Cell wall metabolism: The Yin and Yang of fruit postharvest biology

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For the fruit postharvest physiologist, discussions of cell wall metabolism initially bring to mind the processes associated with the fruit softening that occurs during ripening. Fruit softening traditionally has been equated to the series of apoplast-localized events that lead to textural changes, which are desired by most consumers. Among these events, the enzyme-catalyzed breakdown and solubilization of cell wall polysaccharides are considered to be crucial in most fruit. The goal of this paper is to help us to see fruit softening in terms of a series of mechanistically connected wall metabolism processes that ends with the familiar texture changes, but which may influence a good deal more of the developmental and metabolism transitions that occur as a fruit ripens.

Yin and Yang are terms that describe the apparently opposing, but occasionally complementary, sides of the same entity. The postharvest biologist certainly must apply this perspective to his/her view of the ripening fruit's cell wall metabolism. In general, the most costly fruit postharvest management problems are associated with poorly controlled ripening (the softening aspects, in particular) and losses to pathogens. Our work with tomato fruit demonstrates a Yin/Yang-like connection between (1) the wall metabolism events that control ripening and (2) the processes that convert an unripe, pathogen-resistant tomato to a fully ripe, pathogen-susceptible fruit. A biologist who studies the evolution of Angiosperms would most likely see the ripening fruit's softening and increasing pathogen susceptibility as behaviors that contribute to seed dispersal and the eventual success of well-adapted species. A longer-term goal of this research is to understand the genetic basis of this linkage and then sever it, thus enabling the delivery of ripe and pathogen-free fruit to consumers.

A brief introduction to the plant cell wall

The cell wall *per se* is located in the apoplast, the space "outside" of the plasma membranes of individual cells. Available for a few decades have been models of cell wall organization and composition (Keegstra et al. 1973, Carpita and Gibeaut 1993, McQueen-Mason and Cosgrove 1995). Research has shown that the walls depicted in models, reflect average distributions of different polysaccharides in walls of unspecialized cells; thus, cells that are specialized to perform specific functions may have polysaccharide compositions that differ substantially from those shown by models. However, the bulk of the cells in the tomato fruit pericarp are relatively unspecialized, so the dicot cell wall model provided in figure 1 might be useful for an examination of ripening-associated wall metabolism. The wall has two general structural polysaccharide networks. Cellulose microfibrils (MFs) are assemblages of several β-1,4-linked glucans that tightly associate via many H-bonds to neighboring glucans, MFs are rigid elements that provide the wall's main resistance to applied stress. This resistance is further assured

by hemicellulose polysaccharides (HCs, linear, neutral sugar-rich polysaccharides) that form H-bonds to the surfaces of two or more MFs and essentially lock the MFs in place relative to each other. The MF-HC network can be exemplified by comparing it to the steel bar and wire network that gives strength against sheering stresses to roads and buildings. The second network is composed of a structurally diverse set of simple and complex, galacturonic acid-rich polysaccharides, the pectins. These can be thought of as the concrete that is poured in around the steel and wire to hold that network in place. In addition to their role in cell wall structure, the pectins also determine the wall's porosity. The porosity of the cell wall affects the diffusion of mobile interacting molecules (e.g., enzymes, polysaccharides, hormones, enzyme substrates and products) that participate in reactions to disrupt or reinforce the wall's structure and to assist the plant in its interactions with enemies and friends. The importance of the cell wall environment is emphasized by the presence of membrane-spanning sensors (wall-associated and receptor-like kinases, WAKs and RLKs, respectively; more information in Decreux, and Messiaen 2005; Hématy et al. 2009; Kohorn and Kohorn 2012) that are positioned to monitor the wall's chemical and physical status (Figure 1).

