Plant cell walls: barriers to postharvest disease

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Postharvest Course

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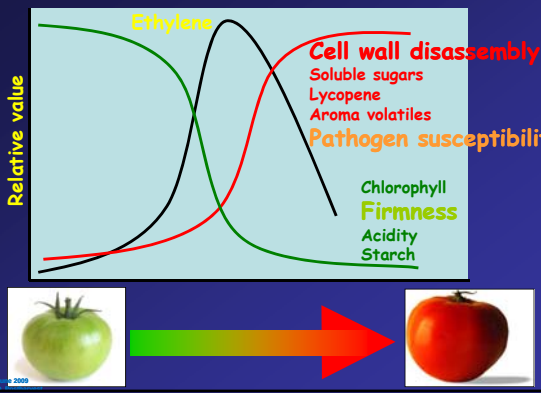
The postharvest commodity

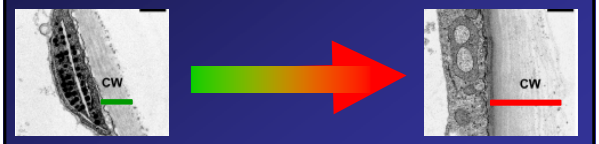
Ripening tomato fruit: an important commodity and a model for climacteric fruit ripening

During fruit ripening

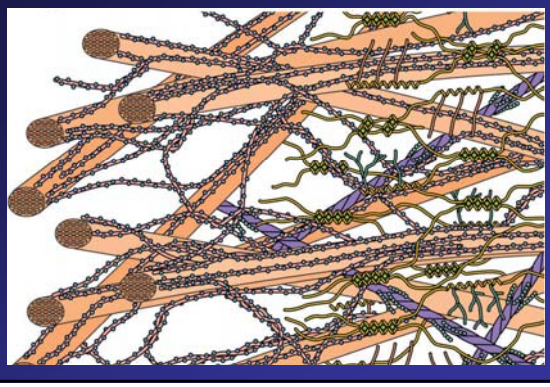


The fruit cell wall changes during ripening

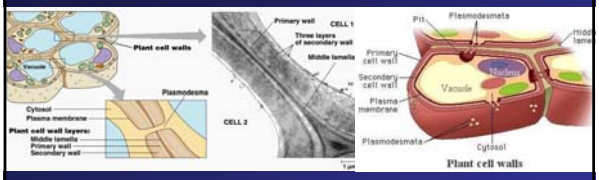
Cell walls in fruit flesh swell
Cell walls are disassembled

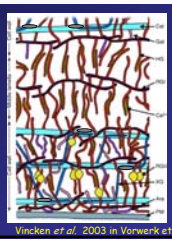


Cell walls are comparable to a fabric woven from many threads



The plant cell wall





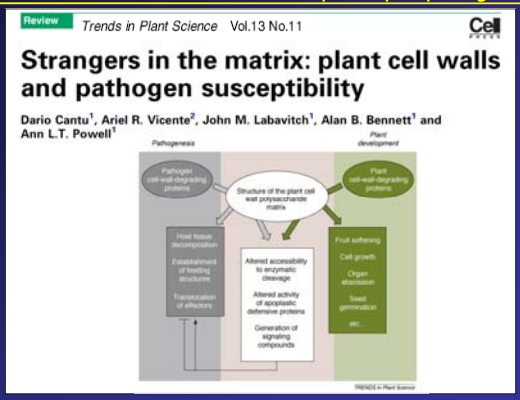
The cell wall polysaccharide matrix

- Cellulose microfibrils — Expansin,
- Hemicelluloses (xyloglucans) — XTH
- Pectin (HGA, RGI, RGII)

Polygalacturonases (PG),
PME, PLs, RGases

Vincken *et al.* 2003 in Vorwerk et al. 2004

Plant cell wall architecture and susceptibility to pathogens



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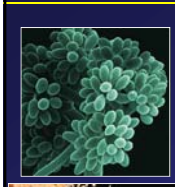
Strangers in the matrix: plant cell walls and pathogen susceptibility

Dario Cantu[1], Ariel R. Vicente[2], John M. Labavitch[1], Alan B. Bennett[1] and Ann L.T. Powell[1]

Botrytis cinerea

The pathogen



Botrytis cinerea (*gray mold*)

