https://doi.org/10.1093/plphys/kiac469 PLANT PHYSIOLOGY 2023: 191: 21–23

Plant Physiology®

News and Views

Killing me softly: A pathogen accelerates fruit ripening and softening to cause disease

Igor Cesarino [1,2,*]

1 Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-090 São Paulo, Brazil
2 Synthetic and Systems Biology Center, InovaUSP, Avenida Professor Lucio Martins Rodrigues, 370, 05508-020 São Paulo, Brazil

*Author for correspondence: icesarino@usp.br

Host–pathogen dynamics change considerably during the course of tissue development; for example, many tissues become more susceptible to necrotrophic pathogens during senescence. In the case of fruits, susceptibility to such pathogens dramatically increases during ripening prior to senescence (Alkan and Fortes, 2015). Although immature and unripe fruits are normally resistant to disease, some fungal pathogens can establish quiescent infections during these early developmental stages (Prusky et al., 2013). During fruit ripening, a complex combination of biophysical, physiological, transcriptional, and biochemical changes occurs, providing a favorable environment that allows the pathogen to emerge from quiescence, switch to the necrotrophic lifestyle, and cause decay of fruit tissues (Prusky et al., 2013; Blanco-Ulate et al., 2016b). Therefore, understanding the molecular mechanisms associated with increased susceptibility to fungal diseases during fruit ripening is essential to help ensure food security.

Given that the cell wall functions as a physical barrier and source of plant defense molecules, the disassembly of this structure during fruit softening is one of the most important contributors to pathogen susceptibility (Brummell, 2006; Prusky et al., 2013; Blanco-Ulate et al., 2016a; Wang et al., 2022). Fruit cell walls are enriched in pectin when compared with hemicellulose and cellulose. During ripening, pectins degrade, the network of hemicellulose and cellulose loosens, and structural proteins release. These changes, in addition to the hydration of the walls of pericarp cells, lead to increased cell wall porosity and fruit softening (Brummell, 2006; Vicente et al., 2007).

The positive relationship between fruit ripening and pathogen susceptibility is supported by the observation that tomato (*Solanum lycopersicum*) genotypes defective for various cell wall degrading enzymes (CWDEs) and related proteins show reduced susceptibility to the necrotrophic pathogen *Botrytis cinerea* (Cantu et al., 2008; Silva et al., 2021). Some studies suggest that fungal pathogens actively induce fruit ripening and cell wall disassembly as an infection strategy. For instance, *B. cinerea* triggers the biosynthesis of the ripening-promoting hormone ethylene and induces the expression of genes encoding CWDEs in unripe tomato fruits (Cantu et al., 2008, 2009; Silva et al., 2021). However, our understanding of the mechanisms by which fungal pathogens induce ripening to cause disease in fruits remains limited.

In this issue of *Plant Physiology*, Silva et al. (2022) report on a comprehensive analysis to determine whether and to what extent *B. cinerea* actively induces ripening-related processes to accelerate infection and promote disease in tomato fruits. To confirm that *B. cinerea* infection accelerates ripening progression, the authors initially assessed the ripening rate in mock-inoculated and *B. cinerea*-inoculated unripe (mature green, MG) fruits 3–6 days post-inoculation (dpi), analyzing three characteristic physiological processes: external color progression, ethylene production, and loss of fruit firmness. They found that *B. cinerea* infection accelerated all three measured parameters. Pathogen-inoculated fruits turned red (Figure 1) and lost firmness significantly faster and showed a dramatic increase in ethylene synthesis when compared with mock-inoculated fruits. These results confirmed that *B. cinerea* infection accelerates ripening processes in tomato fruit.