Diverse manifestations of cell wall metabolism in ripening fruits

For many decades, postharvest researchers have considered the cell wall's disassembly as a key factor of fruit ripening (Brummell and Harpster 2001; Vicente et al. 2007). In the 1970s, Peter Albersheim and colleagues suggested that the attention on the wall metabolism events *per se* was too narrow because it ignored the potential regulatory impacts of oligosaccharides, which are products of the digestion of the cell walls by plant or pathogen enzymes (Ayers et al. 1976). He coined the term *oligosaccharin*, implying that we should think of these pieces of cell walls as if they played hormone-type roles (Albersheim et al. 1996). Subsequently, several studies showed that fragments of pathogen or host plant cell walls could elicit plant anti-pathogen responses even if no pathogens were present (Hahn et al. 1981, Ridley et al. 2001, Osorio et al. 2008, Galletti et al. 2011). Other research suggested that wall-derived oligosaccharides had impacts on plant growth and developmental events, such as cell elongation (reviewed in Ryan and Farmer 1997). These studies suggested that the oligosaccharides that were applied and elicited plant defenses also might influence the normal developmental programs of plant tissues. However, further validation is needed, because in all of theses early situations, the oligosaccharides tested had been generated *in vitro* and not *in planta*.

We thought that ripening tomato fruit might be a good system for studying possible regulatory roles of endogenous cell wall oligosaccharides. A tomato fruit is a bulky tissue that should provide a relatively substantial amount of wall breakdown products. Also, reports for diverse plant systems, including tomato fruit, suggested that pectin-derived oligosaccharides (PDOs) promoted the synthesis of ethylene, the hormone most closely linked with fruit ripening (Baldwin and Biggs 1988, Baldwin and Pressey 1988, Brecht and Huber 1988). Our first work on this topic utilized PDOs generated by an acid "partial" hydrolysis of polygalacturonic acid, a linear polymer of α-1,4-linked galacturonosyl residues with no methyl-esterified carboxyl functions. Former Ph.D. student Alan Campbell showed that these PDOs stimulated the ethylene production of suspension cultured pear fruit cells (Campbell and Labavitch, 1991a). While this was encouraging, the pear cell system did not present any other ripening fruit behaviors.
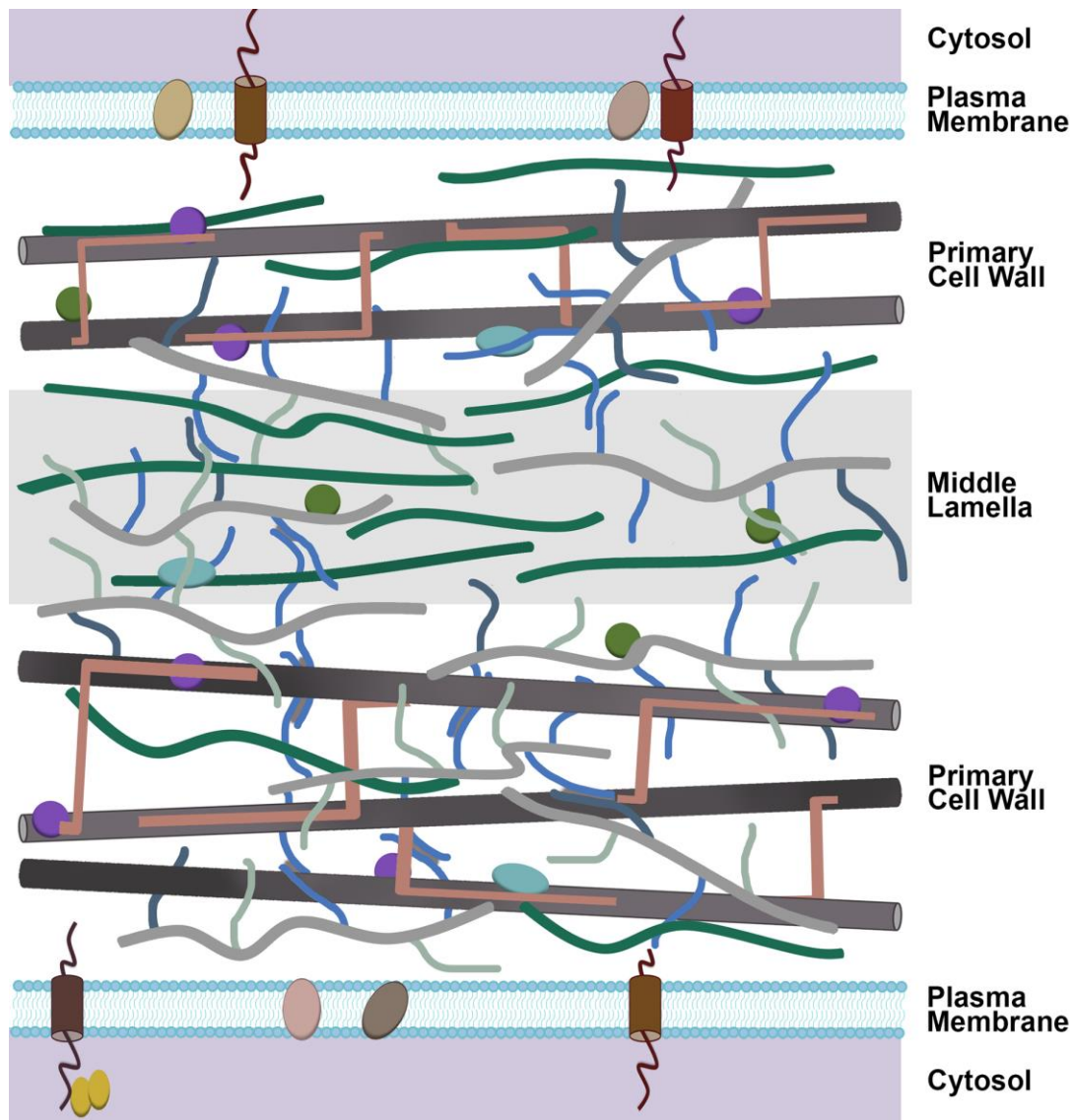
Figure 1. Schematic model of the primary cell wall structure of a dicot plant. The primary cell wall is mainly composed of cellulose, hemicelluloses, pectins and structural proteins. Cellulose microfibrils are represented as slate-gray rods, while hemicelluloses are the light pink-colored connectors that join the cellulose microfibrils together. The middle lamella is the pectin-rich matrix between two adjoining cells. Two major classes of pectin backbones are illustrated: homogalacturonan (HG) backbones are dark-green lines and rhamnogalacturonan-I (RG-I) backbones are gray lines. Two types of RG-I side-branches are shown: branched arabinan (light green lines), linear galactan (blue lines). Cell wall structural proteins are depicted as purple and green circles. Other proteins associated with the wall (e.g., PGIPs) are depicted as cyan ovals. Trans-membrane proteins and receptors (e.g., WAKs and RLKs), which have parts that are in the plasma membrane and extensions into the cell wall, are depicted as brown ovals and cylinders.