Infects more than 200 host plants

Model organism for pathogens that thrive on dying tissues

Genomes of two isolates have been sequenced and annotated

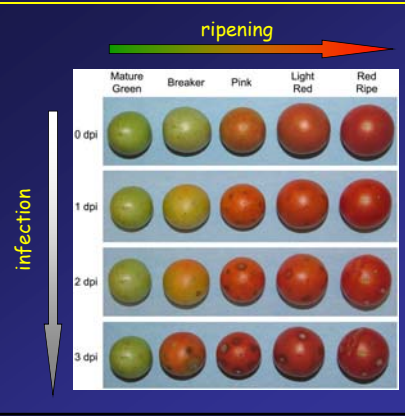
Many knocked-out mutants are available (→ known virulence factors)

Tomato fruit susceptibility to *B. cinerea*

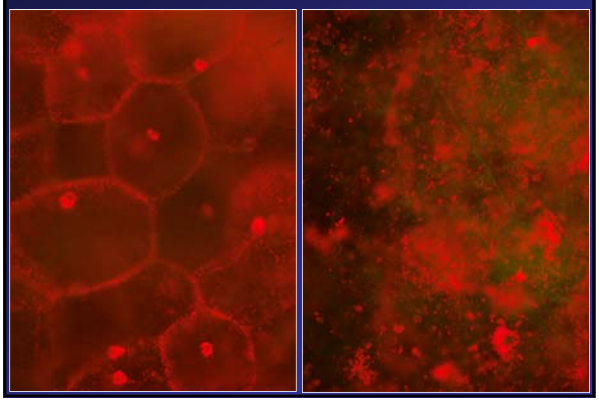
Ripening tomato fruit: pathogen susceptibility coincides with ripening.

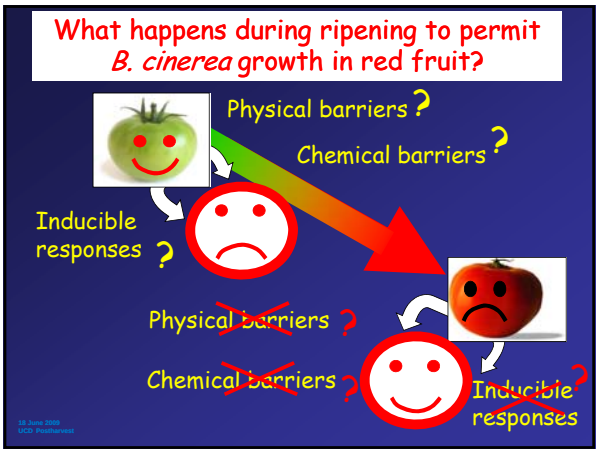
Susceptibility to pathogens increases

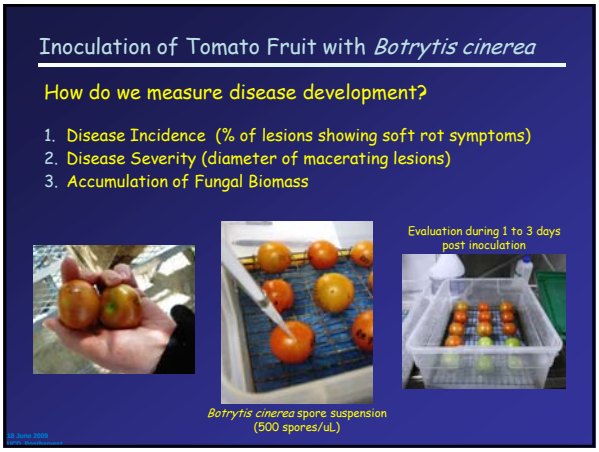
The ripening acquired susceptibility to *Botrytis cinerea*

