infection, such as, during penetration or during active fungal growth (Blanco-Ulate et al. 2014).

Although CAZy proteins secreted by *B. cinerea* are expected to target plant cell wall substrates, they could also remodel the fungal cell wall as the pathogen grows and develops, or they may degrade host cellular contents, including starch and glycosylated compounds (e.g., glycosylated proteins or secondary metabolites) (Faure 2002; Klis et al. 2009; Sha et al. 2009b). CAZy proteins could act on more than one polysaccharide substrate. The conditions in infected tissues and/or the availability of substrates may alter the kinetics and substrate preferences of their activities (Eklöf and Brumer 2010). Identifying common *B. cinerea* CAZy proteins or families expressed during infections of different host tissues predicts plant cell wall polysaccharides that are inevitable targets in multiple host tissues. Determining which CAZy genes are uniquely expressed on particular hosts reveals that *B. cinerea* makes adjustments to its virulence strategies in order to adapt to conditions in its host.

18.3.2 Cell wall polysaccharide targets of B. cinerea CAZy proteins

Pectins

Pectin breakdown during *B. cinerea* infections may increase the cell wall porosity and may facilitate the degradation of other classes of wall polysaccharides by enhancing access of other fungal enzymes to their substrates. Growth of *B. cinerea* may be enabled by its metabolism of sugars released from hydrolyzed pectins (Zhang et al. 2013). Pectins appear to be the main cell wall targets during *B. cinerea* infections, regardless of the host tissue or species (Blanco-Ulate et al. 2014). Enzymes that target pectin backbones include, PGs and rhamnogalacturonases (RGaes; GH28) and pectin/pectate lyases (PL/PELs; PL1 and PL3). Pectin methylesterases (PMEs; CE8) and rhamnogalacturonan esterases (CE12) might cooperate in the effective degradation of pectin backbones (Zhang and Van Kan 2013).

Polygalacturonases hydrolyze the backbone of homogalacturonan (HG; Table 18.1). Exo-PGs remove one D-galacturonic acid monomer at a time from the non-reducing ends of HG; endo-PGs can hydrolyze the polymer at internal sites and release oligogalacturonides or pectin-derived oligomers (PDOs) (Jayani et al. 2005). Of the 11 secreted PGs predicted in the *B. cinerea* genome, at least five are likely exo-PGs and six are endo-PGs (Blanco-Ulate et al. 2014). Most of the endo-PGs (i.e., PG1-6) have been extensively characterized (Wubben et al. 2000;

Reignault 2000; ten Have et al. 2001; Kars et al. 2005a). *B. cinerea* produces particular endo-PGs depending on the conditions in the host and the stage of infection (Wubben et al. 2000; Reignault 2000; ten Have et al. 2001; Kars et al. 2005a; Blanco-Ulate et al. 2014). PG1 is constitutively expressed, PG3 is preferentially produced in acidic conditions, PG4 and PG6 are induced by monomers of galacturonic acid, and PG4 is inhibited by glucose (Kars et al. 2005a; Zhang and Van Kan 2013). Kars et al. (2005a) determined that these PGs have specific substrate preferences and produce distinct pectin-degradation product profiles. For example, PG1, PG2 and PG4 are more effective in the depolymerization of demethylesterified HGs.

The endo-PGs expressed during *B. cinerea* infections have been investigated by characterizing the virulence of single-gene knockout mutants (i.e., *Δpg1-6*) and a double knockout mutant, *Δpg1Δpg2*. The PGs appear to have redundant functions because none of the mutants in single genes result in the total loss of *B. cinerea* virulence. The *Δpg1* mutant is less virulent on leaves of tomato, broad bean, tobacco and Arabidopsis, as well as on apple fruit and unripe tomato fruit (ten Have et al. 1998; Zhang and Van Kan 2013; Blanco-Ulate et al. pers. obs.), supporting the conclusion that it has a major role during plant infections (ten Have et al. 1998 and 2001; Kars et al. 2005a). The virulence of the *Δpg2* mutant is reduced in infections of tomato and broad bean leaves and unripe tomato fruit (Kars et al. 2005a; Blanco-Ulate et al. 2015), but not on tobacco or Arabidopsis leaves (Zhang and Van Kan 2013). Both PG1 and PG2 are required for *B. cinerea* infections in certain plant tissues, as the double mutant *Δpg1Δpg2* is avirulent in unripe tomato fruit, and the other PGs do not seem to complement the missing endo-PG activity (Blanco-Ulate and Dario Cantu, pers. obs.). The single mutants *Δpg3, Δpg4, Δpg5* and *Δpg6* are as virulent as the wild-type strain in leaves of different plant hosts (Joubert et al. 2007; Zhang and Van Kan, 2013), but their ability to cause disease in other plant organs (e.g., stems or fruit) has not been investigated.