To identify genes involved in the accelerated ripening induced by fungal infection, the authors performed RNAseq analysis in mock-inoculated, *B. cinerea*-inoculated, and healthy MG fruits 3 dpi or 3 days post-harvest (dph). This

Received September 7, 2022. Accepted September 13, 2022. Advance access publication October 11, 2022

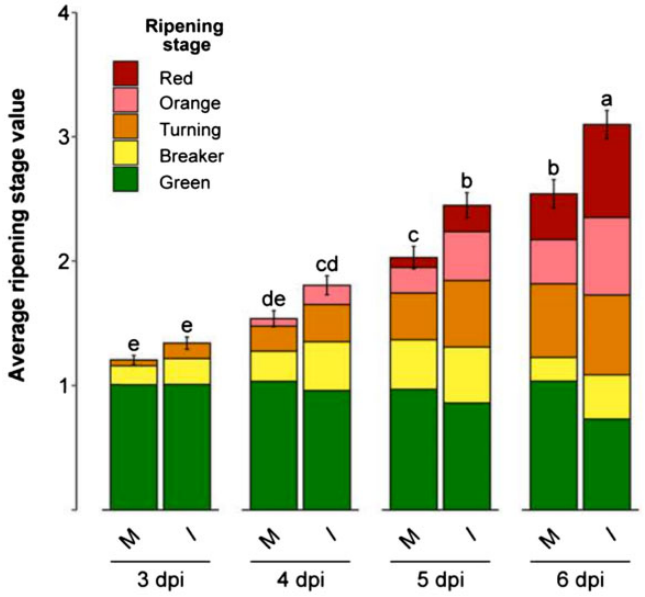

Figure 1 *Botrytis cinerea* accelerates fruit ripening to cause disease. Ripening stage assessed by color in mock-inoculated (M) and pathogen-inoculated (I) MG fruits 3–6 dpi. Colored bars represent the proportion of fruits in each ripening stage according to the color key. This figure was adapted from Figure 1 of Silva et al. (2022). Error bars indicate standard deviation. Letters indicate statistical differences ($P < 0.05$) between each treatment across all dpi using ANOVA followed by Tukey's HSD ($n = 174$). HSD: Honestly Significant Difference.

transcriptomic analysis was complemented with the incorporation of two existing datasets: (i) an identical sample set to the 3 dpi but sequenced at 1 dpi to evaluate genes potentially induced earlier during the inoculation and (ii) samples of healthy fruits at five developmental stages from MG to red ripe (RR) to evaluate ripening-related genes. The authors first focused on the expression of genes related to three key functional categories: carotenoid biosynthesis, ethylene biosynthesis, and CWDEs. Curiously, genes belonging to the first category were not significantly induced upon *B. cinerea* infection, despite the enhanced color progression caused by the fungus. Conversely, *B. cinerea* inoculation resulted in upregulation of genes involved in ethylene biosynthesis, which might trigger the ripening process, and in activation of host CWDE expression, particularly pectin-related enzymes, facilitating the disassembly of the fruit cell walls.

The authors then focused on genes commonly affected by *B. cinerea* infection and the natural ripening process. They performed enrichment analyses of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations and found the most significantly enriched pathways among the commonly upregulated genes were "plant–pathogen interaction" and "proteasome." "Alpha-linolenic acid metabolism" was also enriched and includes genes involved in biosynthesis of jasmonic acid, a hormone that also positively regulates ripening and pathogen responses. Commonly downregulated genes were enriched in various photosynthesis-related pathways, which is in line with decreased fruit photosynthetic capacity due to ripening. The overall overlap between genes induced by *B. cinerea* inoculation and ripening-related genes indicates that the pathogen activates multiple ripening processes.

Given that cell wall disassembly has a major impact on disease outcome by facilitating fungal colonization, the authors performed cell wall glycome profiling to compare the cell wall changes induced by *B. cinerea* inoculation and those that naturally occur during ripening. A total of 112 of 144 monoclonal antibodies (mABs) against diverse epitopes present in distinct cell wall polysaccharides showed significant fold changes for both *B. cinerea* inoculation and ripening, suggesting a high similarity in cell wall polysaccharide changes caused by fungal inoculation and fruit ripening. Remarkably, similar patterns of overrepresented polysaccharide classes among these mABs were observed between the samples, particularly mABs associated with the rhamnogalacturonan I backbone of pectins and the hemicellulose arabinogalactan, and most showed increased binding (positive fold changes). These results indicate that *B. cinerea* inoculation and ripening increase access to pectin polymers, leading to cell wall disassembly.