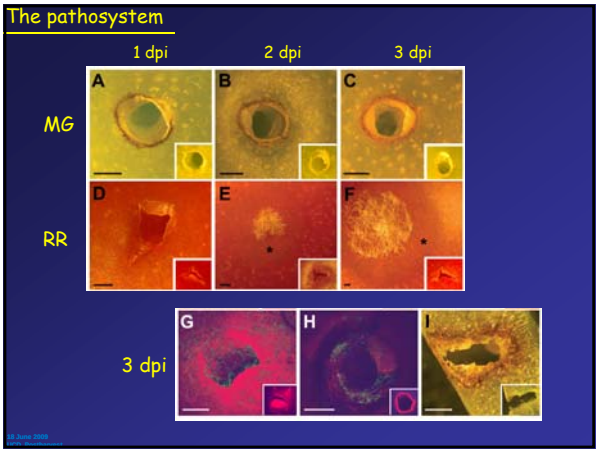


Botrytis cinerea

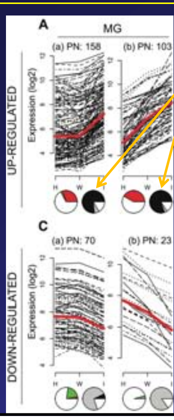



What happens during ripening to permit
B. cinerea growth in red fruit?
Physical barriers ?
Chemical barriers ?
Inducible responses ?
Physical barriers ?
Chemical barriers ?
Inducible responses ?


Inoculation of Tomato Fruit with Botrytis cinerea
How do we measure disease development?
1. Disease Incidence (% of lesions showing soft rot symptoms)
2. Disease Severity (diameter of macerating lesions)
3. Accumulation of Fungal Biomass
Evaluation during 1 to 3 days post inoculation
Botrytis cinerea spore suspension (500 spores/uL)

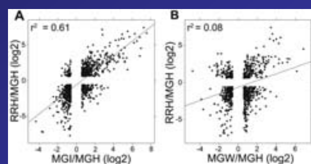

The pathosystem
1 dpi
2 dpi
3 dpi
MG
RR
3 dpi
A
B
C
D
E
F
G
H
I

Botrytis induces expression of ripening functions



Genes induced during normal fruit ripening

MOST OF THE TRANSCRIPTIONAL CHANGES CAUSED BY *BOTRYTIS* ARE ALSO INDUCED BY NORMAL RIPENING



Cantu et al. 2009. Ripening-regulated susceptibility of tomato fruit to *B. cinerea* requires *NOR* but not *RIN* or ethylene. Plant Physiology, in press

Proteome Analysis

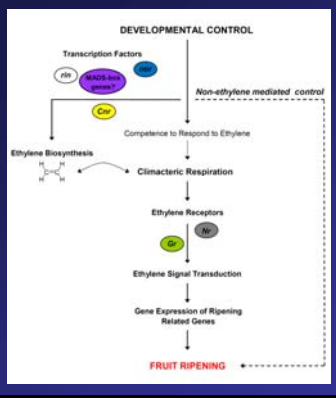
What proteins are in the microenvironment of fruit infected by *B. cinerea*?