Analyses of knockout mutants in putative exo-PGs might provide further information on the cooperative roles of different PGs. In infected bean leaves, *B. cinerea* exo-PG activity increases early in infection, but endo-PGs increase only after host penetration has occurred (Kapat et al. 1998). The temporal deployment and synergistic functions of exo- and endo-PGs has not been broadly analyzed on different host tissues.

PLs and PELs utilize a β-elimination mechanism rather than hydrolysis to degrade HGs. PLs act on HG backbones with high degrees of methylesterification, while PELs are more efficient on HGs with low levels of methylesterification and are calcium-dependent (Jayani et al. 2005). Four genes encoding PLs and four encoding PELs are annotated in the *B. cinerea* genome. The expression of genes encoding putative PLs/PELs is not as high as that of PG genes (PL1/PL3 versus GH28), suggesting that PLs/PELs assist PGs rather than being the primary enzymes for the decomposition of HGs or that PME action (discussed below) reduces the need for enzymes that can digest a methylesterified HG backbone. It is not known whether digestion products of some of these enzymes, such as PLs, alter the expression of other enzymes, such as PGs. Mutants in *B. cinerea* PLs or

PELs have not been developed, thus the impact of these enzymes on virulence is not known.

Methylation and acetylation of the HG backbones can impact the activity of *B. cinerea* endo-PGs and PELs (Kars et al. 2005a). PMEs catalyze the specific demethylesterification of HGs, releasing methanol and acidic HG. PMEs tend to act randomly on methylesterified HG backbones, releasing protons that may favor the activity of particular endo-PGs (Micheli 2001). The *B. cinerea* genome encodes three putative secreted PMEs. PME1 and PME2 have been described; they are constitutively expressed and are stable in a wide range of pHs and temperatures (Reignault et al. 1994 and 2000; Valette-Collet et al. 2003; Kars et al. 2005b). No pectin acetylesterases have been identified in the *B. cinerea* genome, although an RG-I acetylesterase has been noted (discussed below).

Botrytis cinerea PME activity may not always be essential for virulence. Mutations in *PME1* and *PME2* did not affect *B. cinerea* virulence on tomato and grapevine leaves or pear and tomato fruit (Kars et al. 2005b; Blanco-Ulate et al. pers. obs.), although PME1 seems to be necessary for successful infections of apple fruit (Valette-Collet et al. 2003). Kars et al. (2005) proposed that PME3 or other putative PMEs could compensate for the lack of PME activity in the *Δpme1Δpme2* mutant or that PMEs are not necessary for endo-PG action *in planta*. However, it is possible that *B. cinerea* relies on plant PMEs to demethylesterify the HG backbones to make them amenable for subsequent degradation (Raiola et al. 2011; Blanco-Ulate et al. pers. obs.).

The *B. cinerea* genome encodes six possible secreted rhamnogalacturonan hydrolases (RGases), which could cleave rhamnogalacturonan-I (RG-I; Table 18.1) pectin backbones (Blanco-Ulate et al. 2014). The galacturonic acids that make up the RG-I backbone can be methylated or acetylated. Deacetylation by rhamnogalacturonan acetylesterases (RGAEs) is essential for the subsequent action of RGases (Mølgaard et al. 2000). Only one RGAE is predicted in the *B. cinerea* genome and it appears to be relevant for plant cell wall degradation, as it is expressed in multiple hosts (Blanco-Ulate et al. 2014). Trimming of the side branches of the RG-I pectin backbone is an important pre-requisite for hydrolysis (Mutter et al. 1998). Although RG-I is a major part of the "hairy" (i.e., highly branched) regions of pectins in plant cell walls, it is not as abundant as pectins with HG-backbones, which could explain why expression of RGases and RGAEs is low compared to expression of genes encoding PGs and PLs/PELs.

The rhamnogalacturonan-II (RG-II, Table 18.1) pectins are complex and recalcitrant to hydrolysis. RG-II is a major component of the lees in red wine (Vidal et al. 2000); presumably, it accumulates there because the diversity of sugars and glycosidic linkages in its side-groups make its digestion a relatively expensive challenge for microbes.

The *B. cinerea* genome encodes other enzymes that could degrade the diverse side-branches of pectins. These enzymes could cut entire side-branches from the backbone, internally cleave side-branches or remove terminal residues (Zhang and Van Kan 2013). Among these putative genes are four α-arabinofuranosidases (GH GH51, GH54|CBM42 and GH62|CBM13), three β-galactosidases (GH2 and GH35) and two α-L-1,5-arabinanases (GH43 and GH93).