Campbell then developed a very useful test system, explanted tomato pericarp disks (Campbell et al., 1990). Disks cut from a surface-sterilized, unripe tomato fruit could be held in plastic multi-well culture dishes in a humid atmosphere and the disks would display all of the changes typical of a ripening tomato over the course of 7-10 days in culture. Of course the disks displayed a transient increase in ethylene synthesis that we attributed to a wound response, but disk ethylene output returned to baseline before fruit red color development became apparent. More importantly, application of the PDO preparations rapidly stimulated ethylene synthesis and accelerated ripening in the tomato disks. The promotion of ethylene synthesis probably played a role in the accelerated ripening, but some aspects of color change in the PDO-treated disks occurred more rapidly than in disks treated with the ethylene precursor ACC (Campbell and Labavitch, 1991b), suggesting that PDOs had biological impacts *via* signaling pathways that were independent of ethylene. .

This work clearly showed that pectin polysaccharide breakdown products could promote fruit ripening, but the work, like most of the earlier studies, used oligosaccharides that had been generated *in vitro*, rather than in the fruit tissues. Former Ph.D. student Eunice Melotto followed in the project by carefully analyzing the pectins and pectin breakdown products that accumulated in tomato fruits as they began to ripen. She used anion exchange chromatography to isolate a minor fraction (less than 1% of the soluble, uronic acid-containing wall components) that contained PDOs. Based on HPLC analysis, this set of uronic acid-rich oligomers contained molecules similar in size to those from cirus pectin that had stimulated ethylene synthesis and ripening in the pear cell supensions and tomato disks. In another experiment, Melotto incubated extracted tomato fruit polymeric pectins with a pure sample of tomato fruit polygalacturonase (PG, a pectin polymer hydrolase). This incubation generated a series of PDOs similar to those that had been isolated from ripening fruit, and when these endogenous tomato fruit oligosaccharides were applied to pericarp disks, they also promoted ethylene synthesis and ripening (Melotto et al., 1994).