Proteins predicted by transcriptome analysis

Table 3. Identified tomato cell wall-related proteins

Sub-categorie	Gene Id	Putative Function	SignalP	MG Score	MG Spectra	MG Peptide	RR Score	RR Spectra	RR Peptide	*rin* Score	*rin* Spectra	*rin* Peptide
Cell wall related proteins	SGN-U312757	Pectin methylesterase	0.966 Y	937	48	18	6464	2157	98	5940	1839	90
	SGN-U312703	Polygalacturonase	0.992 Y	1027	41	21	6704	1540	107	2278	206	40
	SGN-U312758	Pectin esterase	0.002 N	449	18	8	2248	339	34	1654	171	27
	SGN-U314662	beta-fructosidase	0.000 N	1350	55	27	2515	312	44	1655	72	31
	SGN-U313725	Pectin methylesterase	0.009 N	519	24	11	1624	255	28	1582	305	30
	SGN-U314665	beta-fructosidase	0.000 N	764	45	18	1826	250	31	1125	49	21
	SGN-U318294	Pectin acetylesterase	0.998 Y	62	2	1	1216	106	21	931	99	15
	SGN-U313100	Extensin	0.000 N	64	2	2	907	44	19	602	35	14
	SGN-U319244	alpha-xylosidase	0.000 N	117	4	4	648	39	13	1035	64	21
	SGN-U312614	Arabinogalactan protein	1.000 Y	257	15	4	408	39	7	833	55	14
	SGN-U318232	Pectin esterase	0.252 N	0	0	0	611	31	10	432	26	8
	SGN-U313742	endo-beta-mannanase	0.019 N	0	0	0	662	29	10	172	6	3
	SGN-U320011	alpha-xylosidase	0.000 N	45	1	1	642	20	10	729	28	11
	SGN-U322781	Arabinogalactan protein	0.785 Y	380	13	6	496	19	9	800	31	14
	SGN-U336993	alpha-xylosidase	0.000 N	126	4	3	397	19	8	391	17	8
	SGN-U318102	Germin-like protein	0.982 Y	92	3	2	437	17	8	211	5	4
	SGN-U324003	PG 2A precursor	0.999 Y	269	12	5	250	16	5	393	22	7
	SGN-U319056	beta-galactosidase	0.020 N	22	1	1	536	15	11	73	2	2
	SGN-U313648	Pectin esterase	0.001 N	0	0	0	496	14	10	817	42	13
	SGN-U320073	alpha-galactosidase	1.000 Y	0	0	0	311	14	7	156	5	4
	SGN-U320295	alpha-xylosidase	0.001 N	269	9	8	304	14	7	717	39	14
	SGN-U314144	Glycosyl hydrolase	0.000 N	225	12	5	221	14	5	730	28	13
	SGN-U320398	Arabinogalactan protein	0.999 Y	379	14	7	454	12	8	485	22	9
	SGN-U315107	beta-galactosidase	0.013 N	0	0	0	303	10	5	147	4	4
	SGN-U319599	alpha-galactosidase	0.003 N	0	0	0	285	10	5	288	6	5
	SGN-U314637	Pectin methylesterase	0.986 Y	0	0	0	335	9	5	55	1	1
	SGN-U321036	alpha-glucosidase	0.000 N	0	0	0	317	9	7	346	15	7
	SGN-U313531	Pectin esterase	0.992 Y	0	0	0	286	8	5	145	5	3
	SGN-U316037	Glycosyl hydrolase	0.000 N	0	0	0	256	7	6	225	5	4
	SGN-U316373	beta-Ig-H3 domain-containing protein unknown	0.811 Y	0	0	0	131	4	2	261	8	5
	SGN-U326255	Hydroxyproline-rich glycoprotein	0.932 Y	0	0	0	99	4	2	0	0	0
	SGN-U322726	beta-galactosidase	0.362 N	0	0	0	212	3	3	36	1	1
	SGN-U312953	Expansin 1	0.998 Y	0	0	0	212	3	2	30	1	1
	SGN-U317347	Expansin-like	1.000 Y	0	0	0	134	3	2	68	1	1
	SGN-U319861	beta-fructosidase	0.681 Y	0	0	0	70	3	2	514	19	8
	SGN-U328864	beta-galactosidase	0.000 N	0	0	0	103	2	2	61	2	1
	SGN-U321477	Polygalacturonase	0.000 N	55	1	1	39	2	1	54	4	1
	SGN-U320881	Pectinacetylesterase	0.964 Y	0	0	0	74	1	1	58	2	1
	SGN-U330767	Pectinacetylesterase	0.846 Y	0	0	0	68	1	1	41	1	1
	SGN-U321329	Arabinogalactan protein	0.998 Y	52	1	1	48	1	1	27	1	1
	SGN-U322174	Germin-like protein	0.999 Y	69	1	1	37	1	1	102	2	2
	SGN-U317665	1,3 beta-glucosidase	0.995 Y	0	0	0	0	0	0	0	0	0
	SGN-U316315	Arabinogalactan protein	1.000 Y	0	0	0	0	0	0	249	8	5
	SGN-U319146	beta-D-glucan exohydrolase	0.000 N	0	0	0	0	0	0	63	1	1
	SGN-U345075	beta-D-glucan exohydrolase	0.015 N	0	0	0	0	0	0	59	1	1
	SGN-U312510	Expansin 10	1.000 Y	0	0	0	0	0	0	129	2	2
	SGN-U318084	Extensin	0.889 Y	63	4	1	0	0	0	64	4	1
	SGN-U313233	Pectin methylesterase	0.156 N	0	0	0	0	0	0	96	8	2

What aspects of ripening does *Botrytis* need to infect?



Fruit ripening is a complex network of pathways that result in fruit with many changed properties.