Ara1, an endo-α-L-1,5-arabinanase, cleaves linear arabinans present in RG-I, but is not able to cut branched arabinans (Nafisi et al. 2014). The *Δara1* knockout *B. cinerea* mutant is not capable of degrading 1,5-arabinan *in vitro* and displays a delay in secondary lesion formation when infecting Arabidopsis leaves. However, no differences in virulence in comparison to the wild-type strain were observed when this mutant was introduced on tobacco or tomato leaves. Nafisi et al. (2014) concluded that the role of *ara1* depends on the plant host.

Hemicelluloses

Multiple hemicellulose-modifying enzymes are encoded in *B. cinerea*'s genome (Blanco-Ulate et al. 2014). Cleavage of hemicelluloses may loosen the cell wall by disrupting the hemicellulose-cellulose microfibril network (Fig. 18.1). Relaxation of the wall network may facilitate access to targets by other enzymes of the pathogen. XyG backbones (Table 18.1) are hydrolyzed by endo-acting β-1,4-glucanases or β-glucosidases, which also cleave cellulose (Gilbert 2010). The *B. cinerea* genome encodes one candidate XyG-specific β-glucanase (GH12) and six β-glucosidases (GH3; Blanco-Ulate et al. 2014).

XyG endo-transglycosylases/hydrolases (XTHs) cleave the XyG backbones of hemicelluloses using two mechanisms: 1) XyG endo-transglycosylase (XET) non-hydrolytically cleaves and re-ligates shortened XyG polymers, and 2) XyG endo-hydrolase (XEH) irreversibly hydrolyses the XyG backbone (Eklöf and Brumer 2010). Plant XETs participate in loosening of cell walls (Rose et al. 2002). The *B. cinerea* genome has six candidate XTHs (GH16), and two are expressed in diverse plant tissues (Blanco-Ulate et al. 2014).

Xylans and mannans are present in the primary and secondary walls of many *B. cinerea's* hosts, but they tend to be less abundant than XyGs. Five β-xylanases (GH10, GH10|CBM1, GH11 and GH11|CBM1) and three β-xylosidases (GH43) are predicted in the *B. cinerea* genome (Blanco-Ulate et al. 2014). Expression of the *xyn11A* gene (GH11), encoding a putatively secreted endo-b-1,4-xylanase, is detected when *B. cinerea* infects lettuce leaves and ripe fruit (tomato and grape berries). The *Δxyn11a* mutant is less virulent in tomato leaves and table grape berries (Brito et al. 2006). However, the contribution of Xyn11A to virulence does not depend on its xylan-cleaving activity; it is related to the necrosis in the host caused by the xylanase protein itself (Noda et al. 2010). The involvement of other xylanases in plant cell wall degradation or necrotizing activities has not been studied.

Some of the side-branches along the XyG and xylan backbones (Table 18.1) contribute to the overall strength of the hemicellulose-cellulose microfibril network (Pauly et al. 2013). Removal of these groups might affect the hemicellulose cross-linking properties and favor the breakdown of the hemicellulose backbones. Enzymes that remove the side-branches in these polysaccharides, include β-xylosidases (GH31), α-L-fucosidases (GH95) and other enzymes that are equivalent or similar to those acting on the side groups of pectins as previously described. Furthermore, because the strength of XyG binding to cellulose depends on the integrity of the XyG's side chains modifications of these during pathogenesis can have an impact on wall porosity, hence enzyme access to other wall substrates.

Cellulose

Botrytis cinerea can degrade cellulose *in vitro* and *in vivo* (Verhoeff et al. 1983). Enzymes that might be involved in the degradation of cellulose are expressed by *B. cinerea* during infections (Blanco-Ulate et al. 2014); these include ten endo-β-1,4-glucanases (GH5, GH5|CBM1 and GH45), three cellobiohydrolases (GH6 and GH7), and the six β-glucosidases (GH3). Espino et al. (2005), demonstrated that a mutant with a deletion in *cel5A*, another endo-β-1,4-glucanase, is able to infect tomato leaves and gerbera petals. This gene is among the group of CAZy genes commonly expressed in lettuce leaves and fruit tissues. Evaluating the virulence of this mutant in other hosts may provide some information about the importance of this enzyme in *B. cinerea*-lettuce or *B. cinerea*-fruit interactions.

Glycans attached to proteins

Four putative α-mannosidases, which could release mannose from complex high-mannose N-glycans attached to structural cell wall proteins, have been predicted in the *B. cinerea* genome (Blanco-Ulate et al. 2014). The activity of fungal α-mannosidases in the disassembly of plant cell walls requires further investigation.