This series of studies shows that oligosaccharides with the potential to influence fruit development are generated in unripe tomatoes at the onset of ripening; however, it does not prove that the tomato PDOs actually play a regulatory role in ripening. That test would require manipulations that (1) prevent the endogenous generation and accumulation of PDOs, or (2) eliminate a cellular receptor that enables tomato fruit cells to detect "local" PDO presence and initiate the ripening program.

By the start of the 1990s, a number of groups had cloned the tomato's PG gene and reduced or eliminated its expression as ripening progressed. This work was done to test the role of PG in fruit softening (Sheehy et al. 1988, Smith et al. 1988). The reduced PG tomato lines showed altered patterns of softening, but they still appeared to carry out the other aspects of a typical ripening program. If PDOs generated by PG action on tomato pectins are involved in the regulation of fruit ripening, one would expect that fruit with little or no PG would have displayed substantially altered ripening, but that was not the case.

In the late 1990s, a number of groups interested in fruit ripening had detected the presence of pectate/pectin lyases (PL), enzymes that break down the same pectins that are digested by PG, but cleave them by a different mechanism (β-elimination). PL gene expression and enzyme activity were reported in a number of fruits (Dominguez-

Puigjaner et al. 1997, Marin-Rodriguez et al. 2002, Payasi and Sanwal 2003), including strawberry where suppressed expression of the PL gene in ripening fruit led to much slower fruit softening (Jimenez-Bermudez et al., 2002). We (An et al., 2005) performed a detailed structural analysis of the PDOs that accumulated in *B. cinerea*-infected and healthy, ripening tomatoes. We observed the same patterns of oligosaccharides that we had reported earlier (Melotto et al., 1994), but additional detailed structural analysis showed that some of these PDOs could have been produced by PL action. We expect that the PL-generated PDOs were present in the transgenic fruit with suppressed PG expression. Therefore, if the PL-generated PDOs are active as signals, they also could have influenced the ripening of tomatoes with suppressed PG, even if the PG-generated PDO series was absent. In the past few years, we (Blanco-Ulate et al., unpublished) have identified transcripts with sequences similar to those of known PLs in transcriptome data sets from ripening tomatoes. Two ripening-associated PL genes have been cloned and we will generate transgenic lines in which PL expression is suppressed. Depending on our analysis of the ripening PL-suppressed fruits, we then may cross the PL knock-out lines with lines suppressed in PG and expansin (Exp) expression (discussed in more detail, below; Cantu et al. 2008a) in order to develop a more complete understanding of factors influencing pectin polymer metabolism in ripening tomatoes as well as to test the possible involvement of PG- and PL-generated PDOs in the regulation of ripening.

Testing of this idea at the "cellular signal perception" level, as is now done for ethylene signals with the aid of inhibitors or with natural or engineered tomato mutant lines (Klee and Giovannoni 2011; Pech et al. 2012), is much further in the future. Only recently, data that suggest the nature of a cellular receptor for PDOs have been published (Brutus et al., 2010). These investigators worked with a wall-associated kinase WAK1 from *Arabidopsis thaliana* and used a domain-swap strategy to demonstrate that a WAK chimeric protein could respond to PDOs and activate down-stream expression events. In their system, transgenic plants that over-expressed WAK1 displayed enhanced resistance to the grey mold fungus, *Botrytis cinerea*, similar to defenses expressed by exogenous application of oligogalacturonide-type PDOs. However, the certain identification of apoplast-localized "sensors" for oligosaccharide signals (whether they are important components of endogenous signal systems or merely intriguing distractions) is still to be deciphered.