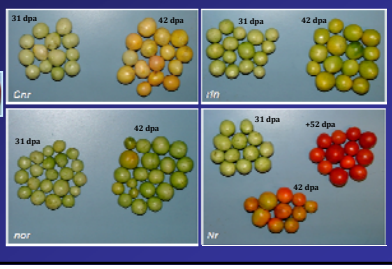
Fruit that do not ripen, might be expected to be resistant to *B. cinerea*.

Tomato Ripening Mutants

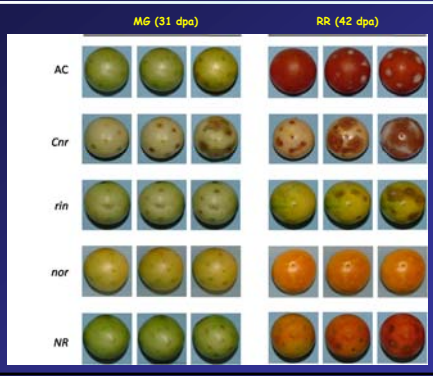
Mutant lines of tomato whose fruit ripening program has been perturbed:

- Fail to ripen as response to ethylene
- Do not turn red*
- Do not soften

cv. 'Ailsa Craig' Background

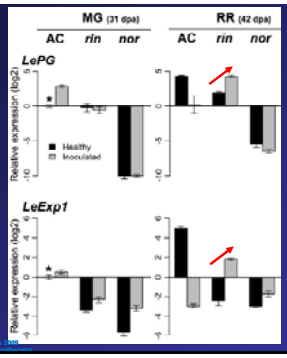


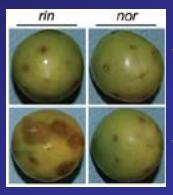
Susceptibility of Tomato Ripening Mutants to *Botrytis cinerea*



Plant cell wall degrading enzymes as susceptibility factors

Cell wall degrading proteins are induced in susceptible but not in resistant mutant fruit





"MG"

"RR"

Botrytis infection promotes ripening but requires only some aspects of ripening

- Most of the transcriptional changes caused by *Botrytis* are also induced by normal ripening.
- Some, but not all, ripening pathways and events render fruit susceptible.
- *LePG* and *LeExp1* are induced only in susceptible fruit and not in the resistant fruit.

What is the role of plant cell wall disassembly in the susceptibility of ripe fruit ?

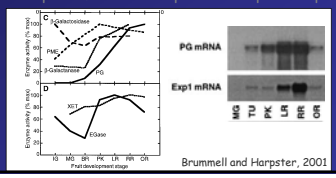
The plant cell wall

Ability of *Botrytis cinerea* to digest cell walls correlates with its virulence:

1. Botrytis has at least 6 PGs (BcPGs) that are differentially expressed both *in vitro* and *in planta* (Wubben et al. 1999 App Env Mic)
2. BcPG1 and BcPG2 are required for full virulence (ten Have et al., 1998 MPMI)
3. Most of these BcPGs show distinct product profiles when assayed *in vitro* (Kars et al. 2005 Plant J)
4. Plant susceptibility to Botrytis correlates with the expression of a protein that inhibits BcPGs called PGIP (PG-inhibiting protein, Powell et al., 2000 MPMI)

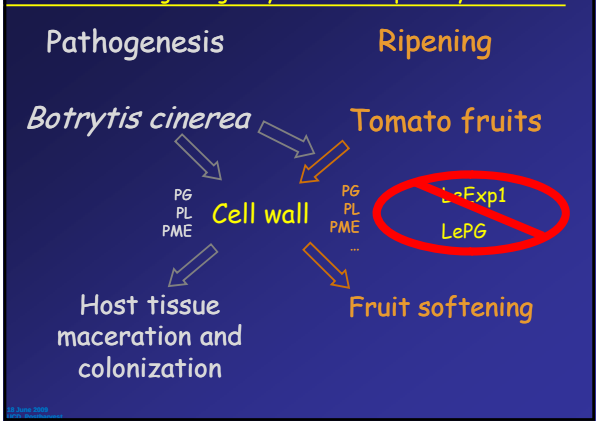
Plants disassemble their own cell walls (e.g., cell growth, fruit ripening)

Plant cell wall degrading proteins modify the composition and structure of the plant cell wall as part of the normal plant developmental programs