18.3.3 Vesicle transport of cell wall modifying enzymes

Botrytis cinerea's secretion of cell wall modifying enzymes is required for virulence. Without the essential exocytosis machinery, *B. cinerea* is not able to deliver cell wall modifying enzymes to targets in plant hosts and, therefore, would not be expected to advance with infections. The *sas1* gene encodes a Rab GTPase, which might be required for vesicle docking and fusion, and, thus, may play a central role in the secretory pathway. A knockout mutant in *sas1* displays suppressed hyphal growth, decreased sporulation and reduced virulence on tomato and apple fruit (Zhang et al. 2014).

The deletion of the *sas1* gene causes the accumulation of transport vesicles at the hyphal tip, a significant reduction of extracellular proteins (e.g., glycoside hydrolases and proteases) and a decrease in PG and xylanase activity when *B. cinerea* is grown in culture. Because expression of genes encoding the main endo-PGs and a xylanase is unaffected in the *Δsas1* mutant, it is likely, that the secretion, not the synthesis, of these hydrolases and proteases is hampered in this mutant (Zhang et al. 2014).

18.4 *Botrytis cinerea* infections influence cell wall modifications by endogenous plant enzymes and proteins

Botrytis cinerea may time its infections to a particular developmental stage of

the host, when the plant tissues or organs are more susceptible (e.g., as tissues senesce or fruit ripen), or, it may actively induce susceptibility in hosts. For example, *B. cinerea*'s infections accelerate the ripening of unripe fruit and activate disassembly of the host cell wall polysaccharide matrix, as described in Chapter 19. *B. cinerea*-derived molecules that induce or suppress the expression of cell wall modifying proteins by the plant host have not been definitively identified but may include plant hormone analogues, small RNAs, protein effectors and/or pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively).

18.4.1 Plant cell wall degrading enzymes and proteins

The cellulose microfibrils of plant cell walls are targeted for degradation during development (e.g., fruit ripening, organ abscission) by the activity of endo-β-1,4-glucanases (EGs) (Minic and Jouanin 2006). Suppressed expression of two tomato EG genes, *SlCel1* and *SlCel2*, reduces the susceptibility of leaves and tomato fruit to *B. cinerea*. The absence of EG activity may limit *B. cinerea* growth and promote the activation of defence responses. Enhanced callose deposition and expression of defence genes, such as *SlPR1* and *SlLoxD,* were observed when *B. cinerea* infects fruit from the double EG-suppressed line (Flors et al. 2007; Finiti et al. 2013).

Mutations in *AtKOR1*, a membrane-bound EG in Arabidopsis, correlate with improved resistance to *B. cinerea i*nfections. *Atkor1-1* mutant plants deposit high levels of callose in response to pathogens. However, the accumulation of callose in the *Atkor-1* mutants may impact signaling networks and hormone homeostasis. High levels of abscisic acid (ABA) and jasmonic acid (JA) were detected in infected *Atkor1-1* plants (Finiti et al. 2013). ABA and JA can have roles in plant susceptibility or resistance to *B. cinerea* (reviewed in Chapter 19).

Expansins are extracellular plant proteins involved in the loosening of the hemicellulose-cellulose microfibril network during cell expansion (i.e., the acid-growth response) and during ripening (i.e., softening). Host plant expansins might facilitate access of *B. cinerea's* cell wall modifying enzymes (e.g., PGs, PLs, EGs et al.) to their substrates (Cantu et al. 2008b). Induction of the ABA-dependent expansin-like gene *AtEXLA2* correlates with increased susceptibility of Arabidopsis leaves to *B. cinerea* (Abuqamar et al. 2013). Leaves of the knockout *exla2* mutant are more resistant to *B. cinerea* and *Alternaria brassicicola*. The absence or suppression of *AtEXLA2* expression activates immune responses via a cyclopentenone oxylipin-signaling pathway. Whether reducing *AtEXLA2* expression leads to reduced cell wall porosity, has not been determined, but may be another reason why *exla2* mutants are more resistant to necrotrophic infections (Abuqamar et al. 2013).

Once healthy tomato fruit begin to ripen, an expansin gene, *SlExp1*, is up-regulated (Rose and Bennett 1999). Concurrently, the expression of a gene encoding a plant PG, *SlPG2A*, is also strongly induced (Bennett and Labavitch

2008). *B. cinerea* precociously activates the expression of *SlExp1* and *SlPG2A* once it establishes an interaction with unripe fruit (Cantu et al., 2009). This observation leads to the conclusion that *B. cinerea* infections of unripe tomato fruit can over-ride endogenous fruit host ripening programs, especially the expression of the cell wall modifying proteins (Cantu et al. 2009; Blanco-Ulate et al. 2015). When either *SlExp1* or *SlPG2A* expression is suppressed in tomato fruit, no change in susceptibility to *B. cinerea* is observed, although pectin depolymerization is reduced (Cooper et al. 1998; Cantu et al. 2008b). However, when the expression of both *SlExp1* and *SlPG2A* is compromised, the typical ripening-associated increase in *B. cinerea* susceptibility of fruit decreases (Cantu et al. 2008b) and these fruit are firmer. The composition and architecture of fruit cell walls may directly impact the ability of *B. cinerea* to grow because less fungal growth is seen in cultures containing cell walls extracted from uninfected *SlPG2A*- and *SlExp1*-suppressed fruit than in cultures with cell walls from control fruit (Cantu et al., 2008b). The activities of *SlPG2A* and *SlExp1*may influence access by other fruit and fungal proteins to their polysaccharide substrates in the cell wall.