Cell wall changes and the ripening tomato fruit's softening

Among the first lines of postharvest investigation that made use of the then new molecular biology techniques were studies of the relationships between the expression of genes encoding cell wall modifying proteins (CWMPs) and aspects of cell wall change in ripening fruits (Christoffersen et al., 1984; Fischer and Bennett, 1991). The relative ease of tomato genetic transformation and production of progeny homozygous for the introduced DNA sequence made that species a postharvest fruit model. The suppression of genes encoding several tomato fruit CWMPs and the subsequent impacts of suppression on fruit softening has been a widely used strategy. The CWMP roles tested in transgenic tomatoes include the ripening-associated endo-β-1,4-glucanase (EGase, Lashbrook et al, 1994), β-galactosidase (Smith et al., 2002), pectin methyl-esterase (PME, Tieman et al., 1992), Expansin (Exp, Brummell et al., 1999), as well as PG

(Sheehy et al., 1998; Smith et al., 1988) . Discussions of how Exp functioned to "loosen the cell walls" to permit turgor-driven cell elongation in seedlings suggested that the protein caused a relaxation of the intra-polysaccharide associations in the wall's MF-HC network (Cosgrove 2000). The clearest slowing of softening in tomatoes with reduced Exp gene expression was observed at the start of ripening (Brummell et al., 1999), while the reduced softening of tomatoes with suppressed expression of PG was most evident at the end of ripening (Sheehy et al., 1998; Smith et al., 1988).   These observations supported the idea that fruit softening starts with processes that weaken the wall's MF-HC networks and ends with processes that modify the pectin networks (Rose and Bennett 1999). This hypothesis led to a series of crosses involving homozygous tomato lines with suppressed expression of PG or Exp and resulted in a line that expressed neither Exp nor PG as they ripened (Powell et al., 2003). The resulting PG/Exp double suppressed fruit softened more slowly than the individual PG- and Exp-suppressed lines (Cantu et al., 2008a). Analysis of the changes in the cell walls of the ripening PG/Exp double suppressed fruit indicated much less pectin change than that observed in fruits with only PG suppression. However, the analysis showed no apparent differences in the solubility, amount or size of the HC polymers isolated from the control (wild-type, normal PG and Exp gene-expressing) and the individual PG- or Exp-suppressed fruit. This led us to conclude that, at least in ripening tomatoes, Exp loosens the general wall structure to facilitate the interaction of other apoplastically localized CWMPs with their wall targets, and that the improved access enhances the breakdown of the cell wall and impacts fruit softening. Therefore, if the role of Exp in fruit softening is largely to enhance access of other CWMPs to their cell wall targets, then it is reasonable to suggest that combining the suppression of Exp with suppression of any fruit CWMP-encoding genes (those mentioned above or, perhaps, others that are identified) might also lead to slowed loss of firmness during ripening. It is also reasonable to imagine that enhancing access of pectin-degrading enzymes to their wall targets would also affect the concentrations and specific structures of PDOs in the fruit apoplast (discussed above) and this might have an impact on fruit softening and, perhaps, other ripening-associated processes.

The ripening tomato fruit's increasing pathogen susceptibility
        The fact that ripening fruits become increasingly susceptible to a range of postharvest pathogens has long posed a problem for postharvest physiologists and pathologists. We can understand why ripening and susceptibility are linked; after all, tomato fruit ripening begins after developing seeds are mature and several aspects of the process that make the fruit attractive to us also make it attractive to organisms that contribute to seed dispersal. However, this recognition of the biological purpose of ripening does not lessen the disappointment and frustration of packers, shippers, retailers, consumers and postharvest biologists with the ripening fruit's enhanced susceptibility of to diverse biotic stresses. Some of the "mechanistic" explanations of the ripening-related increase in susceptibility include: 1) greater amounts of and easier access to plant substrates that support pathogen energy metabolism and growth, 2) loss of pre-formed, small and large defense compounds, 3) changes in the apoplast's chemical environment (e.g., sugars, organic acids, ions, pH), and 4) decrease in the effectiveness of the "barrier" functions of cell walls and cuticles (Saladie et al. 2007, Cantu et al. 2008b; Cantu et al. 2009).   In horticulturally important fruits, all of these factors affecting pathogen

susceptibility are likely to be involved, with some being more important than others, depending on the fruit-pathogen combination being considered.