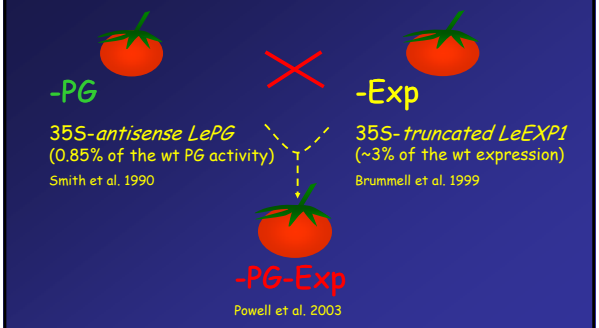


Brummell and Harpster, 2001

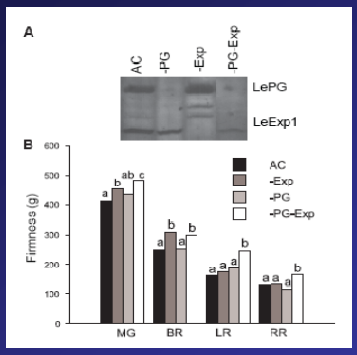
Plant cell wall degrading enzymes as susceptibility factors



What happens if ripe fruit cell wall disassembly is reduced?

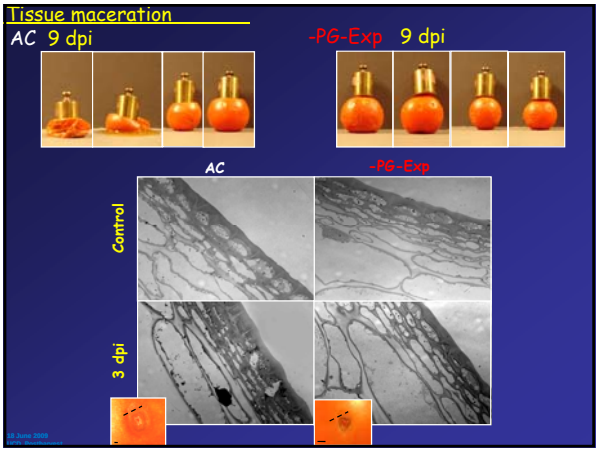


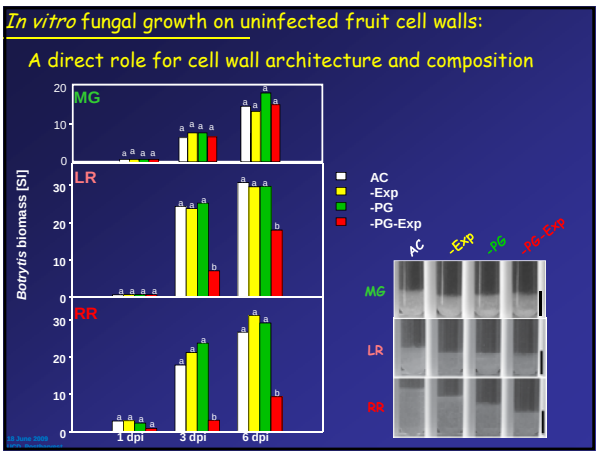
Reduced PG and Exp increase fruit firmness



Cantu et al. 2008. The intersection between cell wall disassembly, ripening and fruit susceptibility to *B. cinerea*. PNAS. 105: 859-864

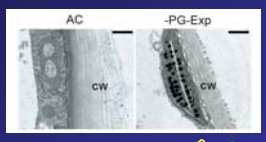

Disease Incidence
AC
-PG-Exp


Tissue maceration
AC 9 dpi
-PG-Exp 9 dpi
AC
-PG-Exp
Control
3 dpi


In vitro fungal growth on uninfected fruit cell walls:
A direct role for cell wall architecture and composition
MG
LR
RR
Botrytis biomass [SI]
1 dpi
3 dpi
6 dpi
AC
-Exp
-PG
-PG-Exp

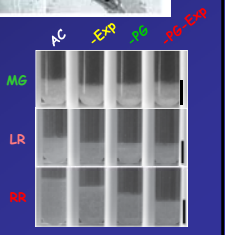
Plant cell wall architecture

How does the cell wall architecture influence the population of apoplastic proteins?