Expansin-like microbial proteins, such as swollenin, have been identified in the cellulolytic fungus *Trichoderma reesei*. Swollenin has an N-terminal fungal type cellulose-binding domain connected by a linker region to its expansin-like domain. This protein is able to disrupt the hemicellulose-cellulose microfibril network without producing detectable amounts of reducing sugars. At present, *B. cinerea* proteins with expansin activities have not been identified. However, *B. cinerea* abundantly produces a cerato-platanin protein (BC1G_02163), which contains an expansin-like domain (Frias et al. 2011; Martellini et al. 2012; Frias et al. 2013).

In Arabidopsis leaves, two cellulose synthase genes, *AtCeSA1* and *AtCeSA3*, have roles in responses to *B. cinerea* (Windram et al. 2012). Plants with mutations of *CeSA3* gene display decreased susceptibility to *B. cinerea*, possibly due to the induction of JA and ethylene synthesis and signaling (Ellis et al. 2002). A mutant in *AtCeSA1* also exhibits increased expression of a JA-inducible gene (i.e., *VEGETATIVE STORAGE PROTEIN*, *VSP1*), suggesting overproduction of JA in this mutant as well. In wild-type Arabidopsis plants, *AtCeSA1* and *AtCeSA3* are down-regulated by *B. cinerea*, possibly as part of the plant's effort to limit pathogen infection (Windram et al. 2012). Mutation of a secondary cell wall regulator, *MYB46*, enhances Arabidopsis resistance to *B. cinerea* (Ramirez et al. 2011a). The *myb46* knockout mutants show a rapid reduction in the expression of cellulose synthase genes, including *AtCeSA1* and *AtCeSA3*, after *B. cinerea* infections. This observation indicates that the timing of *AtCeSA* repression may be important to control *B. cinerea* spread in Arabidopsis leaves (Ramirez et al. 2011b).

18.4.2 Modifications in plant cell wall polysaccharides

Botrytis cinerea infections of Arabidopsis leaves alter the expression of plant host PME genes (Abuqamar et al. 2006). The enhanced expression and activity of AtPME3 has been linked to increased susceptibility to *B. cinerea* and the necrotrophic bacterium *Pectobacterium carotovorum* (Raiola et al. 2011). In response to *B. cinerea*, plant PME expression increases in fruit. The tomato *SlPMEU1* is up-regulated during infections of unripe and ripe tomato fruit, and *SlPME2* increases slightly at the early stages of infections of ripe fruit (Blanco-Ulate and Dario Cantu, pers. obs.).

Plant PMEs may act as susceptibility factors by cooperating with *B. cinerea* PMEs in the extensive demethylesterification of HG backbones that facilitates their further breakdown by fungal or host enzymes (Lionetti et al. 2012). The importance of plant and fungal PMEs during *B. cinerea* infections may depend on the conditions in the host. For example, infections with the *B. cinerea* double mutants *Δpme1Δpme2* and *Δpg1Δpme1* cause a strong induction of the tomato *SlPME1* and *SlPME2* particularly in a tomato fruit lacking *SlPG2A*. Remarkably, these mutant *B. cinerea* strains are as virulent as the wild-type counterparts when introduced to the *SlPG2A*-suppressed fruit. This result suggests that the induction of plant PMEs might compensate for the lack/reduction of fungal PME activity. This compensation may, then, favor the action of *B. cinerea*'s endo-PGs and PELs, particularly, in a fruit host in which the endogenous PG activity is hampered (Blanco-Ulate et al. 2014).

Elevated acetylation of pectins and XyGs in the cell walls of Arabidopsis is associated with susceptibility to *B. cinerea*. A knockout mutation in the *REDUCED WALL ACETYLATION2* (*AtRWA2*) gene, which encodes an *O*-acetyltransferase, results in decreased levels of acetylated cell wall polymers and increased tolerance to *B. cinerea* (Manabe et al. 2011). Because *O*-acetylation could interfere with the hydrolysis of polysaccharides by microbial enzymes (Selig et al. 2009), it is unclear why the *Atrwa2* Arabidopsis mutant is more resistant to *B. cinerea* (Manabe et al. 2011).