In addition to triggering the expression of host CWDEs, *B. cinerea* also employs its own CWDEs, particularly pectin-degrading enzymes, to facilitate infection. To identify fungal CWDEs that likely contribute to host cell wall disassembly, the expression of genes known to encode *B. cinerea* pectin-degrading enzymes was measured in fruits 1 and 3 dpi. Four genes showed high relative gene expression: two encoding polygalacturonases (PGs) and two encoding pectin methylesterases. Various fungal mutant combinations knocking out one or both types of enzymes were tested for their virulence in MG fruits, and only the mutant with a loss-of-function of the two PGs showed a loss of virulence. PGs depolymerize pectin chains by hydrolyzing glycosidic bonds present in the polymeric backbone. The PG double-mutant also failed to substantially induce ripening by 6 dpi, suggesting that it lost critical virulence factors to establish and survive in MG fruits. Additionally, fungal biomass in MG fruits inoculated with the PG double-mutant was only two times greater 20 dpi when compared with 3 dpi, whereas direct inoculation of RR fruits resulted in a much faster growth. These results suggest that *B. cinerea* survival necessarily demands the induction of fruit ripening and that this infection strategy depends on fungal PGs.

The work of Silva et al. demonstrates that fungal induction of fruit ripening enables *B. cinerea* to emerge from quiescence and cause disease in tomato. The observation that the pathogen induces multiple ripening-related processes will allow future identification of ripening-promoting virulence factors, ultimately enabling biotechnological strategies to improve fruit resistance to pathogens.

Conflict of interest statement. None declared.

References

Alkan N, Fortes AM (2015) Insights into molecular and metabolic events associated with fruit response to post-harvest fungal pathogens. Front Plant Sci **6**: 1–14

Blanco-Ulate B, Labavitch JM, Vincenti E, Powell ALT, Cantu D (2016a) Hitting the wall: plant cell walls during *Botrytis cinerea* infections. *In* S Fillinger, Y Elad, eds, Botrytis—The Fungus, the Pathogen and Its Management in Agricultural Systems. Springer International Publishing, Cham, pp 361–386

Blanco-Ulate B, Vincenti E, Cantu D, Powell ALT (2016b) Ripening of tomato fruit and susceptibility to *Botrytis cinerea*. *In* S Fillinger, Y Elad, eds, Botrytis—The Fungus, the Pathogen and Its Management in Agricultural Systems. Springer International Publishing, Cham, pp 387–412

Brummell DA (2006) Cell wall disassembly in ripening fruit. Funct Plant Biol **33**: 103–119

Cantu D, Blanco-Ulate B, Yang L, Labavitch JM, Bennett AB, Powell ALT (2009) Ripening-regulated susceptibility of tomato fruit to *Botrytis cinerea* requires NOR but not RIN or ethylene. Plant Physiol **150**: 1434–1449

Cantu D, Vicente AR, Greve LC, Dewey FM, Bennett AB, Labavitch JM, Powell ALT (2008) The intersection between cell wall disassembly, ripening, and fruit susceptibility to *Botrytis cinerea*. Proc Natl Acad Sci USA **105**: 859–864

Prusky D, Alkan N, Mengiste T, Fluhr R (2013) Quiescent and necrotrophic lifestyle choice during postharvest disease development. Annu Rev Phytopathol **51**: 155–176

Silva CJ, van den Abeele C, Ortega-Salazar I, Papin V, Adaskaveg JA, Wang D, Casteel CL, Seymour GB, Blanco-Ulate B (2021) Host susceptibility factors render ripe tomato fruit vulnerable to fungal disease despite active immune responses. J Exp Bot **72**: 2696–2709

Silva CJ, Adaskaveg JA, Mesquida-Pesci SD, Ortega-Salazar IB, Pattathil S, Zhang L, Hahn MG, van Kan JAL, Cantu D, Powell ALT, et al. (2022) *Botrytis cinerea* infection accelerates ripening and cell wall disassembly to promote disease in tomato fruit. Plant Physiol **191**: 575–590

Vicente AR, Saladié M, Rose JK, Labavitch JM (2007) The linkage between cell wall metabolism and fruit softening: looking to the future. J Sci Food Agric **87**: 1435–1448

Wang D, Yang L, Silva CJ, Sarwar R, Chen Y, Blanco-Ulate B (2022) Pectin-mediated plant immunity. *In* Annual Plant Reviews Online. John Wiley & Sons, Ltd, pp 55–80