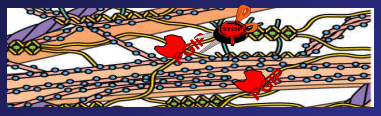
Apoplastic proteins:

- PR (pathogen response) proteins
- Cell wall remodeling proteins
- Defense proteins
- Receptors

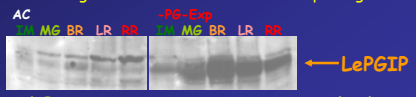


PG and Exp suppression reduces pectin depolymerization.....

What happens to proteins within the fruit cell wall?



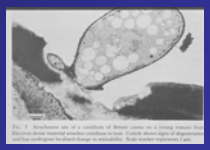
- PG inhibiting proteins (PGIPs)
  - Associated with pectins in the fruit cell wall
  - Inhibit some but not all *B. cinerea* PGs
  - Do not inhibit fruit ripening PGs
  - Present in green/immature fruit without pathogen challenge



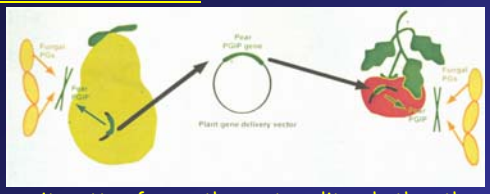
Does PG and Exp suppression cause PGIPs and other anti-fungal proteins to be retained at strategic positions?

Pathogen cell wall degrading functions

- *Botrytis* produces cell wall degrading enzymes (including PGs) to digest host cell walls
- Virulence factors are molecules or processes vital for full pathogen development on its hosts
- For *B. cinerea*, PG is a virulence factor



Another way to reduce cell wall degradation

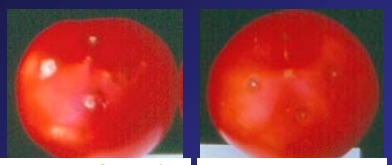


Does it matter for postharvest quality whether the wall-digesting enzymes are from the fruit or pathogen?

Clearly, the fruit's enzymes are important. Are the pathogen's enzymes important? YES! Control measures can target the enzymes that a fungus uses to attack fruit!

Powell et al. 2000. Transgenic expression of pear PGIP in tomato limits fungal colonization. *MPMI* 13: 942-950

Plant inhibition of pathogen cell wall degrading functions



Untransformed tomato (no pear PGIP)

Tomato transformed to express pear PGIP

Thus, PGIP expression reduces *Botrytis* virulence!

Plant cell wall degrading enzymes as susceptibility factors

Simultaneous suppression of fruit PG and Exp targeting the cell wall pectin network

Ripening-related cell wall changes

Inhibition of pathogen PGs, targeting the cell wall pectin network

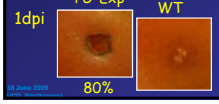
Reduced cell wall disassembly:

Difficult food source

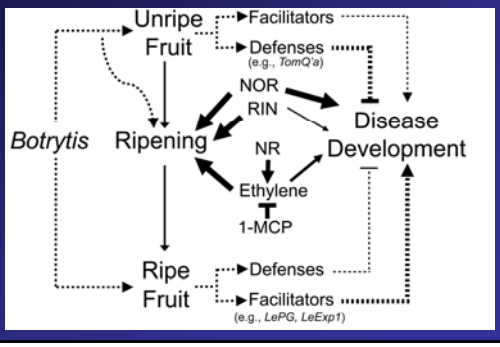
Efficient barrier to fungal penetration

Fungal growth

Inducible defenses



Events in the host tissue have a large impact on the outcome of the interaction between the plant and the pathogen



Key points

- To infect fruit, *B. cinerea* relies on some, but not all of the processes and events that occur during ripening
  - For postharvest improvement, knowing what ripening pathways are necessary for susceptibility is important
- Since *Botrytis* induces ripening in green fruit, the pathogen itself initiates the induction of susceptibility
  - For postharvest improvement, knowing how the fungus induces ripening is important
- Disassembly of the plant cell wall is crucial for susceptibility
  - For postharvest improvement, both plant and pathogen cell wall disassembling functions can be targeted
- Plant responses to a pathogen change during development
  - For postharvest improvement, the ripening stage is important