18.5 Pathogen perception, defences and host cell walls

When *B. cinerea* confronts the cell wall matrix of its host, the ensuing alterations and disassembly of the wall polysaccharides have secondary consequences for the host-pathogen interaction. Changes in the host cell wall may also evoke a switch in *B. cinerea*'s infection strategy. DAMPs, such as fragments generated by digestion of the wall polysaccharides, or *B. cinerea* cell wall modifying proteins that are PAMPs, may trigger defence responses, including fortifications of the host wall. Wall-associated kinases and receptors, which protrude from the host plasma membrane into the extracellular cell wall space, may "sense" that the integrity of the wall has been compromised and relay that

information to the cellular cytoplasmic compartment. Host defence proteins, such as pathogenesis-related (PR) proteins that are exported to the apoplast may reside in the wall matrix and they may become dislodged by infections and, thus, become ineffectual.

18.5.1 Host wall-associated plant defence proteins.

Plant receptors that sense the integrity of the plant cell wall or perceive PAMPs and other effectors protrude from their anchor points in the plasma membrane into the apoplast (Fig. 18.1). How these receptors and signal transmitting proteins participate in host resistance and susceptibility is not entirely known, although several have been shown to be important for resistance or susceptibility to *B. cinerea* in vegetative organs, especially in Arabidopsis. Since the composition of cell walls differs depending on the plant tissues and species (Keegstra 2010), exposure to PAMPs or microbial effectors may be influenced significantly by the wall's composition and architecture. Access to *B. cinerea*-derived signals may be promoted especially as walls are destroyed by *B. cinerea* or disassembled during plant developmental processes, such as ripening and senescence.

Knowing that *B. cinerea* can activate immune responses, suggests that plasma membrane-anchored extracellular receptors may participate in responses to *B. cinerea* (Lai and Mengiste 2013). The PG1 protein itself is recognized as a PAMP by an Arabidopsis RBPG1 receptor that complexes with a Leucine-rich Repeat Receptor-like Kinase (LRR-RLK) that is anchored in the plasma membrane (Zhang et al. 2014).

The extracellular domains of Wall-Associated Kinases (WAKs) interact via carbohydrate binding domains with the pectins in primary cell walls (Fig. 18.1; Decreux and Messiaen 2005; Kohorn and Kohorn 2012) and bind PDOs (Brutus et al. 2010; De Lorenzo et al. 2011). In Arabidopsis leaves, *B. cinerea* small RNAs target a WAK-coding gene (*At5g50290*; Weiberg et al. 2013). Since WAKs bind pectins and pectin degradation is key for *B. cinerea* infections, WAKs might signal the host cell wall that its integrity has been compromised by a fungal attack.

Another plasma membrane anchored protein, SlCOBRA, that has an extracellular cellulose binding domain is expressed abundantly early in tomato fruit development and then declines, in contrast to most WAKs. When SlCOBRA is suppressed, fruit tend to crack extensively and have altered cellulose synthesis and cell wall architecture, over-expression of SlCOBRA improves the postharvest performance of fruit. Expression of tomato cell wall modifying genes, WAKs and other RLKs, such as *Theseus 1* and *Lectin Receptor-like Kinase* is up-regulated by the suppression of SlCOBRA expression; however, it is not clear how SlCOBRA impacts other dynamic changes in plant cell walls (Cao et al. 2012). Also, it is not known whether expression of SlCOBRA or other RLKs like *Theseus 1* or LRR-RLK homologues is altered in response to *B. cinerea*.

In leaves, the cytoplasmic protein, *B. cinerea*-Induced Kinase (BIK), is involved in PAMP triggered immunity to *B. cinerea* through phosphorylation and

signaling that includes ethylene (Laluk et al. 2011). BIK interacts with BRASSINOSTEROID INSENSITIVE 1- ASSOCIATED KINASE 1 (BAK1) to signal for PAMP triggered immunity (Mengiste 2012). BAK1 has an extracellular domain that protrudes into the cell wall space. BAK1 receptors are involved with FLAGELLIN SENSITIVE2 (FLS2) by their common association with BIK1. FLS2 is a receptor of flagellin, an extracellular structural protein produced by bacteria (Chinchilla et al. 2006). Exposure to flagellin results in responses similar to those to fungal elicitors (e.g., chitin, xylanases, ergosterol, mannose-rich glycopeptides) and the wound hormone peptide, systemin (Scheer and Ryan 1999; Meindl et al. 2000). While in Arabidopsis many of the responses to *B. cinerea* may be signaled through the WAK1 receptor, PAMP perception by the BAK1/BIK1 system may also transmit information about infections.