The disassembly of cell walls during *Botrytis*'s infection of ripening tomato fruits: The fruit and pathogen share a strategy

In our work on the interaction of ripening tomato fruit and *Botrytis cinerea* (*Botrytis*, henceforth), most of our focus has been on plant cell wall metabolism. Plant pathologists know that the breakdown of host cell walls is an aspect of the confrontation between the host and pathogen that is initiated soon after infection (Cantu et al. 2008b, Underwood 2012). Furthermore, because the fruit is ripening, it will be active in modifying its own cell walls and this process can provide assistance for the pathogen's development on fruit.

We have tested these ideas in our work on the PG/Exp double suppressed tomatoes. These fruit not only soften more slowly than do wild-type fruits; their *Botrytis* susceptibility is almost abolished as they ripen (Cantu et al., 2008a). Why this should be is not immediately obvious. The *Botrytis* genome contains many genes that encode CWMPs (discussed below, Blanco-Ulate et al. 2014); therefore, why would it need help from the plant's PG and Exp to be effective in establishing infection on a ripening tomato fruit?

One of the answers may be related to a class of pre-formed defense factors, the PG-inhibiting proteins (PGIP; De Lorenzo and Ferrari 2002). These proteins have been identified in many species of plants and often are present at a relatively high level in fruit tissues (De Lorenzo et al. 2001, Di et al. 2006). Our first work with PGIP came serendipitously as we were trying to understand why 'Bartlett' pear fruits became more susceptible to pathogens as they ripened (Abu-Goukh et al. 1983, Abu-Goukh and Labavitch 1983). That study led to the purification of the pear PGIP (pPGIP), a protein that was an inhibitor of PGs from several fruit pathogens, including some but not all, of the PGs from *Botrytis* (Sharrock and Labavitch, 1994; Stotz et al. 2000). Our work with pPGIP eventually led to the cloning of the pPGIP-encoding gene and then a gene encoding the tomato fruit's PGIP (tPGIP, Stotz et al., 1993, 1994). The tPGIP also inhibited some of *Botrytis*'s PGs, but its inhibition was not as strong as that of pPGIP. Our efforts to purify these fruit PGIPs led us to recognize that they were cell wall-associated in unripe fruits, but as the pears or tomatoes ripened their PGIPs lost their cell wall association. Studies of the bean PGIP also indicate that the protein is localized in the apoplast of bean vegetative tissues and is cell wall-associated (Toubart et al.1992). Our early work with pPGIP showed that it selectively bound to pectin-like insoluble matrices (Egli et al., unpublished), an observation that led us to assume that in unripe fruits it was generally associated with the cell wall's pectin polymers.

PGIP expression is induced by infections of *Botrytis* and other pathogens in fruit. We validated that the over-expression of pPGIP reduces *Botrytis* growth on tomato fruit (Powell et al. 2000). Like the other plant PGIPs that have been studied, pPGIP and tPGIP do not inhibit the plant's own PGs. This also seemed logical; after all, the PGIP's job is to protect the pectins against pathogens so that the fruit can contribute to seed development and, subsequently, go through ripening in support of seed dispersal. Thus, one hypothesis to explain the increased pathogen resistance of the PG/Exp-minus tomatoes is tied to the

idea that the fruit PGIP's inhibition of *Botrytis*'s PGs normally will slow down the growth of the pathogen on unripe fruit tissue. However, as ripening begins the fruit's PG, which is not inhibited by PGIP, digests the fruit wall's pectin. This, in turn, disrupts cell wall integrity and the PGIP's binding to the pectin components, eliminating the protection against pathogen attack that the wall generally provides. However, if the ripening fruit does not produce its own PG, the pectin in the fruit remains almost intact, as does the defense provided by the tPGIP. Because the fruit's Exp contributes to the fruit's self-digestion of pectin, when the fruit expression of both PG and Exp is suppressed, the protection may be even stronger, as shown in Cantu et al. (2008).