Plant PR proteins induced in response to pathogens have diverse potential anti-pathogen functions and they accumulate in response to *B. cinerea* (Díaz et al. 2002). Since PR proteins are located in the apoplast, changes in the plant cell walls due to *B. cinerea* infections may impact the efficacy of these anti-pathogen proteins.

18.5.2 Oligosaccharides

PDOs accumulate in the cell wall as a consequence of the pathogen-induced breakdown of pectin polysaccharides. PDOs can be recognized by WAK receptors that, then, signal plant responses, including defences (Hahn et al. 1981; Ridley et al. 2001, Galletti et al. 2011). Only de-esterified PDOs induce defence responses that enhance resistance to *B. cinerea* (Spadoni et al. 2006; Osorio et al. 2008), suggesting that WAKs may discriminate classes of PDOs. For example, over-expression of the PME, *FaPE1*, in strawberry fruit leads to the accumulation of defence-eliciting PDOs (Osorio et al. 2008; Osorio et al. 2011). During *B. cinerea*-plant interactions, the perception of PDOs could cause increases in ethylene and reactive oxygen species (ROS) (Campbell and Labavitch 1991; Bellincampi et al. 2000; Galletti et al. 2008), changes in membrane polarization and ion fluxes (Mathieu et al. 1991; Thain et al. 1995), elevated expression of defence-related genes and accumulation of phytoalexins (Davis et al. 1986).

Signaling for plant responses to pathogens through PDOs involves mitogen-activated protein kinases (MAPKs; chapter 17), at least in Arabidopsis leaves. MPK3 and MPK6 are activated by PDOs, flg22 and other PAMPs. Mutations in *MPK3* increase susceptibility of leaves and while a knockout of *MPK6* does not affect basal resistance, it does suppress PDO- and flg22-induced resistance to *B. cinerea*. This observation suggests that MPK6 may be more important for responses to *B. cinerea* (Galletti et al. 2011). Weinberg et al. (2013) demonstrated in Arabidopsis that *B. cinerea* releases small RNAs that reduce expression of MAPKs involved in defence.

18.5.3 Proteins that interfere with B. cinerea–induced cell wall disassembly

One class of plant proteins associated with the cell wall that is known to affect the ability of *B. cinerea* to infect fruit and leaves is the PG inhibiting proteins, PGIPs. Defence-activating PDOs are thought to accumulate to higher levels when pathogen PGs are inhibited by plant PGIPs *in vitro* (De Lorenzo et al. 1994; Reymond et al. 1995; De Lorenzo and Ferrari 2002; Casasoli et al. 2009). PDOs, including those that result from PG and PL activities, have been isolated from developing *B. cinerea* lesions on infected tomato fruit (An et al. 2005). PGIPs can bind pectins and when pectin disassembly is reduced by suppression of the ripening PG in tomato fruit, the PGIP protein is retained in the cell wall matrix (Powell pers. obs.). Expression of PGIP-encoding genes is high in unripe fruit and declines as fruit ripen; however, although the protein is stable, it is probably less tightly associated with the looser wall matrix of ripe fruit (Powell et al. 2000). PGIPs inhibit microbial PGs, including some but not all of *B. cinerea*'s PGs (Sharrock and Labavitch 1994; Joubert et al. 2007). Particular amino acids in the PGIP LRRs determine which PGs are inhibited (Stotz et al. 2000). PGIP expression is induced by infections with *B. cinerea* and other pathogens. Over-expression of PGIPs reduces *B. cinerea* growth on ripe tomato fruit (Powell et al. 2000). In some situations, expression of PGIPs can cause alterations in the polysaccharides within the wall matrix (Nguema-Ona et al. 2013b), further altering the extracellular environment that *B. cinerea* encounters.

Proteins that are inhibitors of plant PMEs (called PMEIs), limit *B. cinerea* growth in vegetative tissues (Lionetti et al. 2007, 2014). Since tomato fruit PMEs are abundantly induced by *B. cinerea*, induced PMEIs may participate in plant responses to the infections.

Plants express other proteins that inhibit fungal host cell wall modifying enzymes. Tomato fruits produce an endo-β-glucanase inhibitor, XEGIP, which suppresses the activities of xyloglucan β-1,4 endoglucanases (XEGs) of the fungus *Aspergillus aculeatus* (Qin et al. 2003; York et al. 2004). Expression of XEGIP decreases during fruit ripening. Other glucanase inhibitors are serine proteases; loss of the Arabidopsis Unusual serine Protease Inhibitor (UPI) results in increased resistance to *B. cinerea* (Laluk et al. 2011).

18.5.4. Plant cell wall fortifications

Fortifications of host plant cell walls are common responses that can limit pathogen progress. Localized structural reinforcements of the cell wall are usually accomplished by secretion of cross-linking phenolic compounds and subsequent accumulation of apoplastic polymers that are recalcitrant to degradation (Underwood 2012; Finiti et al. 2013). During *B. cinerea* infections, the localized fortifications of host cell walls can restrain fungal growth and impede degradation

of wall components that serve as nutrient sources for the pathogen (Van Baarlen et al. 2007).

Asselbergh et al. (2007) proposed that deposition of phenolic compounds and cross-linkage of cell wall structural proteins may limit *B. cinerea* infections of leaves in the *sitiens* tomato mutant, an ABA-deficient tomato line. The *sitiens* leaves also have increased methylesterification of the pectins in their walls, which may limit the activity of *B. cinerea* endo-PGs and PLs. Thus, the higher levels of methylesterified pectins and the wall reinforcements may result in the improved resistance of *sitiens* mutant leaves to *B. cinerea* (Curvers et al. 2010). In another study, Arabidopsis mutants with low levels of phenolic monomers (i.e., monolignols) failed to mount efficient defences against *B. cinerea* infections (Lloyd et al 2011).

In young tomato fruit, accumulation of the polymers callose and suberin has been linked to the formation of "ghost spots" (i.e., small necrotic lesions, usually surrounded by a white halo), which appear to restrain *B. cinerea* growth (de Leeuw 1985). In addition, Cantu et al. (2009) demonstrated that suberin and lignin are deposited and H_2O_2 accumulates when unripe tomato fruit display resistance to *B. cinerea*.

When *B. allii* attempts to penetrate the epidermal cells of onions, granular deposits of reaction material (RM) form on the cell wall. Feruloyl-3′-methoxytyramine (FMT) and feruloyltyramine (FT) are the main components of the RM; while other phenolics, such as coumaroyl glucose, coumaroyltyramine (CT) and 2-hydroxy-2-(4-hydroxyphenyl) ethylferulate, are minor constituents. The formation of RM is associated with early increases in peroxidase activity. FMT, FT or CT are not antifungal compounds *per se*, thus these phenolics probably contribute to resistance because they prevent or retard cell wall degradation (McLusky et al. 1999).

18.6 Outlook for improving resistance by altering host cell walls

The plant cell wall is a complex and dynamic structure that provides important functions for the integrity of plant tissues. *B. cinerea* has evolved a wide array of virulence mechanisms that target multiple components of the cell walls of its hosts. The confrontation between *B. cinerea* and its hosts alters structural and biochemical aspects of the plant cell walls. *B. cinerea*'s virulence activities have substantial impacts on host cell wall integrity and ultimately on whether the outcome is resistance or susceptibility (Fig. 18.2).

Multiple approaches using plant and *B. cinerea* mutants as well as transgenic plants have identified processes targeting the plant cell wall by enzymes expressed by the host and by *B. cinerea*. However, comprehensive studies of the progressive disassembly and remodeling of plant cell wall polysaccharides during plant development or *B. cinerea* infections, but also on the interaction between plant and fungal enzymes and cell wall components, are still needed to expand our knowledge of the molecular basis of plant susceptibility and *B. cinerea* virulence.

Forward screens of genetic variants in natural, mapping, or mutagenized populations in plants and in *B. cinerea* may identify master regulators of processes that impact the cell walls of plant hosts. Reverse genetic approaches may be beneficial once the sequence of degradation of key cell wall targets and recognition of the direct and indirect consequences of wall modification in particular hosts is characterized. Particular attention should be paid to the developmental stage of the potentially susceptible host tissues in order to develop strategies that will effectively limit damage caused by *B. cinerea*.

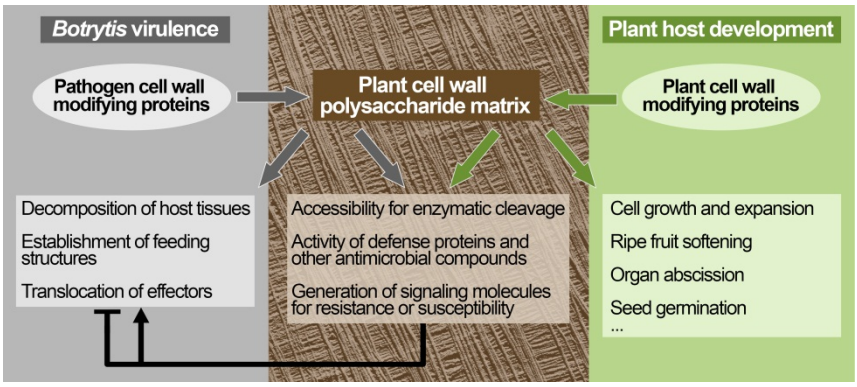

Fig. 18.2 Summary of the cell wall implications during plant-*B. cinerea* interactions (Reproduced and adapted, with permission, from Cantu et al. 2008a).

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