A second hypothesis about why pathogen defense is stronger in the PG/Exp-minus fruit is related to the fact that fruit ripening is associated with increasing susceptibility. If PDOs do play a "local" role in promoting ripening by stimulating the synthesis of ethylene (as discussed above), then it is easy to imagine that when pectin metabolism is reduced, either because no fruit PG is made or because *Botrytis*'s PGs are inhibited by PGIP (or both), ripening would slow down. Furthermore local concentrations of ethylene could be reduced: with reduced PG action there would be lower concentrations of PDOs, so that oligosaccharide signal impact on ethylene synthesis could be reduced.. If less ethylene is present the fruit's shift into "system 2" ethylene synthesis will be delayed as will the progress of fruit ripening, along with the associated increase in pathogen susceptibility. (**Note**: While it is clear that the suppression of PG and Exp expression in ripening tomatoes is coupled to elevated fruit defense against *Botrytis*, these hypotheses have not been rigorously tested in other tomato fruit-pathogen interactions.)

Cell walls are barriers that restrict the colonization of plant tissues by invaders, but also are important reservoirs of energy-rich sugars for pathogen growth. Fungal pathogens, such as *Botrytis*, secrete a large repertoire of enzymes to disrupt the polysaccharides in their plant host's cell walls (van Kan et al. 2006). We have described the annotation of putative secreted Carbohydrate-Active enZymes (CAZymes) identified in the *Botrytis* genome (strain B05.10 v.1; Amselem et al. 2011), which may be important for the degradation of the cell walls of ripening fruit (Table 1; Blanco-Ulate et al. 2014). CAZymes are proteins that are predicted to participate in polysaccharide and oligosaccharide disassembly (i.e., carbohydrate binding modules, esterases, glycoside hydrolases and polysaccharide lyases) or polysaccharide synthesis and assembly (i.e., glycolsyltransferases; Cantarel et al., 2009). Using a transcriptomic approach (i.e., RNASeq), we identified *Botrytis* "CAZyme"-encoding genes that were expressed by the pathogen when infecting ripe grape berries and tomatoes. We also compared the expression levels of the *Botrytis* CAZymes expressed in fruits with those expressed during infections of lettuce leaves. This allowed us to determine if the pathogen's wall disruption strategy varied when the infection target was a vegetative or fruit tissue. While primary cell walls contain a combination of pectins, HC and cellulose microfibrils, available data indicate that fruit cell walls are relatively enriched in pectin polymers while lettuce leaves have less pectin and more HC and cellulose (Wagstaff et al. 2010; Nunan et al. 1998; Lunn et al. 2013). On the three hosts, *Botrytis* expressed a common group of 229 potentially secreted CAZymes, which included pectin backbone-modifying enzymes, HC-modifying proteins, enzymes that might target pectin and HC side-branches, and enzymes that could degrade cellulose (Blanco-Ulate et al. 2014). We found

Table 1. *Botrytis* CAZymes with potential roles in plant cell wall disassembly (Adapted from Blanco-Ulate et al. 2014)

Plant cell wall target	CAZyme subfamily	Functional annotation	Number of potentially secreted # of proteins
Pectin backbones	GH28	Polygalacturonases	11
	PL1	Pectin lyases	4
	PL1, PL3	Pectate lyases	4
	CE8	Pectin methylesterases	3
	GH28, GH105|GH28	Rhamnogalacturonan (RG) hydrolases	6
	GH78	α-L-Rhamnosidases	2
	CE12	RG acetylesterases	1
Hemicellulose backbones	GH3	β-Glucosidases	6
	GH12	Xyloglucan (XyG)-specific β-glucanases	1
	GH16, GH16|CBM18	Glucanases and XyG transglycosylase/hydrolases	11
	GH10, GH11, GH10|CBM1, GH11|CBM1	β-Xylanases	5
	GH43	β-Xylosidases	3
	GH5|CBM1	β-Mannosidases	1
	GH26, CBM3|GH26|CBM35|GH44	β-Mannanases	2
Cellulose	GH5, GH5|CBM1, GH45	1,4-β-Glucanases	10
	GH6|CBM1, GH6|CBM2, GH7, GH7|CBM1	Cellulose 1,4-β-cellobiosidases	5
Polysaccharide side-chains	GH2, GH35	β-Galactosidases	3
	GH31	α-Xylosidases	2
	GH43, GH93	α-L-1,5-Arabinanases	2
	GH47, GH92	α-Mannosidases	4
	GH51, GH54|CBM42, GH62|CBM13	α-Arabinofuranosidases	4
	GH53	Arabinogalactan β-galactosidases	1
	GH95	α-L-Fucosidases	1
	GH115	Xylan α-1,2-glucuronosidases	1

that 36 genes encoding putative CAZymes with secretion signals were expressed exclusively when *Botrytis* interacted with ripe fruit. The results of this study indicated that *Botrytis* targets similar wall polysaccharide networks on fruit and leaves, particularly the pectin components. Therefore, the diversity of the *Botrytis* pectin-degrading enzymes may be partly responsible for its wide host range.

We previously reported that *Botrytis* infections in unripe tomatoes induced the premature expression of plant CWMPs involved in fruit softening Cantu et al. 2009). In particular, genes encoding enzymes that target pectin backbones, such as PG and rhamnogalacturonan hydrolases, appear to be up-regulated as a result of *Botrytis* infection (Cantu et al. 2009; Blanco-Ulate et al., unpublished). These observations suggest that fungal and plant CWMPs cooperate in the effective disassembly of the plant cell wall during *Botrytis* infections of tomato fruit; but also make it clear that the ripening fruit's enzymatic machinery promotes extensive pectin degradation, thus treating *B. cinerea* as a welcome guest!

What we need to know now

The overall aim of our studies is to dissect how the tomato fruit coordinates and performs all of the metabolic and structural biology aspects of ripening and how these regulatory and developmental events affect susceptibility to post-harvest pathogens. This information would help us determine if there is a way to disconnect (1) the events that are required for maximizing the storage potential and fruit flavor and nutritional qualities of harvested fruits from (2) the changes in ripening fruits that result in their deterioration and loss on the way to a consumer's table. We discussed aspects of cell wall metabolism in pathogen-free tomato fruits that have begun to ripen and the wall changes that occur when ripening fruit are infected by *Botrytis*. It is fair to conclude that both tomato fruit and *Botrytis* focus their attention on the fruit's pectin polysaccharides and use many of the same types of enzymes to modify these substrates. Although the pathogen is likely to digest cell wall polysaccharides more completely in order to support its need for energy and biomass production, the fruit's CWMP enzymes are produced jn abundance and cause major changes in the fruit cell wall structures. An important component of the fruit's major pectin-digesting enzymes (i.e., PG) is needed by *Botrytis* to support its early establishment on fruit that have begun to ripen. This suggests that the wall modification processes that contribute to the ripe fruit texture enjoyed by consumers are biochemically very like the processes that support disease development. Before we can separate fruit susceptibility from softening, we, as post-harvest biologists, must understand the regulatory aspects of fruit ripening. We have evaluated the pathogen susceptibility phenotypes of tomato fruit with mutations in the main ripening-related transcription factors, *RIN* (*RIPENING INHIBITOR*, a MADS-box transcription factor, TF), *NOR* (*NON-RIPENING*, a NAC domain TF) and *CNR* (*COLORLESS NON-RIPENING*, an SPB-box TF) (Cantu et al., 2009, Blanco-Ulate et al., unpublished). We have transcriptomic data that suggest roles for several plant hormones (ethylene, abscisic acid, salicylic acid and jasmonic acid) in ripening and infected tomatoes (Blanco-Ulate et al. 2013) and, of course we have a continuing interest in aspects of cell wall metabolism. However, ripening is a coordination of many metabolic and structural/developmental pathways. Our co-author Ann Powell and several others have reported on the important

enhancements of tomato fruit nutritional and other characteristics that are influenced by the *Golden 2-like* transcription factor as ripening-associated events convert chloroplasts to chromoplasts (Powell et al., 2012). Giovannoni, Rose, Seymour and other colleagues are delving into the roles of other tomato transcription factors and the impacts on fruit ripening (Gapper et al. 2014; Seymour et al. 2013). Furthermore, a much larger community of molecular biology researchers is learning more details of the mechanisms and players involved in the complex regulatory networks that control most aspects of plant development and metabolism. Perhaps a strategy to separate tomato ripening and softening from the ripening fruit's increasing susceptibility to pathogens will not be identified; however, what we learn during the quest should have important impacts on fundamental biology and postharvest applications that are useful for all of us.

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